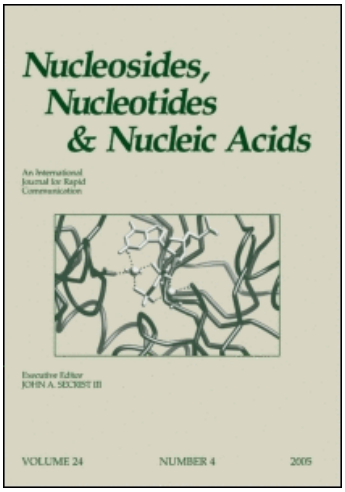

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Synthesis and Biological Evaluation of Antisense Oligonucleotides Containing Modified Pyrimidines

Y. S. Sanghvi[a]; G. D. Hoke[a]; M. C. Zounes[a]; S. M. Freier[a]; J. F. Martin[a]; H. Chan[a]; O. L. Acevedo[a]; D. J. Ecker[a]; C. K. Mirabelli[a]; S. T. Crooke[a]; P. D. Cook[a]
[a] ISIS Pharmaceuticals, Carlsbad, California, U.S.A.

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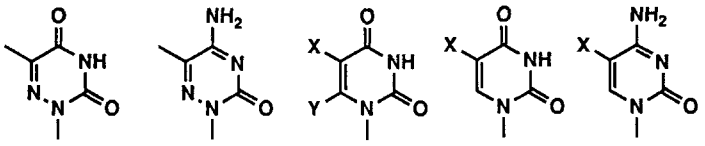
SYNTHESIS AND BIOLOGICAL EVALUATION OF ANTISENSE OLIGONUCLEOTIDES CONTAINING MODIFIED PYRIMIDINES

Y. S. Sanghvi*, G. D. Hoke, M. C. Zounes, S. M. Freier, J. F. Martin, H. Chan, O. L Acevedo, D. J. Ecker, C. K. Mirabelli, S. T. Crooke, and P. D. Cook

ISIS Pharmaceuticals, 2280-B Faraday Avenue, Carlsbad, California 92008, U.S.A.

Abstract: Antisense oligodeoxynucleotides (ODNs) containing certain pyrimidines modified at the 5 and/or 6-position(s) of the heterocycle were synthesized. The ODNs were evaluated for their resistance to nuclease degradation in serum, hybridization properties, and ability to activate RNase H.

Oligodeoxynucleotides (ODNs) targeted as antisense inhibitors of gene expression have increasingly become the focus of various strategies[1] to improve therapy for diseases such as cancer and AIDS. The current knowledge of antisense ODNs rest mainly on the modification of the phosphorus atom of the sugar backbone, providing structural classes such as phosphorothioates and methylphosphonates. Interestingly, the use of modified bases in antisense ODNs has received very little attention. We have begun to investigate alternative sites for chemical modifications of ODNs and have synthesized and evaluted ODNs containing pyrimidines modified at the 5 and 6-position(s). We were interested in enhancing the nuclease stability of ODNs without compromising their ability to hybridize.



General structure of pyrimidine modifications incorporated in to ODNs

We have chosen a short segment of 5-lipoxygenase 5'-untranslated region as a target molecule for our present studies. The target selection is based on several criteria, which includes reasonable representation of A, T, G, and C, lack of hairpin structures, and is a complementary sequence to a therapeutic target. Several antisense ODNs were synthesized with a chain length of 16-bases, wherein one to five bases were replaced by a modified pyrimidine.

Chemistry: Several novel monomers (for e.g. 6-aza T, 6-aza dC, 6-Me dU, and 5,6-diMe dU), synthesized in our laboratory, were suitably protected by conventional methods for their incorporation into antisense ODNs by an automated DNA synthesizer. An average coupling yield of 85-98% was obtained for all ODNs made. The ODNs were purified by preparative HPLC and final purity was checked by gel electrophoresis.

Hybridization: A variety of ODNs were evaluated for their hybridization properties at 4 μM concentration of each strand in 100 mM NaCl. Replacement of natural pyrimidine bases of a DNA of one strand of a mixed sequence Watson-Crick duplex resulted in a defined structrual activity relationship. When the 5-methyl group of T was replaced by F, Br, or I group, there was no significant effect on the stability of the duplex . Whereas addition of a methyl group (5,6 or 6-position) to dU, or T or a 6-azapyrimidine replacing T destabilized the duplex 2-5° C depending on the position of substitution within the sequence. Incorporation of 5-Br or 5-Me dC in place of a dC enhanced the stability of the DNA duplex.

Nuclease resistance: Among all of the ODNs studied, 6-aza T modified compounds were found to be most resistant towards nucleolytic degradation. When 6-aza T 3'-capped ODNs were assayed in fetal calf serum for nuclease degradation, a 12-fold increase (4 hr vs 0.33 hr) in half life over parent, unmodified ODNs, was observed.

Ribonuclease H cleavage: It has been shown that mRNA is cleaved by RNase H at the site of the RNA/DNA heteroduplex. This is thought to play a role in the mechanism of action of antisense ODNs. In the present study we have used a 6-aza T modified antisense ODN to 5-lipooxygenase mRNA and have clearly demonstrated that the modification did not alter the ability of E. *Coli* RNase H to cleave the mRNA strand.

Moleculer modeling: We calculated the Watson-Crick base pairing electrostatic interactions of A-T (-5.06 Kcal/mole) and A-6-aza T (-4.50 Kcal/mole), using the MOPAC (AM1) molecular orbital program. The X-ray crystal structure of 6-aza T has been shown to be present in an *anti* conformation. These two observations taken together suggested that replacing T with 6-aza T in an antisense ODN may not compromise the hybridization properties of the duplex.

Conclusions: Our results suggest that 6-aza T modified antisense ODNs are significantly resistant to nuclease degradation, while retaining hybridization properties similar to the parent unmodified ODNs. Furthermore, 6-aza T modified ODNs form heteroduplexes which activate RNase H degradation of the RNA. Further biochemical and pharmacological evaluation of 6-aza T and related modified ODNs is in progress.

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