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Design of a Hairpin Polyamide, ZT65B, for Targeting the Inverted CCAAT Box (ICB) Site in the Multidrug Resistant (*MDR1*) Gene

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*A novel hairpin polyamide, ZT65B, containing a 3-methylpicolinate moiety was designed to target the inverted CCAAT box (ICB) of the human multidrug resistance 1 gene (*MDR1*) promoter. Binding of nuclear factor-Y (NF-Y) to the ICB site upregulates* MDR1 *gene expression and is, therefore, a good target for anticancer therapeutic agents. However, it is important to distinguish amongst different promoter ICB sites so that only specific genes will be affected. All ICB sites have the same sequence but they differ in the sequence of the flanking base pairs, which can be exploited in the design of sequence-specific polyamides. To test this hypothesis, ten ICB-containing DNA hairpins were designed with different flanking base pairs; the sequences ICBa and ICBb were similar to the 3′-ICB site of* MDR1 *(TGGCT). Thermal-denaturation studies showed that ZT65B effectively targeted ICBa and ICBb ($\Delta T_M = 6.5$ and 7.0 °C) in preference to the other DNA hairpins (<3.5 °C), with the exception of ICBc (5.0 °C). DNase I-footprinting assays were carried out with the topoisomerase IIα-promoter sequence, which contains five ICB sites; of these, ICB1 and ICB5 are similar to the ICB site of* MDR1. *ZT65B was found to selectively bind ICB1 and ICB5; footprints were not observed with ICB2, ICB3, or ICB4. A strong, positive induced ligand band at 325 nm in CD studies confirmed that ZT65B binds in the DNA minor groove. The selectivity of ZT65B binding to hairpins that contained the* MDR1 *ICB site compared to one that did not (ICBd) was confirmed by surface-plasmon studies, and equilibrium constants of 5×10^6–1×10^7 and $4.6\times10^5\ M^{-1}$ were obtained with ICB1, ICB5,and ICB2 respectively. ZT65B and the previously published JH37 (J. A. Henry, et al.* Biochemistry ***2004***, 43, *12249–12257) serve as prototypes for the design of novel polyamides. These can be used to specifically target the subset of ubiquitous gene elements known as ICBs, and thereby affect the expression of one or a few proteins.*

Introduction

Overexpression of the multidrug resistance 1 (*MDR1*) gene has long been implicated in the resistance of many cancers to chemotherapeutic agents.[1–3] The *MDR1* gene product, P-glycoprotein, is an ATP-dependent transporter that can actively pump a wide variety of unrelated small molecules out of the cell.[1] In some instances multidrug resistance is intrinsic to the cancer and in others it is acquired upon exposure of the cancer cells to a specific drug, such as doxorubicin.[2] In either case, inhibition of the *MDR1* gene and its product is necessary for the suppression of cancer-cell resistance to otherwise effective drugs. Direct inhibition of P-glycoprotein is not ideal because basal levels of functional protein in the colon epithelium and at the blood–brain barrier have been shown to be necessary in the body's defense against natural toxins.[4] Disruption of these normal functions of P-glycoprotein could lead to increased toxicity in an organism. Therefore, inhibition of upregulated transcription of the *MDR1* gene offers an attractive option for drug discovery.[2]

Expression of the *MDR1* gene is regulated by a variety of transcription factors.[2,5] Exposure to ultraviolet radiation[6] and various chemotherapies[7] lead to upregulation of the *MDR1* gene by nuclear factor-Y (NF-Y). This upregulation occurs by binding of NF-Y to the inverted CCAAT box (ICB; sequence: 5′-ATTGG) in the *MDR1* promoter.[6–8] In addition, NF-Y mediates the expression of P-glycoprotein according to levels of histone-modifying enzymes;[9] this implicates NF-Y involvement in

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cell division and proliferation. Additional evidence that links NF-Y to cell replication is its regulation of topoisomerase IIα (topo IIα) expression. Binding of NF-Y to the second of five ICB sites (ICB2) in the topo IIα promoter downregulates expression of the protein at cell confluence.[10] In some cancers this results in nondividing (confluent) cells that are resistant to topo IIα-targeting drugs, such as etoposide.[10–12] Therefore, disruption of NF-Y binding to the promoter is a potential target for anticancer therapeutic agents. The drug ecteinascidin 743 (ET 743) has been shown to inhibit the induced transcription of *MDR1* by a variety of mechanisms that include interactions with NF-Y and other transcription factors, such as SP-1, that bind regions proximal to the ICB site.[7] ET 743 is promising in that it does not influence basal levels of P-glycoprotein expression, but it might inhibit induced transcription too broadly and thereby cause increased toxicity.

NF-Y binds to the ICB of the *MDR1* promoter in the minor groove[13] and is a good target for polyamides, which also bind to the DNA minor groove. In order to specifically aim at this ICB sequence, a target site that partially overlaps the ICB has been identified and is underlined in the sequence: 5′-ATTGGCT. Similar sequences have also been found to flank the 3′ end of ICB1 and ICB5 sites of the topo IIα promoter (ATTGGCT and ATTGGCA, respectively). Even though a similar approach was initially reported by Dervan and co-workers for the inhibition of a variety of transcription factors,[14] the work was not focused on the binding of NF-Y to an ICB site within the *MDR1* promoter. We have designed a polyamide that specifically binds the *MDR1* ICB site, with the goal of tightly regulating NF-Y-activated gene expression. The sequence selectivity of polyamides can be adapted by altering their pyrrole (Py) and imidazole (Im) heterocyclic content so as to specifically target the TGGCT sequence. These heterocyclic groups stack in the DNA minor groove as dimers—one group from each strand of the hairpin polyamide recognizes one base in a pair (Figure 1 A, right). Py dimers recognize A–T or T–A base pairs (denoted A/T in target sequences), Im–Py dimers bind G–C base pairs, Py–Im binds C–G base pairs, and Im–Im recognizes G–T or T–G base pairs.[15–25] We have recently used this approach to design the polyamide hairpin JH-37 (Figure 1 A, inset) and have successfully targeted the sequence TTGGT, which corresponds to ICB2 and ICB3 of the topo IIα promoter.[26]

Although N-terminal, nonformylated imidazoles have been reported,[27] the synthetic yields are typically low. Therefore, we sought an alternative heterocyclic group for the imidazole to increase synthetic yields. Previous research[21] has shown that addition of a picolinic acid to the N terminus of netropsin yields a molecule that recognizes the G/C containing sequence TGTCA and the A/T-rich sequence, AAATTT, with nearly equal affinities. Addition of an imidazole to the N terminus of netropsin recognizes TGTCA only. The recognition of two different sequences by picolinate–netropsin was explained by rotation of the pyridyl–carboxamide bond by ~180°, which results in the presentation of two different sequence-recognition elements to the minor groove. In one position, the nitrogen of the picolinate faces the minor groove; this results in the recognition of guanosine, and thus the picolinate behaves like an imidazole (Figure 1 B). Imidazole and pyrrole moieties commonly used in polyamides have methyl groups that limit the ring in one position. Consequently, upon binding to DNA, the methyl group is positioned away from the minor groove. We therefore utilized the same strategy with a methylated picolinic acid (MePic) to prevent this ring from rotating. We hypothesized that the 3-methylpicolinate moiety would bind such that the methyl group would be preferentially placed away from the minor groove (Figure 1 B), much like methylimidazole groups. CD studies with a simple polyamide, MePicPyIm, have shown that the MePic–Im pairing indeed behaves like an Im–Im pair in its ability to specifically recognize T–G base pairs.[28] Thus, a polyamide hairpin was designed with an N-terminal methylpicolinic acid that stacked opposite the C-terminal pyrrole so as to recognize a G–C base pair (Figure 1 A).

Here we report the synthesis of a novel 3-methylpicolinate-containing hairpin (ZT65B: Figure 1 A) that targets the sequence (A/T)GGC(A/T). Ten ICB-containing DNA hairpins were designed to test the specificity of ZT65B (Figure 1 C). Sequences ICBa and ICBb contained the cognate (A/T)GGC(A/T) sequence for ZT65B; ICBa contains the 5′-TGGCT sequence of the *MRD1* ICB site. Eight additional DNA hairpins were designed: three had five base pairs that flanked the 3′-end of the ICB site (ICBc–e). The remaining DNA hairpins had five base pairs that flank the 5′-end of the ICB site (ICBf–j). The selectivity of ZT65B for these ICB sites over other sequences has been tested by thermal DNA-denaturation experiments, DNase I footprinting, surface-plasmon resonance (SPR), and CD.

Results and Discussion

Synthesis and solubility

The synthesis of ZT65B is shown in Scheme 1 and is similar to that reported for JH37.[26] The polyamide hairpin has been characterized by FTIR, 500 MHz ^{1}H NMR spectroscopy, and mass spectrometry (see Experimental Section). Coupling of the N-terminal methylpicolinic acid **1** with the tripyrroleamine **2** in the presence of PyBOP gave ZT65B in 25% yield. ZT65B is completely soluble in aqueous solutions. However, its absorbance at 309 nm dropped significantly within 12 h. Filtration studies with stock solutions of ZT65B (~500 μM) were performed by using a 0.2 micron membrane. These studies showed that considerable amounts of polyamide were retained on the membrane when samples were older than 12 h but not when they were fresh. This indicates that ZT65B formed larger particles and did not degrade (data not shown). ZT65B is in fact prone to aggregation due to stacking associations of the aromatic rings in the hairpin polyamide.

Thermal denaturation studies

The sequence specificity of ZT65B was tested against ten DNA sequences (Figure 1 C) by using UV-monitored thermal-denaturation experiments. The ability of a molecule to stabilize duplex DNA so that it requires more heat to denature, is generally proportional to the affinity with which the compound

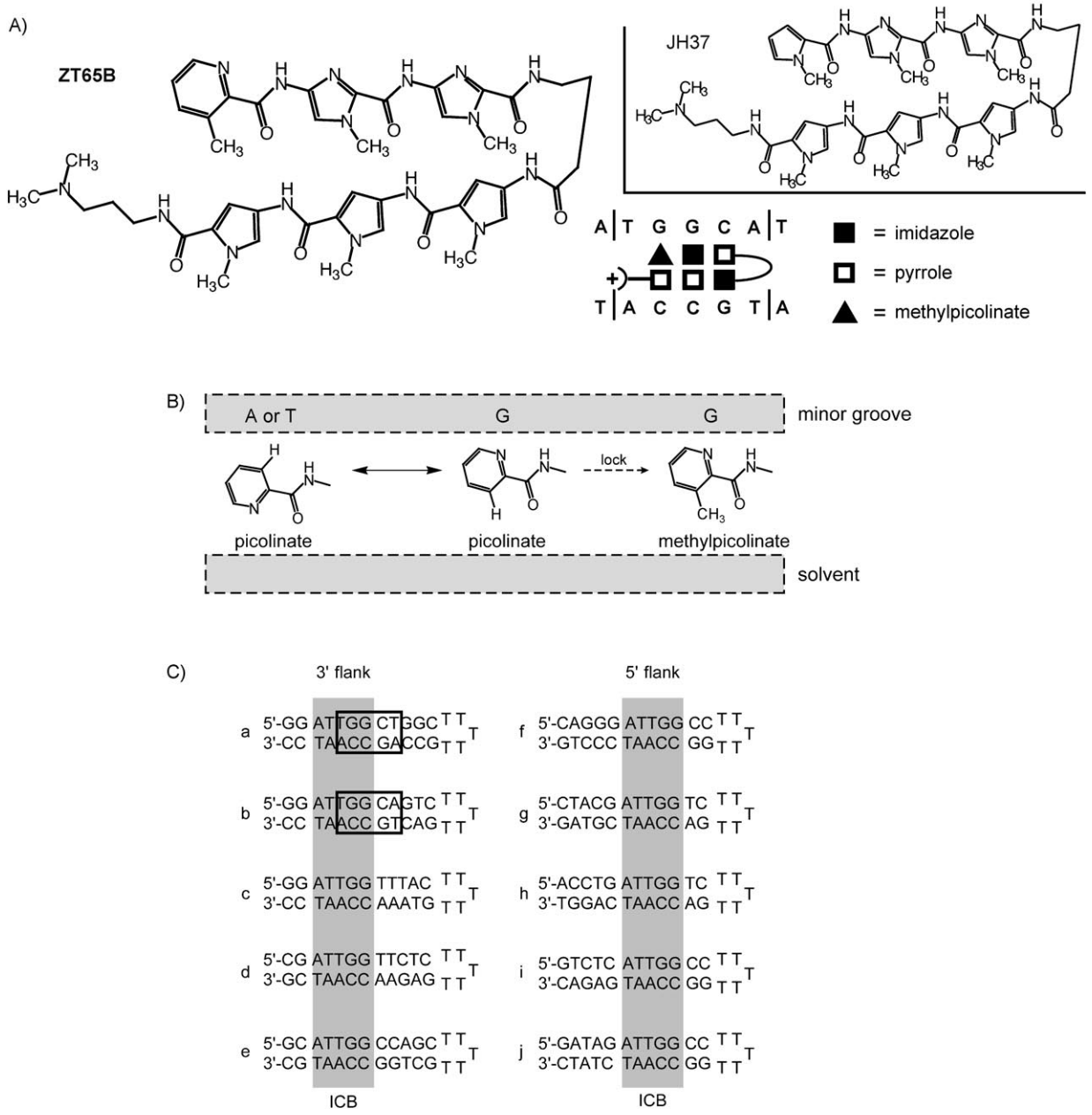


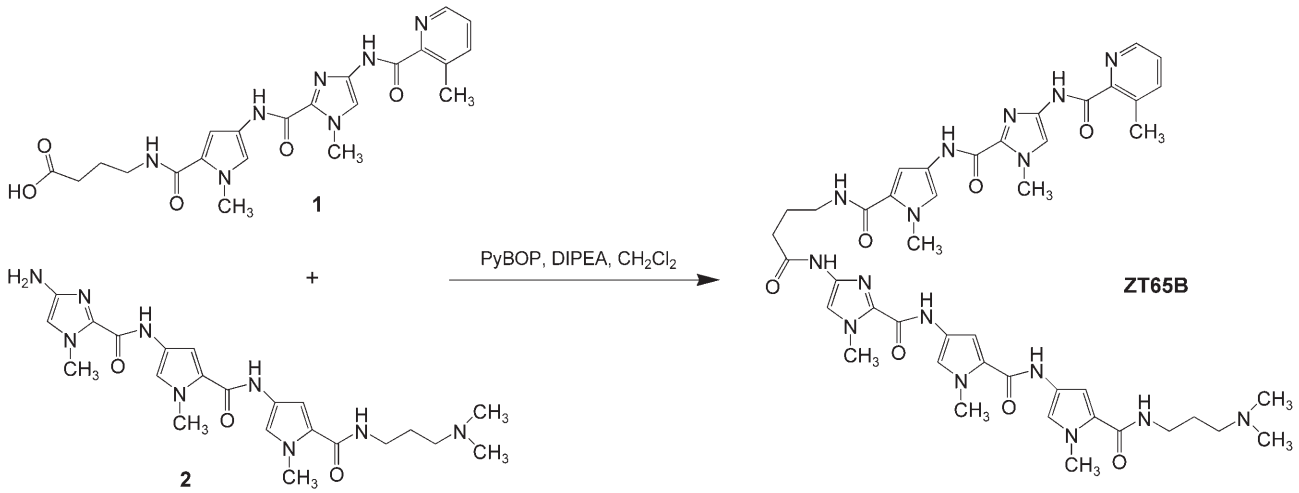
Figure 1. ZT65B, its target sequence, sequence recognition by picolinates, and model DNA hairpins. A) The cationic hairpin polyamide, ZT65B (MePic-Im-Py-turn-Im-Py-Py), and its proposed recognition sequence (right); JH-37 is depicted in the inset. B) Picolinate recognition of A or T, its recognition of G after ring flipping,[21] and prevention of ring flipping by using methylpicolinate. The positions of the minor groove and solvent are labeled and highlighted (gray boxes). C) Ten synthetic hairpins that include ICB sites and different flanking sequences (gray boxes). Five of the hairpins have extended 5′-flanking sequences and five have extended 3′-flanking sequences. The ZT65B target site is boxed (ICBa and ICBb).

binds the DNA.[27] In addition, the hyperchromic effect allows easy discernment between double- and single-stranded DNA at 260 nm. The ΔT_M values represent the differences between denaturation of the DNA–ZT65B complex and the DNA on its own. The binding of ZT65B to ten different sequences was readily assessed by this method (Table 1). Both ICBa and ICBb exhibited higher ΔT_M values (6.6 and 6.8 °C, respectively) than the other eight sequences (< 3.5 °C), with the exception of ICBc, which showed a significant ΔT_M (5.2 °C).

ZT65B selectively recognized the (A/T)GGC(A/T) sequence that is present in ICBa- and ICBb-DNA hairpins, and which corresponds to the single ICB site in the *MDR1* promoter. ZT65B also binds the sequence TGGTT. One possible explanation is that this sequence is just one base pair different from the cognate sequence and that the Py–Im pairing closest to the β-alanine linker can accommodate a T–A base pair. Ultimately, with this ΔT_M method, ZT65B is found to selectively recognize the sequences TGGCA and TGGCT, and to a lesser extent TGGTT.

DNase I footprinting

The sequence selectivity of ZT65B was further characterized with DNase I footprinting. The sense strand was 5′-^{32}P radiolabeled for the visualization of the ICB4 and ICB5 sites; the anti-



Scheme 1. Synthesis of ZT65B: the N-terminal methylpicolinic acid **1** was dissolved in CH_2Cl_2 and coupled with the tripyrroleamine **2** in the presence of PyBOP and DIPEA. ZT65B was obtained in 25% yield.

Table 1. ΔT_M values [°C].

	5′ flank		5′ flank
ICBa	6.5	ICBb	7.0
ICBc	5.0	ICBd	3.5
ICBe	3.0	ICBf	2.0
ICBg	3.5	ICBh	2.5
ICBi	2.0	ICBj	3.0

sense strand was likewise labeled for the visualization of the remaining ICB sites of the topo IIα promoter (Figure 2). The concentrations of ZT65B that were used in this experiment indicate the amount of compound necessary to protect the DNA from DNase I cleavage. Protection of the 3′ flank of ICB1, which is identical to ICBa, is initially visible with 3 μM ZT65B; ICB5, which is analogous to ICBb, shows a footprint upon addition of at least 5 μM ZT65B. Three other regions are protected from DNase I cleavage by ZT65B (Figure 2). Each of these sites contains the (A/T)GGC(A/T) cognate sequence and no other cognate sequences are present on the length of the footprinted DNA. The protected region adjacent to ICB3 is visible in both gels. These protected regions are sufficiently separated from ICB2, ICB3, and ICB4 to demonstrate that ZT65B is selective for those sites that are flanked by CT or CA on the 3′-end. An additional region (denoted by a triangle) was protected that does not contain the (A/T)GGC(A/T) cognate sequence. However, in conjunction with the thermal-melt data discussed above, it is apparent that ZT65B can bind sites that differ by a single base pair from (A/T)GGC(A/T). This protected region centers on the sequence 5′-ATCCT, for which the complementary strand is 5′-AGGAT. These sequences are not universally recognized because ICB3 has the sequence 5′-TGGTT and is not protected by ZT65B from DNase I cleavage. Thus, when ICB sites are placed in direct competition, ZT65B preferentially binds to regions that contain the (A/T)GGC(A/T) sequence and not to other sites.

Surface plasmon resonance

The steady-state binding affinities and stoichiometries of ZT65B for ICBa, ICBb, and ICBd were determined by using SPR. The sensorgrams for the titration of ZT65B to biotin immobilized DNA hairpins are shown in Figure 3. The steady-state response of ZT65B binding to each of these DNA hairpins is shown in Figure 3D. The binding affinities to ICBb and ICBd were determined to be 4.8×10^6 and $4.6\times10^5\,M^{-1}$, respectively. The proximity of the calculated response units (RU; see ref. [23]) to the observed values, and the good-data fit by the monomeric isotherm indicate that ZT65B binds to ICBd and ICBb as a monomer.

ZT65B binds to ICBa and we have observed binding of a second molecule of ZT65B to the hairpin DNA. The RUs observed at high concentrations are approximately twice the calculated values for the binding of a single ZT65B molecule. In addition, the data are best fit by using a two-site isotherm rather than a monomeric fit. The first molecule of ZT65B binds very strongly ($K_1=1.2\times10^7\,M^{-1}$) while the second molecule has much lower affinity for the DNA ($K_2=2.6\times10^5\,M^{-1}$). A similar phenomenon was observed by SPR and isothermal microcalorimetry for the binding of JH-37 to hairpin DNA.[26] The binding of the first molecule to its cognate sequence had an affinity of $K=2.8\times10^7\,M^{-1}$, that of the second molecule was ~100-fold weaker, as measured by SPR.[26] The interactions of this second molecule with the DNA are not understood, but are consistent with nonspecific, weak interactions between the cationic polyamide and polyanionic DNA.[26] Thus, the reported binding affinity of ZT65B to ICBa is the value that corresponds to the strong initial binding. Interestingly, dissociation of ZT65B from ICBa and ICBb is visibly slower than from ICBd (Figure 3A–C). This observation is in good agreement with the ten-fold stronger binding affinities observed with ICBa and ICBb over ICBd.

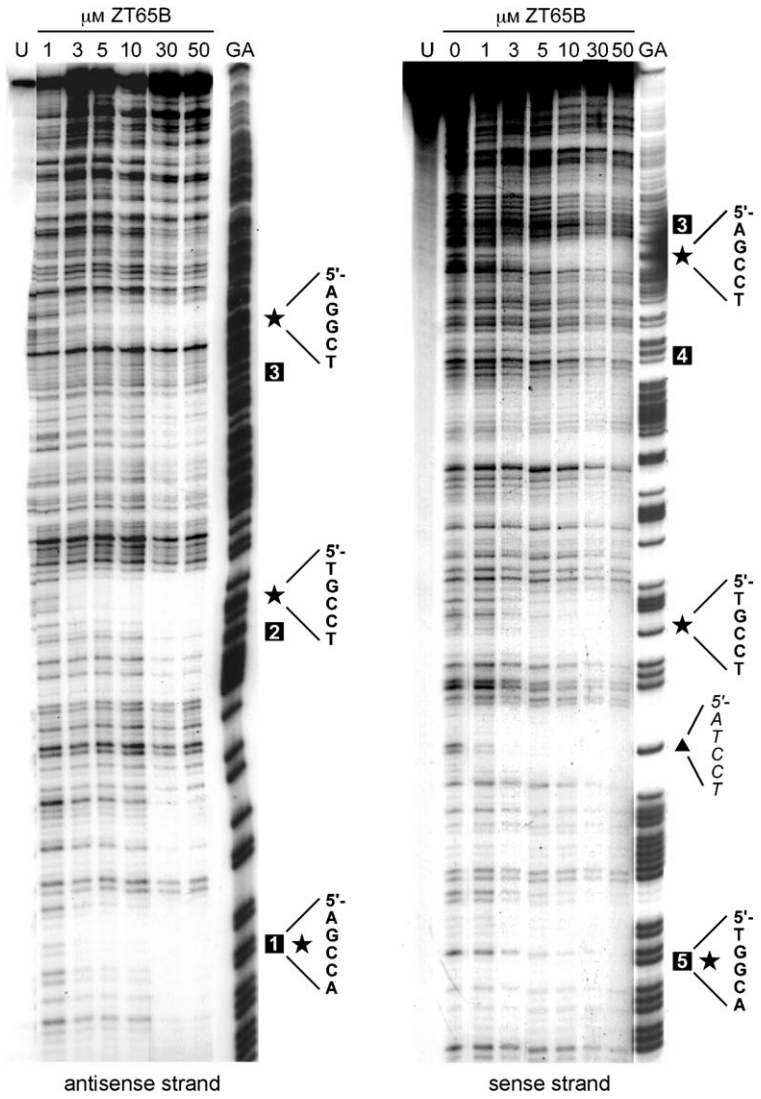


Figure 2. DNase I footprinting protection patterns of ZT65B with the topo IIα promoter. The antisense strand was 5′-^{32}P radiolabeled for the visualization of ICB1, ICB2, and ICB3; the sense strand was radiolabeled for the visualization of ICB4 and ICB5 of the topo IIα promoter. This sequence has been previously published.[32] These ICB sites correspond to ICBa, ICBd, ICBc, ICBe, and ICBb, respectively; U is undigested DNA; GA denotes the sequencing lanes. The topo IIα promoter ICB sites are marked by black boxes with white numbers; protected regions that center on the sequence (A/T)GGC(A/T) are denoted by stars and the protected region that does not contain this sequence is denoted by a triangle.

Circular dichroism titration studies

A series of CD experiments was performed to determine the binding mode of ZT65B to ICBa, ICBb, and ICBd (Figure 4). The induction of a new band at ~320 nm demonstrates that ZT65B binds in the DNA minor groove.[24,27,29] In addition, each set of titrations results in an isodichroic point at ~300 nm; this indicates that ZT65B binds to DNA by a single mechanism.

The maximum response for the induced peak corresponds to the saturation of DNA binding sites, which can be reported as the molar ratio (ZT65B:DNA) required to reach the maxima. These molar ratios do not indicate binding stoichiometry because it is unlikely that three molecules of ZT65B will bind to one twelve base-pair DNA hairpin. The overestimation in binding stoichiometry in CD studies is likely due to equilibration of the free and bound states, and is more apparent for compounds with modest binding affinities.[30] Moreover, ZT65B exhibits monomeric binding to ICBb and ICBd, as determined by SPR. The CD data exhibit a maximum response for ZT65B binding to ICBa, ICBb, and ICBd at molar ratios ranging from ~2.5:1 to ~3.5:1 (ZT65B:DNA).

Conclusion

We have successfully synthesized a novel hairpin polyamide with an N-terminal 3-methylpicolinate moiety. This heterocycle has been previously reported in the context of triheterocyclic polyamides.[28] Coupling of 3-methylpicolinic acid to the growing polyamide was more straightforward than incorporation of a nonformylated imidazole and resulted in yields that are typical of the solution-phase synthesis of polyamide hairpins.

The biophysical studies discussed herein have shown that ZT65B selectively binds the (A/T)GGC(A/T) site found in the ICBa and ICBb DNA hairpins in preference to other sequences tested. Binding of ZT65B to the alternative sequence, (A/T)GG(A/T)T, was also observed, but not universally detected as was apparent by the lack of ICBd and ICB2/3 recognition in thermal-melting and DNase I-footprinting assays, respectively. Recognition of ICBa and ICBb is achieved by partial binding of this sequence and the base pairs that immediately flank its 3′-end. Thus, ZT65B could have potential for selective targeting of the single ICB site of the *MDR1* gene. ICB sequences and their complement, CCAAT, are ubiquitous in promoters that lack the TATA box and have been identified by mRNA analysis in at least 67% of human genes.[31] Thus, identifying specific sites that flank the ICB sequence in different promoter regions is a viable method for affecting the expression of only a few genes, while limiting the influence of the compound on the expression of other genes that contain similar recognition sites. The selective targeting of ICB sites was previously reported with the specific targeting of ICB2 in the topo IIα promoter by JH-37.[26] ZT65B is a good model compound for affecting the expression of P-glycoprotein; in vitro and in vivo biological studies that test the inhibition of NF-Y binding to the *MDR1* ICB site are ongoing.

Experimental Section

Synthesis of ZT65B: The nitro precursor to amine **2** (80 mg, 0.14 mmol) was reduced in chilled methanol (15 mL) over 30% Pd on carbon. The mixture was filtered, co-evaporated with dry CH_2Cl_2 (3×) to give amine **2**. The carboxylic acid **1** (88 mg, 0.18 mmol) was dissolved in freshly distilled CH_2Cl_2 (30 mL) and added to amine **2**. PyBOP (100 mg, 0.19 mmol), dry diisopropylethyl amine

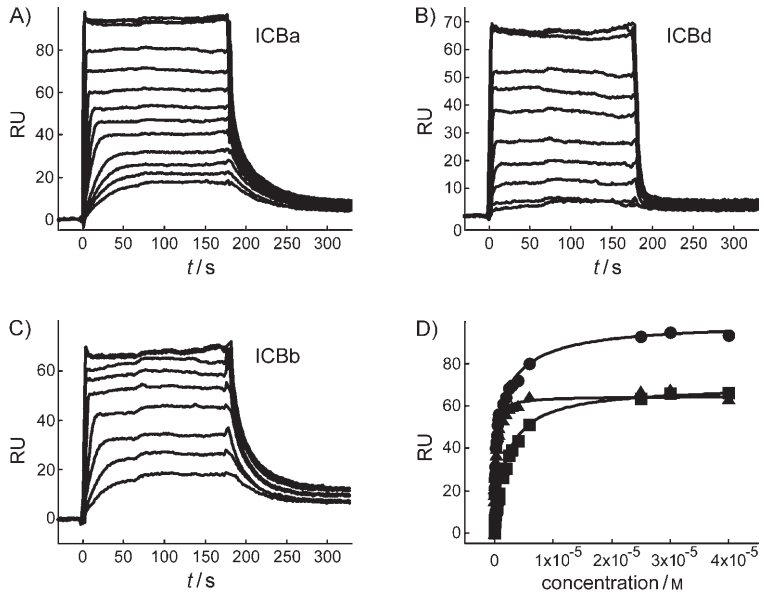


Figure 3. SPR of ZT65B binding to several ICB sequences. Sensorgrams of ZT65B binding to A) ICBa, B) ICBd, and C) ICBb. D) Steady-state binding affinity for ICBa (●), ICBd (■), and ICBb (▲); RU = response units. Equal amounts of DNA hairpins were not loaded; in order to account for this, data were normalized for the respective DNA sequences in the absence of ZT65B.

(DIPEA; 0.07 mL, dried over NaOH) were added to the mixture, and stirred at room temperature for 60 h. The coupling was confirmed by TLC, washed with NaOH (2 M, 20 mL) and extracted with CH_2Cl_2 (four times). The organic extracts were dried with Na_2SO_4, filtered, concentrated by rotator evaporation, and column purified. The product, ZT65B, was eluted with between 15 and 17.5% MeOH/$CHCl_3$ (32 mg of a light yellow solid, 25%): m.p. 190–200 °C; TLC (30% MeOH/$CHCl_3$): $R_f = 0.57$; IR (Nujol): $\nu = 3372$, 3060, 1709, 1651, 1531, 1307, 1258, 1209, 1165, 969, 723 cm^{-1}. UV (water): $\lambda_{max} = 309$ nm ($\varepsilon = 36000\ M^{-1}cm^{-1}$); ^1H NMR (500 MHz, [D_6]DMSO): $\delta = 10.45$ (s, 1H), 10.28 (s, 1H), 10.23 (s, 1H), 9.95 (s, 1H), 9.87 (s, 1H), 8.53 (d, 4.0, 1H), 8.04 (br t, 1H), 7.82 (t, 4.0, 1H), 7.64 (s, 1H), 7.58 (d, 4.0, 1H), 7.44 (s, 1H), 7.34 (br t, 1H), 7.24 (s, 1H), 7.21 (s, 1H), 7.19 (s, 1H), 7.17 (s, 1H), 7.02 (s, 1H), 6.98 (s, 1H), 6.85 (s, 1H), 4.01 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H), 3.79 (s, 6H), 3.19 (q br, 2H), 2.66 (s, 3H), 2.52 (q br, 2H), 2.35 (m, 4H), 2.20 (s, 6H), 1.79 (quintet, 6.0, 2H), 1.67 (quintet, 6.0, 2H); TOF-MS (electrospray) *m/z* (relative intensity): 919 ($[M+H]^+$, 65); exact mass for $C_{44}H_{55}N_{16}O_7$: calcd 919.4440, found 919.4442.

Aqueous solubility of ZT65B: Solutions of ZT65B were tested under a variety of conditions by monitoring the UV/Vis absorbance spectra as a function of time. The change in wavelength maximum from 309 nm and height of the peak maximum when concentration was accounted for, indicated that the compound aggregated and after a period of two days solid particulates were visible. ZT65B was captured on a 0.2 μm membrane only after the compound had been dissolved in aqueous solution for at least 12 h. The best results were obtained when the compound was stored for less than 12 h at room temperature in distilled, deionized water.

DNase I footprinting: The DNA fragment utilized corresponded to the −489 to −10 positions in the topo IIα promoter. The footprinting experiments were performed as described in ref. [22].

Biophysical studies (thermal denaturation, CD titration, and SPR): These studies were performed on a Cary 3 spectrophotometer, JASCO J-710 spectropolarimeter, and a BIACORE 3000, respectively. The thermal melting experiments were carried out in sodium phosphate (10 mM), EDTA buffer (1 mM) with no extra NaCl; the CD and SPR experiments were performed in sodium phosphate (10 mM), EDTA (1 mM), NaCl (200 mM) buffer. The CD and SPR experiments were performed at room temperature (27 °C). The three types of assay were carried out by using the model 12 base-pair DNA hairpins (Figure 1). The experiments were otherwise performed and analyzed as described in ref. [22].

DNA hairpins: The DNA hairpins were chemically synthesized with a 5′-biotin group and AE-HPLC purified by Qiagen, Inc. The DNAs were resuspended in MES20 (pH 6.2) to concentrations of about 50 μM base pairs and used without further purification.

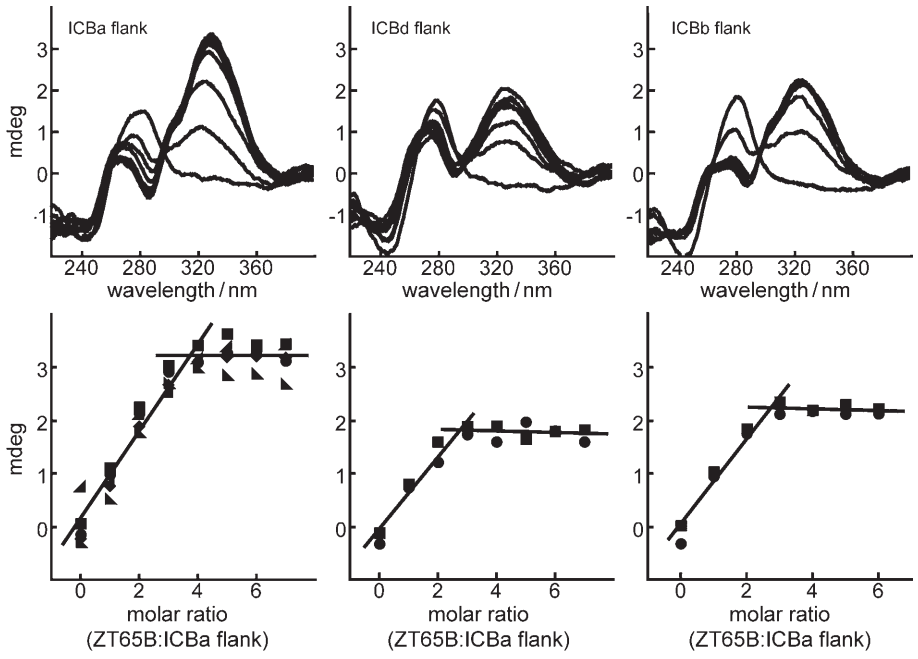


Figure 4. CD studies of ZT65B binding to ICBa, ICBd, and ICBb. Sample spectra with increasing ZT65B titrated in 1:1 molar ratios ZT65B/DNA are shown in the top row. The height of each induced peak at 326, 327, and 327 nm were plotted as a function of the molar ratios in the bottom row. Different experiments are indicated with different symbols.

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Keywords: antitumor agents · DNA recognition · multidrug resistance · polyamides · surface plasmon resonance

[1] V. Ling, *Cancer Chemother. Pharmacol.* **1997**, *40*, S3 – 8.
[2] K. W. Scotto, R. A. Johnson, *Mol. Interventions* **2001**, *1*, 117 – 125.
[3] J. Sun, Z.-G. He, G. Cheng, S.-J. Wang, X.-H. Hao, M.-J. Zou, *Med. Sci. Monit.* **2004**, *10*, RA5 – 14.
[4] A. H. Schinkel, *Semin. Cancer Biol.* **1997**, *8*, 161 – 170.
[5] M. Sukhai, M. Piquete-Miller, *J. Pharm. Pharm. Sci.* **2000**, *3*, 268 – 280.
[6] Z. Hu, S. Jin, K. W. Scotto, *J. Biol. Chem.* **2000**, *275*, 2979 – 2985.
[7] D. Friedman, Z. Hu, E. A. Kolb, B. Gorfajn, K. W. Scotto, *Cancer Res.* **2002**, *62*, 3377 – 3381.
[8] R. Sundseth, G. MacDonald, J. Ting, A. C. King, *Mol. Pharmacol.* **1997**, *51*, 963 – 971.
[9] S. Jin, K. W. Scotto, *Mol. Cell. Biol.* **1998**, *18*, 4377 – 4384.
[10] R. J. Isaacs, A. L. Harris, I. D. Hickson, *J. Biol. Chem.* **1996**, *271*, 16741 – 16747.
[11] J. C. Wang, *Annu. Rev. Biochem.* **1996**, *65*, 635 – 692.
[12] H. Wang, Z. Jiang, Y. W. Wong, W. S. Dalton, B. W. Futscher, V. T. Chang, *Biochem. Biophys. Res. Commun.* **1997**, *237*, 217 – 224.
[13] A. Ronchi, M. Bellorini, N. Mongelli, R. Mantovani, *Nucleic Acids Res.* **1995**, *23*, 4565 – 4572.
[14] L. A. Dickinson, R. J. Gulizia, J. W. Trauger, E. E. Baird, D. E. Mosier, J. M. Gottesfield, P. B. Dervan, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 12890 – 12895.
[15] J. G. Pelton, D. E. Wemmer, *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 5723 – 5727.
[16] T. J. Dwyer, B. H. Geierstanger, Y. Bathini, J. W. Lown, D. E. Wemmer, *J. Am. Chem. Soc.* **1992**, *114*, 5911 – 5919.
[17] J. G. Pelton, D. E. Wemmer, *J. Am. Chem. Soc.* **1990**, *112*, 1393 – 1399.
[18] M. L. Kopka, C. Yoon, D. Goodsell, P. Pjura, P. E. Dickerson, *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 1376 – 1380.
[19] J. W. Lown, K. Krowicki, U. G. Bhat, A. Skorobogaty, B. Ward, J. C. Dabrowiak, *Biochemistry* **1986**, *25*, 7408 – 7416.
[20] K. Kissinger, K. Krowicki, J. C. Dabrowiak, J. W. Lown, *Biochemistry* **1987**, *26*, 5590 – 5595.
[21] W. S. Wade, M. Mrksich, P. B. Dervan, *J. Am. Chem. Soc.* **1992**, *114*, 8783 – 8794.
[22] P. B. Dervan, *Bioorg. Med. Chem.* **2001**, *9*, 2215 – 2235.
[23] M. A. Marques, R. M. Doss, A. R. Urbach, P. B. Dervan, *Helv. Chim. Acta* **2002**, *85*, 4485 – 4517.
[24] E. R. Lacy, K. K. Cox, W. D. Wilson, M. Lee, *Nucleic Acids Res.* **2002**, *30*, 1834 – 1841.
[25] E. R. Lacy, B. Nguyen, M. Le, K. K. Cox, C. O'Hare, J. A. Hartley, M. Lee W. D. Wilson, *Nucleic Acids Res.* **2004**, *32*, 2000 – 2007.
[26] J. A. Henry, N. M. Le, B. Nguyen, C. M. Howard, S. L. Bailey, S. M. Horick, K. L. Buchmueller, M. Kotecha, J. A. Hochhauser, W. D. Wilson, M. Lee, *Biochemistry* **2004**, *43*, 12249 – 12257.
[27] E. R. Lacy, N. M. Le, C. A. Price, M. Lee, W. D. Wilson, *J. Am. Chem. Soc.* **2002**, *124*, 2153 – 2163.
[28] P. B. Uthe, A. M. Staples, M. Turlington, J. B. Jones, K. N. Blackmon, S. L. Bailey, K. L. Buchmueller, M. Lee, *Heterocycl. Commun.* **2005**, *11*, 163 – 166.
[29] R. Lyng, A. Rodger, B. Norden, *Biopolymers* **1992**, *32*, 1201 – 1214.
[30] K. L. Buchmueller, A. M. Staples, C. M. Howard, S. M. Horick, P. B. Uthe, N. M. Le, K. K. Cox, B. Nguyen, K. A. O. Pacheco, W. D. Wilson, M. Lee, *J. Am. Chem. Soc.* **2005**, *127*, 742 – 750.
[31] Y. Suzuki, T. Tsunoda, J. Sese, H. Taira, J. Mizushima-Sugano, H. Hata, T. Ota, T. Isogai, T. Tanaka, Y. Nakamura, A. Suyama, Y. Sakaki, S. Morishita, K. Okubo, S. Sugano, *Genome Res.* **2001**, *11*, 677 – 684.
[32] R. J. Isaacs, A. L. Harris, I. D. Hickson, *J. Biol. Chem.* **1996**, *271*, 16741 – 16747.

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