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*Aza*Hx, a novel fluorescent, DNA minor groove and G·C recognition element: Synthesis and DNA binding properties of a *p*-anisyl-4-*aza*-benzimidazole-pyrrole-imidazole (*aza*Hx-PI) polyamide

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ABSTRACT

The design, synthesis, and DNA binding properties of *aza*Hx-PI or *p*-anisyl-4-*aza*-benzimidazole-pyrrole-imidazole (**5**) are described. *Aza*Hx, 2-(*p*-anisyl)-4-*aza*-benzimidazole-5-carboxamide, is a novel, fluorescent DNA recognition element, derived from Hoechst 33258 to recognize G·C base pairs. Supported by theoretical data, the results from DNase I footprinting, CD, ΔT_M, and SPR studies provided evidence that an *aza*Hx/IP pairing, formed from antiparallel stacking of two *aza*Hx-PI molecules in a side-by-side manner in the minor groove, selectively recognized a C–G doublet. *Aza*Hx-PI was found to target 5′-ACGCGT-3′, the Mlu1 Cell Cycle Box (MCB) promoter sequence with specificity and significant affinity (K_{eq} 4.0 ± 0.2 × 10^7 M^{-1}).

The design of small molecules capable of entering cells, concentrating in the nucleus, recognizing specific DNA sequences, and affecting gene expression continues to be at the forefront of life sciences and medical research.[1] One class of DNA, minor groove and sequence selective binding molecules that continues to receive significant attention is the imidazole (I) and pyrrole (P) polyamide analogs of distamycin **1** (Fig. 1) and netropsin.[2] It has been shown that replacement of the pyrrole moiety of distamycin with imidazole, which contains a nitrogen atom on the DNA facing-side of the molecule, is capable of accommodating the guanine-2-amino group that protrudes from the floor of the minor groove, and forming a hydrogen bond. This structural modification has enabled the polyamides, which bind as an antiparallel, side-by-side, stacked dimer, to target DNA base pairs. Specifically, a P/P pairing binds to either an A/T or T/A base pair, an I/P pairing binds G/C, a P/I pairing binds C/G, and the positively charged C-terminus binds to either A/T or T/A. Dervan's group has introduced the hydroxypyrrole to further distinguish between an A/T or a T/A base pair. However, due to the difficulty in the synthesis and stability of hydroxypyrrole, it has not been widely used.[3] Our group has reported that the formamido group of distamycin behaved similarly to a pyrrole moiety in terms of sequence recognition, and it conferred enhanced binding affinity over its non-formamido-pyrrole counterpart.[4] Furthermore, the I/I pairing was reported by us to target the T/G or G/T mismatched base pair, although it could also tolerate either a G/C or C/G base pair.[5] As examples of sequence specific polyamides, f-IPI **2**[6] and PIPI **3**[6d] bind specifically to 5′-ACGCGT-3′, which corresponds to the Mlu1 Cell Cycle Box (MCB) sequence found in the control region of the human Dbf4 gene promoter. Expression of the Dbf4 gene is essential for the growth of several human cancers.[7]

Polyamides have been investigated extensively for their biological activity, including the control of gene expression in cells and

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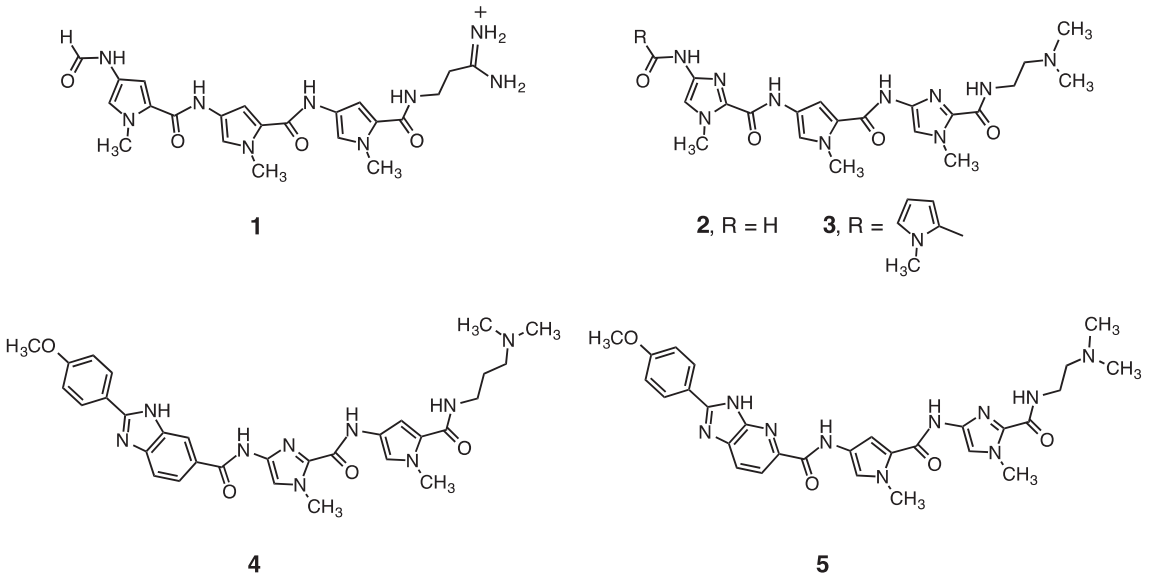


Figure 1. Structures of distamycin **1**, f-IPI **2**, PIPI **3**, Hx-IP **4**, and *aza*Hx-PI **5**.

anticancer activity in vivo. Some encouraging results have been reported recently;[8] however, challenges remain in moving the field forward. These include the need to develop molecules that are of low molecular weight, yet demonstrate sequence specificity, high binding affinity, and good water solubility. In addition, these molecules must readily enter the nucleus and efficiently block gene expression in cells. In order to study nuclear uptake an added advantage would be to develop molecules that are inherently fluorescent so they can be directly observed by microscopy. To address these challenges our group has developed a novel class of small diamino polyamides that contain orthogonal aminoalkyl groups on the N1 positions of P or I.[9] These diamino polyamides exhibited high DNA binding affinity, sequence specificity, water solubility, and propensity to enter cells. Our group is also addressing the issue of fluorescence by introducing the Hx moiety, 2-(*p*-anisyl)benzimidazole-5-carboxamide).[10] Hybrids of Hx with P and I-polyamides, called Hx-amides, were found to bind in a side-by-side, stacked, and antiparallel manner in the minor groove. Hx spans two base pairs, and pairing it with P and I-polyamides was found to recognize DNA base pairs in a predictable manner in which Hx mimicked two consecutive pyrrole units 'P–P' or formamido-pyrrole 'f–P'.[10] As an example, Hx-IP **4** binds selectively to 5′-A/T-A/T-C-G-A/T-A/T-3′ (see Fig. 2A), including 5′-TACGAT-3′, a sequence located in the 5′-flank of the Inverted CCAAT Box-2 (ICB-2) promoter site of the human topoisomerase IIα gene. Hx-IP is able to block the binding of the repressive transcriptional factor NF-Y to ICB-2 thereby activating the expression of the topoisomerase IIα gene in confluent cancer cells.[11]

In this communication, we report the design of a second generation Hx-amide that contains the novel *aza*Hx moiety, which should mimic the DNA binding properties of a 'P–I' or 'f–I' unit. This should expand the ability of Hx-amides to recognize G/C containing sequences. With its resemblance to Hx,[10] Hoechst 33258,[12] and related benzimidazole structures,[13] the *aza*Hx moiety should be fluorescent upon UV excitation. Interestingly, it has also been recently reported that groups with nitrogen acceptors, such as *aza*benzimidazole in *aza*Hx and pyridine, when added to classical AT specific cationic heterocyclic compounds can confer GC binding selectivity on those compounds as with the polyamides reported here.[14] It is clear that a new generation of fluorescent minor groove binding compounds is possible with substitutions of this type. To test these ideas, the synthesis of *aza*Hx-PI **5** was undertaken and reported herein. According to the DNA binding model of *aza*Hx-PI **5** in the minor groove of DNA, depicted in Figure 2B, its dimer is predicted to bind the MCB sequence 5′-ACGCGT-3′ in a similar manner as f-IPI **2**[6] and PIPI **3**.[6d]

As depicted in Scheme 1, *aza*Hx-PI **5** was synthesized by coupling of 2-(*p*-anisyl)-4-*aza*-benzimidazole-5-carboxylic acid

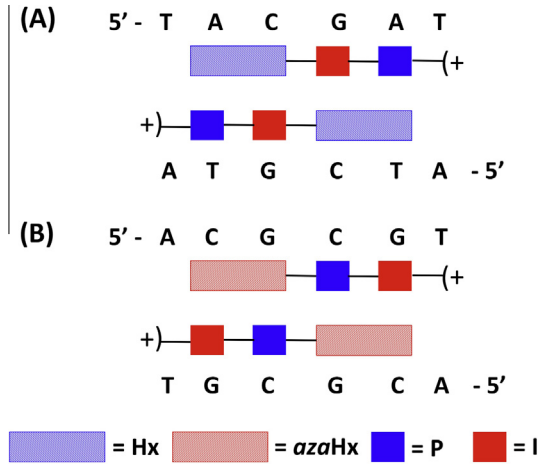
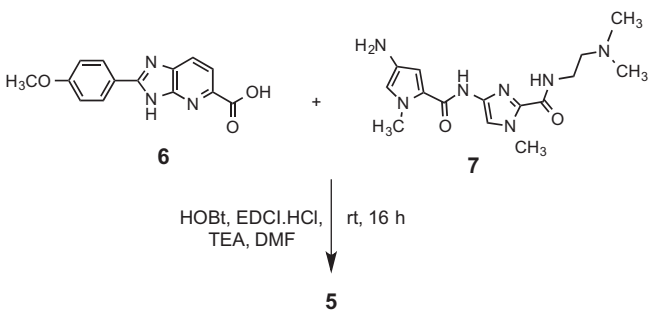


Figure 2. Binding of Hx-IP **4** and *aza*Hx-PI **5** to their respective cognate DNA sequences.



Scheme 1. Synthesis of *aza*Hx-PI, **5**.