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Recognition of Homopyrimidine Mismatches by Distance-Constrained Macrocyclic bisintercalators.

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ABSTRACT

Binding of three macrocyclic bisintercalators to mismatch-containing duplexes was analyzed by thermal denaturation experiments, electrospray mass spectrometry studies (ESI-MS) and fluorescent intercalator displacement (FID) titrations. The macrocyclic bisintercalators bind to duplexes containing mismatched thymine bases with high selectivity over the fully matched one and affinity in the submicromolar range (Kd). The FID results also demonstrate that the macrocyclic naphthalene derivative **BisNP** preferentially binds to pyrimidine–pyrimidine mismatches compared to all other possible base mismatches. This ligand also efficiently competes with a DNA enzyme (M.TaqI) for binding to a duplex with a TT-mismatch.

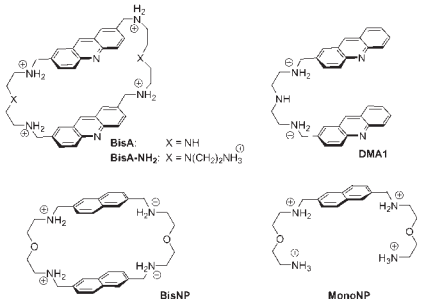
INTRODUCTION

Base mispairs can be hazardous to the cell in altering its ability to transfer the information content of DNA.[1] In particular, mismatches may result in point mutations which are potentially harmful, depending on where they occur in the genome. Consequently, every organism has evolved a variety of control and repair strategies based on complex enzymatic machineries responsible for the maintenance of DNA integrity Therefore, studies aimed at a deeper understanding of the recognition of mismatches by repair enzymes have raised continued attention for more than a decade. Several models have been proposed to rationalize the mechanisms of mismatch recognition, but these are still poorly understood. Given the complexity of these processes the task is highly challenging and requires several approaches, such as genetic, biochemical and chemical ones. In particular, a chemical tool for studying mismatch recognition is represented by small molecules that, similar to the mismatch-recognizing enzymes, can bind base mispairs with a high selectivity over fully paired DNA. Such mismatch-binding ligands may eventually interfere with the repair systems with negative or positive consequences, leading to inhibition or promotion of repair, and thus display high therapeutic potential.

Thus, in the past decade several series of mismatch-recognizing agents have emerged. Among these are molecular systems that operate via intercalation, such as rhodium-based metalloinsertors,[1] or via bisintercalation, such as bisnaphthyridine derivatives.[2] Minor groove binders such as imidazole-rich polyamides have also been shown to selectively bind to the GT mismatched sites. In another approach, we have shown that a macrocyclic bisacridine compound (**BisA**, Scheme 1) recognizes base-pairing defects, like abasic sites and thymine-containing mismatches, such as TT, TC and, to a lesser extent, TG-mismatched base pairs, via a putative threading bisintercalation mode.[3,4]

RESULTS AND DISCUSSION

In order to get deeper insight into the interaction of



Scheme 1: Structures of macrocyclic ligands and acyclic control compounds used in this study.

macrocyclic compounds, such as **BisA**, with mismatches, we carried out a systematic study aimed at the determination of structural factors that determine the binding, as well as the stoichiometric and thermodynamic parameters of the binding event. To achieve this goal, we extended the macrocyclic series by several analogues of **BisA** (scheme 1) and studied their mismatch-binding properties by a number of biochemical and spectroscopic methods. All tested macrocyclic ligands strongly stabilize the mismatch-containing duplexes, whereas their effect on the fully matched duplex **12-TA** is much less pronounced (figure 1).

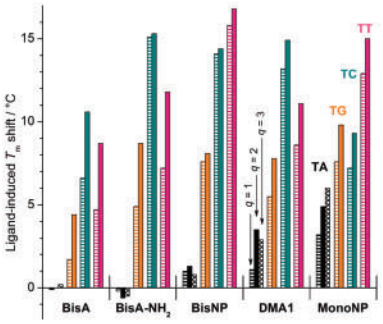


Figure 1. Ligand-induced changes of melting temperature (ΔT_m) of the fully matched (**12- TA**: black) and mismatch-containing 12 bp duplexes (**12-TG**: orange, **12-TC**: cyan, **12-TT**: magenta bars) at ligand-to-duplex ratios of $q = 1$ (horizontally hatched bars), $q = 2$ (filled bars) and $q = 3$ (cross-hatched bars, only for **12-TA** duplex); [**12-TX**] = 3 µM; estimated error in T_m determination is ±1.0 °C.

In contrast both control compounds, especially **MonoNP**, show strong stabilization of the fully matched duplex **12-TA** (ΔT_m = +1.1 and +3.2 °C at $q = 1$ for **DMA1** and **MonoNP**, respectively) which was further increased as the ligand concentration was raised (for **MonoNP** ΔT_m = +6.0 °C at $q = 3$). Altogether, thermal denaturation data indicate that control compounds have much lower selectivity for the mismatched as compared with the macrocyclic ligands. ESI-MS assay (data not shown) confirmed that BisNp is the best TT binder with a Kd in the nanomolar range (70nM).

The interaction of **BisNP** with other mismatches was further analyzed by FID screening of all the 16 possible base combinations (Figure 2). This experiment confirmed the high selectivity for homopyrimidine mismatches (TT, CC, and TC).

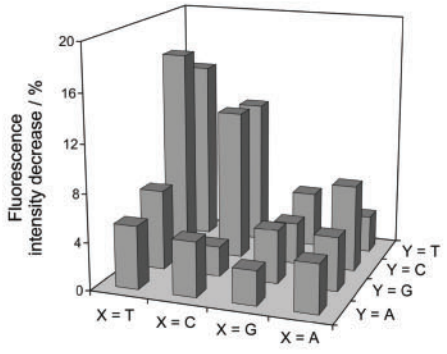


Figure 2: Relative fluorescence intensity decrease (excitation at 520 nm and emission at 615 nm) upon addition of **BisNP** (c = 120 nM) to all 16 **17-YX** duplexes (17bp duplexes, 100 nM each) in the presence of ethidium bromide (1 µM).

Finally we show that BisNP is able to significantly interfere with binding of the DNA methyltransferase M.TaqI, which recognizes a TT mismatch in its recognition sequence with nanomolar affinity.[5]

CONCLUSION

Recognition of DNA mismatches is under a focus of interest as this may give clues about the initial DNA recognition event(s) triggering the complex repair process. In addition, binding of mismatches by small molecules may provide novel therapeutic alternatives to anticancer therapies. The macrocyclic family studied in the present work thus represents a new class of very efficient and selective DNA mismatch binders. Therefore, these remarkable properties make our compounds valuable candidates to further investigate potential interference with repair enzymes that directly bind mismatched DNA.

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