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Sequence-Specific Base Pair Mimics Are Efficient Topoisomerase IB Inhibitors

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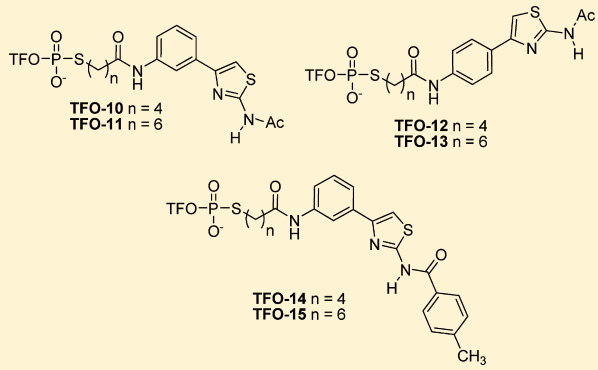
Supporting Information

ABSTRACT: Topoisomerase IB controls DNA topology by cleaving DNA transiently. This property is used by inhibitors, such as camptothecin, that stabilize, by inhibiting the religation step, the cleavage complex, in which the enzyme is covalently attached to the 3′-phosphate of the cleaved DNA strand. These drugs are used in clinics as antitumor agents. Because three-dimensional structural studies have shown that camptothecin derivatives act as base pair mimics and intercalate between two base pairs in the ternary DNA−topoisomerase−inhibitor complex, we hypothesized that base pairs mimics could act like campthotecin and inhibit the religation reaction after the formation of the topoisomerase I−DNA cleavage complex. We show here that three base pair mimics, nucleobases analogues of the aminophenyl-thiazole family, once targeted specifically to a DNA sequence were potent topoisomerase IB inhibitors. The targeting was achieved through covalent linkage to a sequence-specific DNA ligand, a triplex-forming oligonucleotide, and was necessary to position and keep the nucleobase analogue in the cleavage complex. In the absence of triplex formation, only a weak binding to the DNA and topoisomerase I-mediated DNA cleavage was observed. The three compounds were equally active once conjugated, implying that the intercalation of the nucleobase upon triplex formation is the essential feature for the inhibition activity.



DNA topoisomerases control DNA topology through a transient cleavage of the phosphate backbone.[1] Type I topoisomerases (topo I) act by cleaving only one strand of the DNA, while type II topoismerases (topo II) act by cleaving both DNA strands. In all cases, the enzyme forms a transitory cleavage complex in which the catalytic tyrosine is covalently linked to the phosphate of the DNA at the cleavage site, leaving a free OH end. Topoisomerases are ubiquitous and essential for cells.[2] In human cells, topo IB and topo II are the target of commonly used antitumor agents such as camptothecin (CPT)[3] and etoposide,[4] respectively. These agents act by stabilizing the cleavage complex, mostly by inhibiting the religation reaction that maintains the integrity of the DNA genome. Thus, they generate DNA strand breaks that poison the cell and induce cell death. In 2002, the crystal structure of the ternary complex of human topo IB covalently linked to DNA in the presence of topotecan, a derivative of CPT, was determined[5] (Protein Data Bank entries 1K4S and 1K4T). The drug was found intercalated in the DNA and behaved as a base pair increasing the distance between the base pair at position −1 of the cleavage site and the one at position +1 from 3.6 to 7.2 Å with a twist of 10°. This increased the distance between the 5′-OH group of the downstream cleaved DNA strand from the 3′-tyrosylphosphoryl bond that keeps the DNA attached to the enzyme, thus inhibiting the religation reaction. Interestingly, according to the authors, the intercalation can occur only after the cleavage complex between the topo IB and the DNA is formed. Several other inhibitors, even of different chemical families, have been crystallized in the cleavage complex, and all are found intercalated in the DNA at the cleavage site.[6,7]

In the past, we developed sequence-specific topoisomerase poisons upon covalent attachment of the inhibitors to sequence-specific DNA ligands, such as triplex-forming oligonucleotides (TFOs) or minor groove ligands.[8,9] We demonstrated that the DNA ligand positions the inhibitors specifically in the proximity of its binding site and stabilizes its interaction with the DNA−topoisomerase cleavage complex, leading to sequence-specific DNA cleavage.[10−13] It is worth noting that we applied this strategy to inhibit site-specifically topo IB and induce a sequence-specific DNA cleavage using an inactive camptothecin derivative

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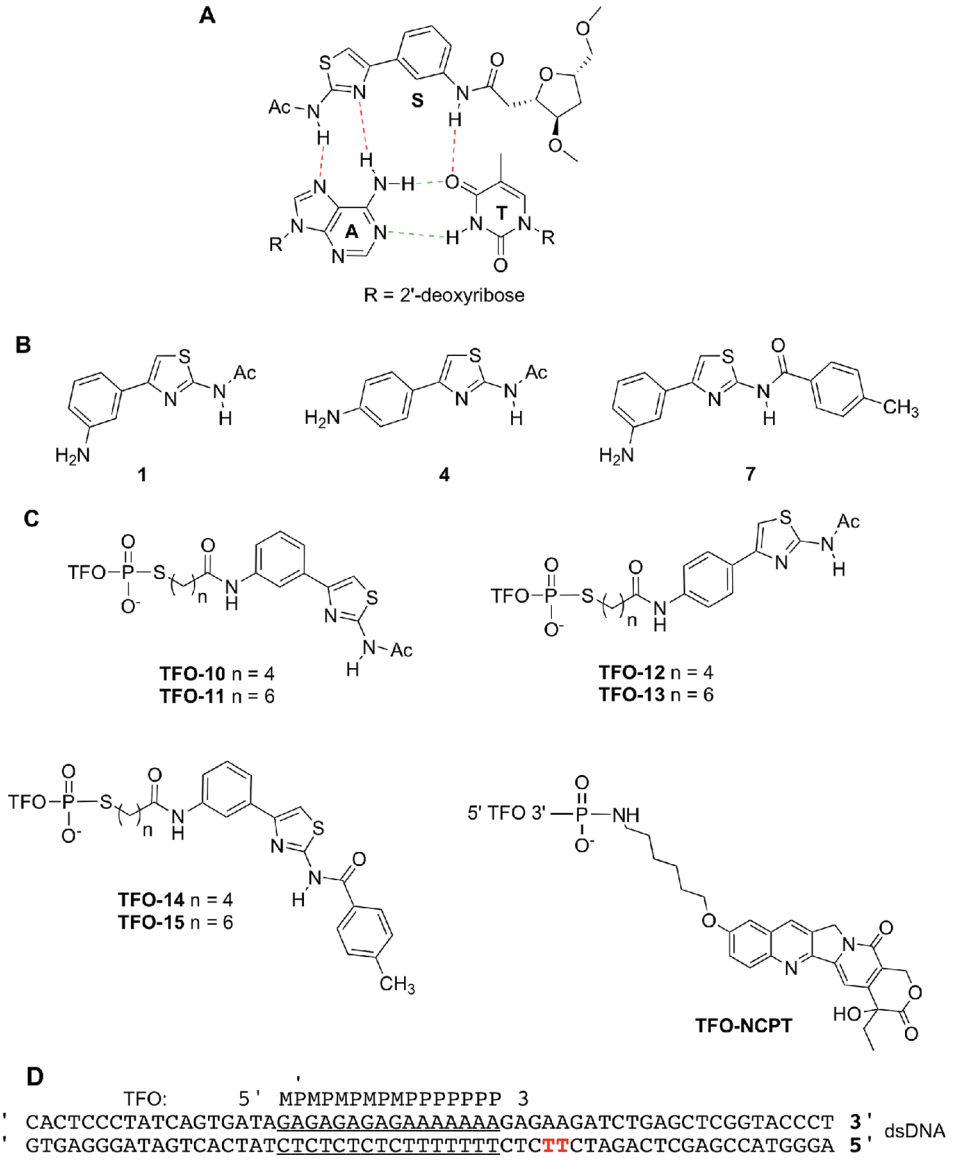

Figure 1. (A) Model proposed for the specific recognition of an AT base pair by nucleobase **S**. (B) Nucleobase analogues synthesized in this work. (C) Chemical structures of the TFO conjugates, where P stands for 5-propynyldeoxyuridine and M for 5-methyldeoxycytidine. (D) Sequences of the TFO and of the DNA target (the triplex site is underlined). The topo IB cleavage site is colored red.

(i.e., lacking one potential H-bond in the cleavage complex).[14] This showed that it is possible to render a molecule otherwise inactive into a good and specific topo IB inhibitor by conjugation to a DNA ligand, such as a TFO.

The formation of a DNA triplex with oligonucleotides is limited to oligopurine·oligopyrimidine sequences, and in an effort to overcome this limitation and to improve the stability toward mixed purine/pyrimidine target sequences, a number of artificial nucleic acids have been designed and synthesized.[15−21] In this context, we have demonstrated that a novel nucleoside analogue [**S** (Figure 1A)], when incorporated into a pyrimidine motif TFO, can effectively circumvent a purine·pyrimidine base pair interruption in an oligopyrimidine·oligopurine sequence.[16,22] Indeed, **S** is an extended heterocyclic ring system (substituted aminophenylthiazole) previously designed for the recognition of inverted AT base pairs by specific Hoogsteen H-bonding in the major groove of the DNA duplex (Figure 1A). Several structure−affinity assays argue for this mode of interaction.[16,22] However, in some cases, nucleobase **S** exhibits less selectivity because it also recognizes CG, depending on the pH and the triplex sequence, which maintain the H-bonding network and the cooperativity of the Hoogsteen base pairing. This low selectivity was attributed to an intercalative mode of recognition. Therefore, we expected that **S** intercalation would be highly favored when **S** is attached to an oligonucleotide via an ended linker by abolishing the H-bonding network.

Because modified nucleobases of **S** could act as base pair mimics, we hypothesized that they could fit perfectly in the DNA−topo IB cleavage complex, when positioned by a DNA ligand, and fulfill the requirements for an intercalating poison as observed for the camptothecin analogues in the crystal structure.[5,6] We thus designed and synthesized triplex-forming oligonucleotides conjugated to artificial nucleobases **1**, **4**, and **7** (Figure 1B) inspired by the structure of **S** nucleoside, via linker with different lengths [$(CH_2)_n$ where n = 4 or 6 (conjugates **TFO-10**−**TFO-15** in Figure 1C)]. We then studied their binding properties with respect to the target duplex as well as

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their ability to inhibit topo IB activity in a sequence-specific manner.

EXPERIMENTAL PROCEDURES

Chemistry. All oligonucleotides were purchased from Eurogentec (Seraing, Belgium). The duplex target dsDNA has the following sequence (the triplex site is underlined): 5′-CACTCCCTATCAGTGATAGAGAGAGAGAAAAAAGAGAAGATCTGAGCTCGGTACCCT-3′/3′-GTGAGGGATAGTCACTATCTCTCTCTCTTTTTTTCTCTTCTAGACTCGAGCCATGGGA-5′. Solvents and most of the starting materials were purchased from Aldrich and Alfa Æsar. High-resolution mass spectra (HRMS) were obtained with a LTQ Orbitrap hybrid mass spectrometer with an electrospray ionization probe (Thermo Scientific, San Jose, CA) by direct infusion from a pump syringe to confirm the correct molar mass and high purity of compounds. The ^{1}H and ^{13}C NMR (200 and 50 MHz, respectively) spectra were recorded on a Bruker AC 200 spectrometer. Chemical shifts are expressed as parts per million from tetramethylsilane. Splitting patterns have been designated as follows: s (singlet), d (doublet), t (triplet), and m (multiplet). Coupling constants (J values) are listed in hertz. Reactions were monitored by analytical thin-layer chromatography, and products were visualized by exposure to UV light. VWR silica gel (230−400 mesh) was used for column chromatography. Dichloromethane, methanol, cyclohexane, and ethyl acetate employed as eluents in column chromatography were distilled on a rotary evaporator prior to being used. High-performance liquid chromatography analysis was conducted on a reverse phase column (Thermo, RP C18, 250 mm × 4.6 mm, 5 μm) using a 0 to 100% gradient of water and acetonitrile with UV detection at 260 nm.

2-*N*-Acetylamino-4-(4-nitrophenyl)thiazole (3). To a solution of 1-bromo-4-nitroacetophenone (**2**, 2.5 g, 10 mmol) in ethanol (60 mL) was added the *N*-acetylthiourea (1 equiv, 1.24 g). The reaction mixture was stirred at 80 °C for 30 min and then left to cool to room temperature. The precipitate was filtered and washed with an ethanol/ether mixture leading to 2.2 g of compound **3** (98% yield) obtained as a yellow solid: R_f = 0.7 (9:1 CH_2Cl_2/MeOH); ^{1}H NMR (DMSO-d_6) δ 2.17 (s, 3H), 7.70 (s, 1H), 8.13 (d, 2H, J = 9.0), 8.30 (d, 2H, J = 9.0); ^{13}C NMR (DMSO-d_6) δ 22.9, 112.7, 124.6, 126.8, 140.7, 146.8, 146.9, 158.9, 169.2; mass spectrum (ESI) m/z 264.04376 $(M + H)^+$ ($C_{11}H_{10}N_3O_3S$ requires m/z 264.04429).

2-*N*-Acetylamino-4-(4-aminophenyl)thiazole (4). A solution of compound **3** (2.20 g, 8.37 mmol) in a mixture of acetic acid and ethanol (1:1, v/v, 50 mL) was stirred under a hydrogen atmosphere in the presence of palladium on activated carbon (10%) for 3 h. After removal of the catalyst by filtration through a pad of Celite, the filtrate was concentrated under reduced pressure and the product crystallized in ethyl ether to give 1.85 g of pure compound **4** (95% yield) obtained as a white solid: R_f = 0.54 (9:1 CH_2Cl_2/MeOH); ^{1}H NMR (DMSO-d_6) δ 2.15 (s, 3H), 5.26−5.28 (br s, 2H), 6.60 (d, 2H, J = 8.6), 7.17 (s, 1H), 7.56 (d, 2H, J = 8.4); ^{13}C NMR (DMSO-d_6) δ 22.9, 103.5, 114.1, 122.8, 127.0, 148.9, 150.1, 157.8, 168.7; mass spectrum (ESI) m/z 234.06985 $(M + H)^+$ ($C_{11}H_{12}N_3OS$ requires m/z 234.07011).

2-*N*-Benzoylamino-4-(3-nitrophenyl)thiazole (6). To a solution of 2-amino-4-(4-aminophenyl)thiazole (**5**, 1.0 g, 4.52 mmol) in anhydrous pyridine (40 mL) was added 4-methylbenzoyl chloride (1.2 equiv, 720 μL, 5.43 mmol). The mixture was stirred for 4 h at room temperature, washed twice with water (2 × 20 mL), and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography (97:3 CH_2Cl_2/acetone) led to 1.41 g of compound **6** (92% yield) obtained as a yellow solid: R_f = (1:1 CH_2Cl_2/methanol); ^{1}H NMR (DMSO-d_6) δ 2.41 (s, 3H), 7.38 (d, 2H, J = 8.2), 7.70−7.90 (m, 1H), 8.02 (s, 1H), 8.07 (d, 2H, J = 8.2), 8.15−8.22 (m, 1H), 8.35−8.45 (m, 1H), 8.59 (m, 1H), 8.83 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 21.5, 111.4, 120.6, 122.7, 124.3, 128.6, 129.4, 129.5, 130.7, 132.2, 136.3, 143.4, 147.0, 148.7, 150.0, 159.4, 165.6; mass spectrum (ESI) m/z 340.07471 $(M + H)^+$ ($C_{17}H_{14}N_3O_3S$ requires m/z 340.07559).

2-*N*-Benzoylamino-4-(3-aminophenyl)thiazole (7). A solution of compound **6** (1.41 g, 4.15 mmol) in a mixture of acetic acid and ethanol (1:1, v/v, 50 mL) was stirred under a hydrogen atmosphere in the presence of palladium on activated carbon (10%). The mixture was stirred for 3 h. After removal of the catalyst by filtration through a pad of Celite, the filtrate was concentrated under reduced pressure and the product crystallized in ethyl ether to give 1.22 g of pure compound **7** (95% yield) obtained as a white solid: R_f = 0.54 (9:1 CH_2Cl_2/MeOH); ^{1}H NMR (DMSO-d_6) δ 2.20 (s, 3H), 6.60−6.68 (m, 1H), 7.15−7.25 (m, 3H), 7.46 (d, 2H, J = 8.2), 7.56 (s, 1H), 8.15 (d, 2H, J = 8.2); ^{13}C NMR (DMSO-d_6) δ 21.5, 108.0, 111.7, 114.0, 114.1, 128.6, 129.5, 129.7, 135.4, 143.1, 149.2, 150.3, 158.7, 165.5; mass spectrum (ESI) m/z 310.10144 $(M + H)^+$ ($C_{17}H_{16}N_3OS$ requires m/z 310.10141).

General Procedure for the Synthesis of 5-Bromovaleric Acid (8) and 7-Bromoheptanoic Acid (9). To a solution of ethyl 5-bromovalerate or ethyl 7-bromoheptanoate (1.30 mmol) in methanol (5 mL) was added 2 N aqueous LiOH (0.6 mL, 1.30 mmol), and the mixture was stirred at room temperature for 30 min. The reaction mixture was then concentrated, redissolved in ethyl acetate, washed with 2 N aqueous HCl and brine, and finally dried over $MgSO_4$. Filtration and concentration under vacuum gave the crude products **8** and **9**, respectively, as white solids. These compounds were then used directly in the following reactions without further purification.

General Procedure for the Synthesis of Compounds 10, 12, and 14. To a solution of 5-bromovaleric acid (**8**, 150 mg, 0.742 mmol) in anhydrous dichloromethane (5 mL) were added HOBt (100 mg, 0.742 mmol) and EDAC (142 mg, 0.742 mmol). After the mixture had been stirred at room temperature under argon for 20 min, compound **4**, **7**, or **10** was added (0.485 mmol), and the mixture was stirred for an additional 3 h. The reaction mixture was then washed twice with water (2 × 15 mL) and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography (1:1 cylclohexane/EtOAc) led to compounds **10**, **12**, and **14** in 65, 70, and 68% yields, respectively.

*N-[3-(2-Acetamidothiazol-4-yl)phenyl]-5-bromopentanamide (**10**):* *Rf* = 0.25 (1:1 cyclohexane/EtOAc); ^{1}H NMR (DMSO-d_6) δ 1.65−1.90 (m, 4H), 2.16 (s, 3H), 2.36 (t, 2H, J = 7.0), 3.57 (t, 2H, J = 6.4), 7.30−7.40 (m, 2H), 7.50−7.60 (m, 2H), 8.28 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 22.9, 24.1, 32.1, 35.2, 35.6, 108.3, 117.1, 119.0, 121.0, 129.3, 135.1, 140.0, 149.1, 158.3, 169.0, 171.3; mass spectrum (ESI) m/z 398.03522 $(M + H)^+$ ($C_{15}H_{19}N_3O_2{}^{81}BrS$ requires m/z 398.03554).

*N-[4-(2-Acetamidothiazol-4-yl)phenyl]-5-bromopentanamide (**12**):* *Rf* = 0.46 (1:1 cyclohexane/EtOAc); ^{1}H NMR (DMSO-d_6) δ 1.65−1.90 (m, 4H), 2.15 (s, 3H), 2.36 (t, 2H, J = 7.0), 3.57 (t, 2H, J = 6.4), 7.47 (s, 1H), 7.64 (d, 2H, J = 7.6), 7.81 (d, 2H, J = 8.8); ^{13}C NMR (DMSO-d_6) δ 22.9, 24.1, 32.1, 35.2, 35.7, 106.3, 119.5, 126.4, 129.6, 139.2, 148.9, 158.2, 169.0, 171.2; mass spectrum (ESI) m/z 398.03519 ($C_{16}H_{19}O_2N_3{}^{81}BrS$ requires m/z 398.03554).

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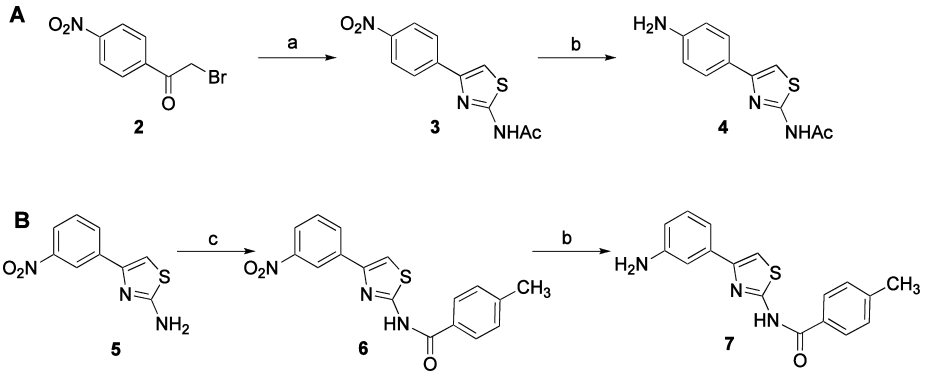
Scheme 1. [a]



[a]Reagents: (a) *N*-acetylthiourea, EtOH, 80 °C, 30 min; (b) H_2, Pd/C, AcOH/EtOH (1:1, v/v), 3 h; (c) 4-methylbenzoyl chloride, pyridine, 4 h.

*N-{4-[3-(5-Bromopentanamido)phenyl]thiazol-2-yl}-4-methylbenzamide (**14**):* *Rf* = 0.59 (1:1 cyclohexane/EtOAc); ^{1}H NMR (DMSO-d_6) δ 1.65−1.90 (m, 4H), 2.30−2.40 (m, 5H), 3.56 (t, 2H, *J* = 6.4), 7.47 (s, 1H), 7.30−7.45 (m, 4H), 7.55−7.65 (m, 2H), 8.04 (d, 2H, *J* = 8.2), 8.33 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 21.1, 23.5, 24.1, 32.1, 33.0, 35.1, 35.6, 109.0, 117.2, 119.1, 121.1, 128.6, 129.2, 129.5, 135.3, 140.0, 143.2, 149.5, 159.0, 165.5, 171.2, 174.6; mass spectrum (ESI) *m*/*z* 474.06671 ($C_{22}H_{23}O_2N_3{}^{81}BrS$ requires *m*/*z* 474.06684).

General Procedure for the Synthesis of Compounds 11, 13, and 15. To a solution of 7-bromoheptanoic acid (**9**, 200 mg, 0.965 mmol) in anhydrous dichloromethane (5 mL) were added HOBt (130 mg, 0.965 mmol) and EDAC (185 mg, 0.965 mmol). After the mixture had been stirred at room temperature under argon for 20 min, compound **4**, **7**, or **10** was added (0.644 mmol), and the mixture was stirred for an additional 3 h. The reaction mixture was then washed twice with water (2 × 15 mL) and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography (1:1 cyclohexane/EtOAc1) led to compounds **11**, **13**, and **15** in 75, 73, and 80% yield, respectively.

*N-[3-(2-Acetamodothiazol-4-yl)phenyl]-7-bromoheptanamide (**11**):* *Rf* = 0.33 (1:1 cyclohexane/EtOAc); ^{1}H NMR (DMSO-d_6) δ 1.65−1.90 (m, 4H), 2.16 (s, 3H), 2.36 (t, 2H, *J* = 7.0), 3.57 (t, 2H, *J* = 6.4), 7.30−7.40 (m, 2H), 7.50−7.60 (m, 2H), 8.28 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 22.9, 24.1, 32.1, 35.2, 35.6, 108.3, 117.1, 119.0, 121.0, 129.3, 135.1, 140.0, 149.1, 158.3, 169.0, 171.3; mass spectrum (ESI) *m*/*z* 424.06891 ($C_{18}H_{23}O_2N_3BrS$ requires *m*/*z* 424.06889).

*N-[4-(2-Acetamodothiazol-4-yl)phenyl]-7-bromoheptanamide (**13**):* *Rf* = 0.45 (1:1 cyclohexane/EtOAc); ^{1}H NMR (DMSO-d_6) δ 1.65−1.90 (m, 4H), 2.16 (s, 3H), 2.36 (t, 2H, *J* = 7.0), 3.57 (t, 2H, *J* = 6.4), 7.30−7.40 (m, 2H), 7.50−7.60 (m, 2H), 8.28 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 22.9, 24.1, 32.1, 35.2, 35.6, 108.3, 117.1, 119.0, 121.0, 129.3, 135.1, 140.0, 149.1, 158.3, 169.0, 171.3; mass spectrum (ESI) *m*/*z* 426.06662 ($C_{18}H_{23}O_2N_3{}^{81}BrS$ requires *m*/*z* 426.06684).

*N-{4-[3-(7-Bromoheptanamido)phenyl]thiazol-2-yl}-4-methylbenzamide (**15**):* *Rf* = 0.61 (1:1 cyclohexane/EtOAc); ^{1}H NMR (DMSO-d_6) δ 1.30−1.45 (m, 4H), 1.60−1.70 (m, 2H), 1.75−1.85 (m, 2H), 2.33 (t, 2H, *J* = 7.3), 2.40 (s, 3H), 3.54 (t, 2H, *J* = 6.7), 7.30−7.45 (m, 4H), 7.55−7.65 (m, 2H), 8.04 (d, 2H, *J* = 8.2), 8.32 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 16.4, 21.4, 25.3, 27.7, 28.1, 32.5, 35.6, 36.6, 109.0, 117.2, 119.0, 121.0, 128.6, 129.5, 135.2, 140.0, 143.2, 149.6, 150.9, 152.9, 158.9, 165.5, 171.6, 174.6; mass spectrum (ESI) *m*/*z* 502.09811 ($C_{24}H_{27}O_2N_3{}^{81}BrS$ requires *m*/*z* 502.09814).

Synthesis of the Conjugates. The conjugates were synthesized from the 3′-thiophosphate TFO as described previously.[23] TFO-NCpt is described in ref 23.

All conjugates were analyzed by UV spectrophotometry and mass spectrometry. **TFO-10**: ES-MS [M − H]$^-$ found 5475.0, calcd 5474.1. **TFO-11**: ES-MS [M − H]$^-$ found 5503.9, calcd 5502.7. **TFO-12**: ES-MS [M − H]$^-$ found 5475.8, calcd 5474.1. **TFO-13**: ES-MS [M − H]$^-$ found 5502.7, calcd 5502.7. **TFO-14**: ES-MS [M − H]$^-$ found 5551.1, calcd 5550.2. **TFO-15**: ES-MS [M − H]$^-$ found 5579.2, calcd 5578.2.

Biology. *Topo IB-Mediated DNA Cleavage.* Sequence-specific topo 1B-mediated DNA cleavage was assessed as described in ref 11 at 30 °C and pH 7.2.

DNase I Footprinting. The samples were prepared for topo IB-mediated DNA cleavage and DNase I digestion, and analysis was conducted as described in ref 24.

Fluorescence Intercalation Displacement Assay (FID). Each well of a 96-well plate was loaded with Tris buffer containing ethidium bromide {130 μL of a 0.575 μM solution in buffer [0.05 M Tris, 100 mM NaCl, and 10 mM $MgCl_2$ (pH 7.4)]}. To each well was added 10 μL of previously hybridized dsDNA (final concentration of 0.500 μM) followed by the compound (10 μL of a solution in Tris buffer at increasing concentrations of 1.5, 7.5, 112.5, 300, and 1500 μM, leading to final concentrations of 0.1, 0.5, 7.5, 20, and 100 μM, respectively). When TFO was added, the protocol was slightly modified. Tris buffer was added first (110 μL of 0.05 M Tris, 100 mM NaCl, and 10 mM $MgCl_2$) followed by DNA (10 μL, final concentration of 0.5 μM) and TFO (10 μL, final concentration of 5 μM). After incubation for 2 h at 25 °C to yield the triplex, ethidium bromide was added (10 μL, 7.5 μM solution in Tris buffer, final concentration of 0.5 μM) followed by the compound to be analyzed (10 μL of a solution in Tris buffer at increasing concentrations of 1.5, 7.5, 112.5, 300, and 1500 μM, leading to final concentrations of 0.1, 0.5, 7.5, 20, and 100 μM, respectively). In both cases, after incubation at 25 °C for 30 min, each well was read (average of 20 readings) on a GeniosPro Tecan fluorescence plate reader (excitation at 515 nm, emission at 595 nm) in duplicate with two control wells (no agent, 100% fluorescence; no DNA, 0% fluorescence). Fluorescence readings are reported as the percentage of fluorescence relative to control wells.

RESULTS

Synthesis of Nucleobase Derivatives 1, 4, and 7 (Scheme 1). The three nucleobases chosen for this study are reported in Figure 1B. Compound **1** [2-acetamido-4-(3-aminophenyl)thiazole] corresponds to the previously published

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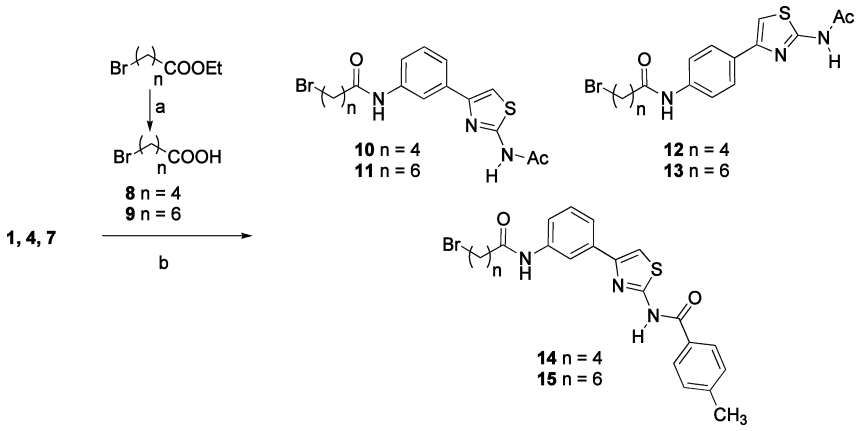
Scheme 2. [a]



[a]Reagents: (a) LiOH, methanol/water, 30 min; (b) HOBt, EDAC, CH_2Cl_2, 3 h.

nucleobase of nucleoside **S** and has been synthesized following the reported procedure, starting from commercial 3-nitroacetophenone.[16,22,25] Compounds **4** and **7** are new analogues of **1**, conceived to mimic a base pair and useful for studying the influence of the position and nature of the substituents of the nucleobase on the interaction with target DNA. Compound **4** [2-acetamido-4-(4-aminophenyl)thiazole] is an analogue of **1** but bears the amino group in the para instead of in the meta position. This derivative has been synthesized following the procedure used for compound **1** (Scheme 1A). In this case, the 1-bromo-4-nitroacetophenone (**2**) is coupled with *N*-acetylthiourea in ethanol leading to the 2-*N*-acetylamino-4-(4-nitrophenyl)thiazole (**3**) in 98% yield. The following reduction of the nitro group by catalytic hydrogenation led to the desired compound **4** in 95% yield. The third nucleobase [**7** (Scheme 1B)] was obtained in two steps starting from known 2-amino-4-(3-nitrophenyl)thiazole (**5**) by reaction of the amino group in the presence of benzoyl chloride in dichloromethane to yield 2-*N*-benzoylamino-4-(3-nitrophenyl)thiazole (**6**) in 92% yield. Compound **7** was obtained in 95% yield by catalytic hydrogenation in the presence of palladium on carbon.

The synthesized nucleobases **1**, **4**, and **7** were conjugated to a TFO to study their ability to inhibit topo IB when positioned site-specifically on the DNA sequence. The DNA target sequence, its corresponding TFO, and the geometry and structure of the cleavage site have been optimized previously.[26] The distance between the nucleobase and the oligonucleotide chain was modulated using two appropriate linkers of four and six atoms (Scheme 2) according to previous results.[11] 5-Bromovaleric acid (**8**) and 7-bromoheptanoic acid (**9**), obtained after hydrolysis of commercially available ethyl esters in the presence of LiOH in methanol, were coupled with the nucleobases in the presence of HOBt and EDAC in dichloromethane. This led to the desired amide compounds **10**−**15** in 65−80% yield, ready to be coupled to the TFO.

Synthesis of TFO−Nucleobase Conjugates. The conjugates between compounds **10**−**15** and the 16-mer TFO illustrated in Figure 1C (**TFO-10**−**TFO-15**) were synthesized following the procedure that we have recently optimized.[23] To increase the stability of the triple-helical structure at pH 7.2 and 37 °C, thymidine was substituted with 5-propynyldeoxyuridine (P) and deoxycytidine with 5-methyldeoxycytidine (M).[10,27] The DNA target sequence is reported in Figure 1C: the TFO binding site is underlined and the topo I cleavage site colored red.

Cleavage and Binding Activity. The conjugates and nucleobases were tested for their ability to inhibit topo IB. As shown in Figure 2A, the conjugates, once bound to the DNA [DNase I footprinting (Figure 2A, lanes 18−25)], induced an efficient and sequence-specific DNA cleavage mediated by topo IB on the 3′ side of the triplex at a T↓T site (arrow, lanes 7, 9, 11, 13, 15, and 17) like the corresponding conjugate of a camptothecin derivative, TFO-NCpt (lane 5), used as a control. DNA cleavage was observed at concentrations as low as 0.05 μM (as shown for **TFO-10** in Figure 2B, lane 8). The conjugates showed a comparable efficacy (between 10 and 30%, depending on the DNA fragment preparation). The length of the linker had a small effect on the cleavage efficiency of conjugates **TFO-15** and **TFO-14** with a mean increase of ∼20% in the cleavage for the six-carbon linker (**TFO-15**). The nature of the nucleobase had little effect; still the best was nucleobase **7**, bearing an aromatic substituent, present in conjugates **TFO-15** and **TFO-14**. The nucleobase analogues alone 20 times more concentrated than the conjugates (Figure 2A, lanes 6, 8, 10, 12, 14, and 16) are weak inhibitors, while the TFO alone induced little modification (lane 3).

DNase I footprinting confirmed that the conjugates are bound to the DNA target (Figure 2A, lanes 20−25). In addition, there is a clear increase in the digestion pattern of the DNase I at the 3′ end of the triplex−duplex junction where the nucleobases are positioned by the TFO. This exaltation is not observed in the presence of the triplex alone (lane 19). This seems to suggest that the base mimics are intercalated at the 3′ triplex−duplex junction[28] and thus could exercise their poisoning activity of topo IB in the same manner that was demonstrated for campthotecin analogues. To test the binding of the base mimics to DNA, we performed fluorescence intercalation displacement assays (Figure 3). Figure 3A shows the binding of compound **14** to the DNA occurs only once the triplex was formed on the DNA target. The same was valid for all compounds (Figure 3B), while the compounds alone bound only weakly to the DNA target as assessed by DNase I footprinting experiments (Figure 3C).

Finally, we investigated the effect of the conjugation of the TFO to **10**−**15** on the stability of the triplex. C_{50} values were determined by measuring DNA binding of the TFO alone or conjugated to the radiolabeled DNA target in gel shift analysis. While camptothecin conjugates destabilize the triple-helical structure,[8] these compounds only slightly alter the stability of the triplex as shown by the C_{50} values listed in Table 1.

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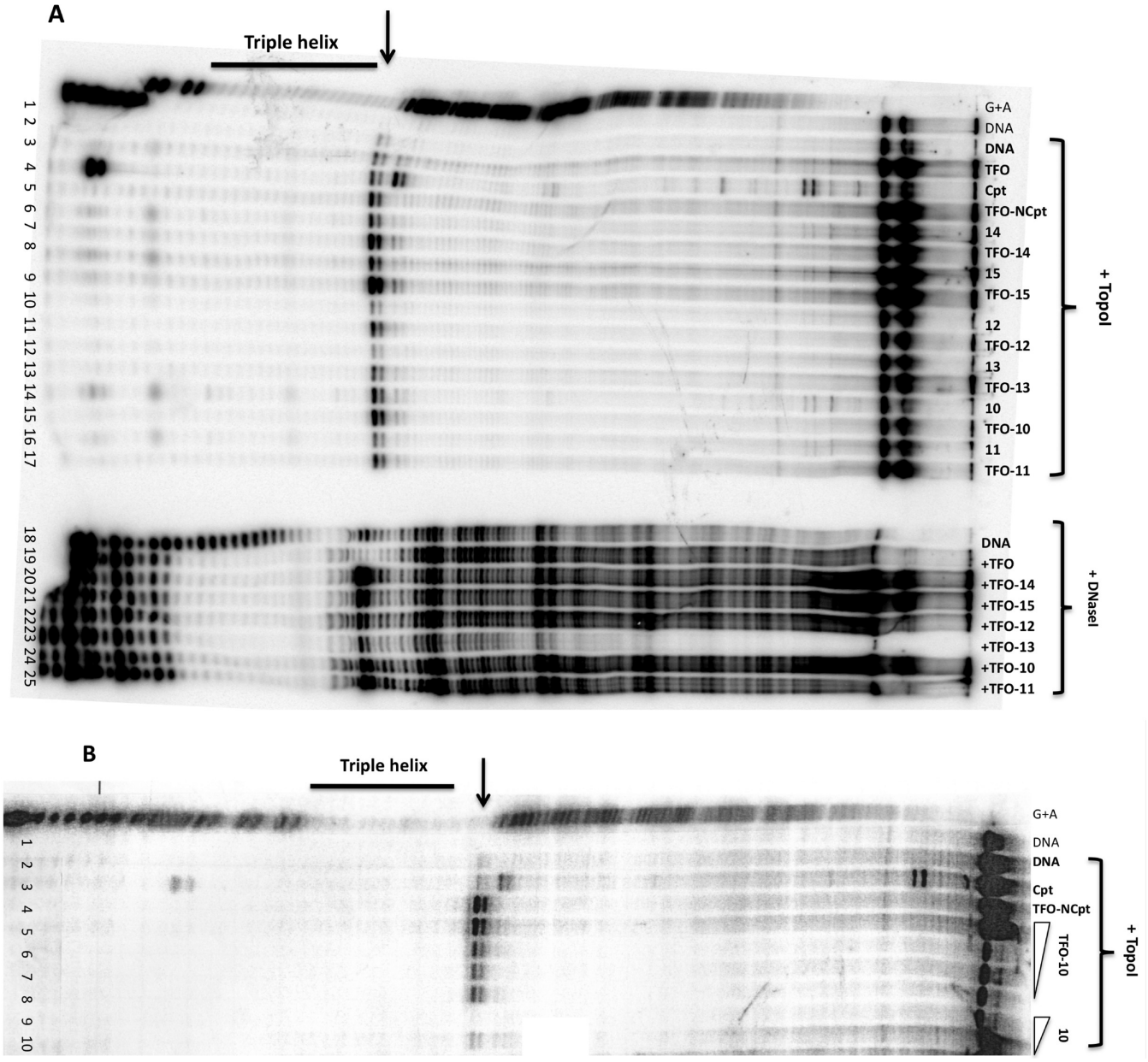

Figure 2. DNA cleavage assays with topo IB and DNase I footprinting. Cleavage products were resolved on a 8% polyacrylamide gel containing 7 M urea. Adenine/guanine-specific Maxam–Gilbert chemical cleavage reactions were used as markers (lane G+A). (A) The target duplex alone (lane 1) was incubated with 10 units of topo IB (lane 2) and in the presence of 5 μM TFO (lane 3), 10 μM Cpt (lane 4), 5 μM TFO-NCpt (lane 5), 100 μM **14** (lane 6), 5 μM **TFO-14** (lane 7), 100 μM **15** (lane 8), 5 μM **TFO-15** (lane 9), 100 μM **12** (lane 10), 5 μM **TFO-12** (lane 11), 100 μM **13** (lane 12), 5 μM **TFO-13** (lane 13), 100 μM **10** (lane 14), 5 μM **TFO-10** (lane 15), 100 μM **11** (lane 16), or 5 μM **TFO-11** (lane 17). The DNA target was digested with DNase I (lane 18) after incubation with 5 μM TFO (lane 19), **TFO-14** (lane 20), **TFO-15** (lane 21), **TFO-12** (lane 22), **TFO-13** (lane 23), **TFO-10** (lane 24), or **TFO-11** (lane 25). (B) The target duplex alone (lane 1) was incubated with 10 units of topo IB (lane 2) and in the presence of 10 μM Cpt (lane 3), 5 μM TFO-NCpt (lane 4), 1, 0.5, 0.1, and 0.05 μM **TFO-10** (lanes 5–8, respectively), or 1 and 100 μM **10** (lanes 10 and 9, respectively).

DISCUSSION

The determination of the crystal structure of the DNA–topo IB–topotecan ternary complex[5] prompted us to design new topoisomerase inhibitors that bear the same features observed for camptothecin derivatives: the molecule should (i) intercalate as a base pair in the target DNA and (ii) be kept firmly in the cleavage complex. On one hand, nucleoside **S** (Figure 1A), bearing an aminophenyl-thiazole derivative as a nucleobase, was developed by our group to recognize a TA inversion in a DNA oligopurine·oligopyrimidine sequence during triplex formation.[16] This derivative is able not only to form three specific hydrogen bonds with the TA base pair but also to mimic a base pair when integrated into a triple-helical structure. On the other hand, we have demonstrated that 20-deoxycamptothecin, lacking an important interaction for the stabilization of the topo IB cleavage complex and thus resulting in an inactive inhibitor of topo IB, could become again a topo IB poison upon conjugation to a TFO.[14] This is due to the fact that the DNA ligand moiety of the conjugates binds to the DNA at its target site,[29,30] positions the drug at this site, and keeps it in the cleavage complex, thereby inducing a specific topo IB-mediated DNA cleavage.

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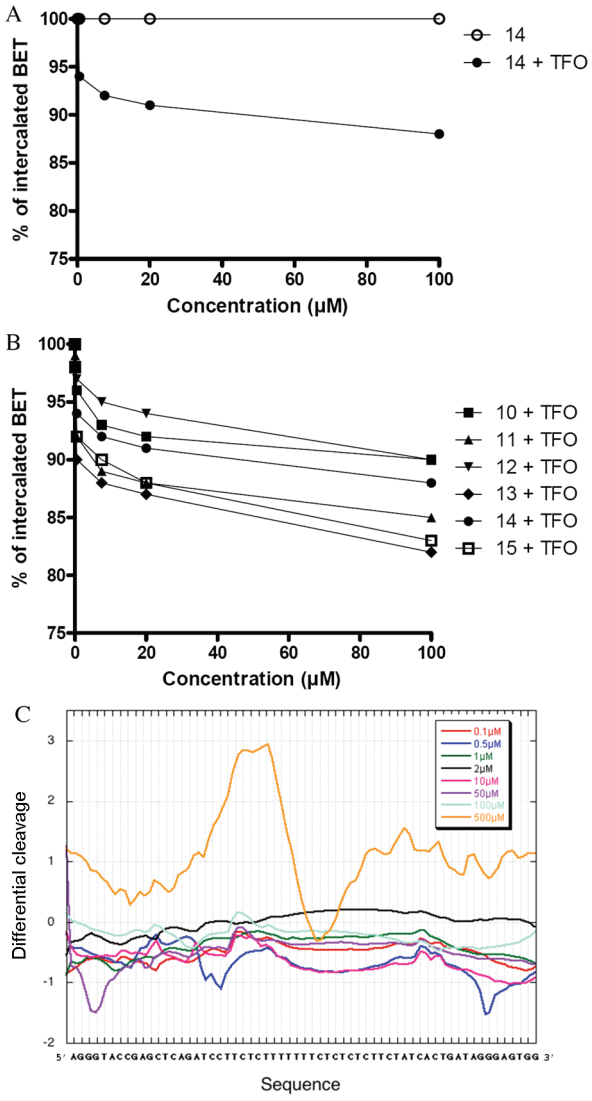

Figure 3. Binding of the unconjugated compounds, followed by fluorescence displacement assay and DNase I footprinting. (A) Percentage of fluorescence obtained after intercalation of BET in double-stranded DNA (dsDNA) in the presence of increasing concentrations of compound **14** in the presence (●) or absence (○) of TFO. (B) Percentage of fluorescence obtained after intercalation of BET in dsDNA in the presence of TFO and increasing concentrations of compounds **10**–**15**. (C) Differential cleavage plots comparing susceptibility of the labeled DNA fragment to DNase I cutting in the presence of compound **14** at increasing concentrations (0.1, 0.5, 1, 2, 10, 50, 100, and 500 μM). Negative values correspond to a ligand-protected site, and positive values represent enhanced cleavage. Vertical scales are in units of ln(fa) − ln(fc), where fa is the fractional cleavage at any bond in the presence of the ligand and fc is the fractional cleavage of the same bond in the control given closely similar extents of overall digestion. Each line drawn represents a three-bond running average of individual data points, calculated by averaging the value of ln(fa) − ln(fc) at any bond with those of its nearest neighbors. Only a zoom of the region of the triplex site analyzed by densitometry is shown.

On the basis of these previous results, we hypothesized that base pair mimics could act as campthotecin analogues, when positioned and kept in the cleavage complex by triplex formation, and inhibit the religation reaction after the formation of topo I–DNA cleavage complex. Accordingly, the conjugation of the base pair mimic to a TFO is essential. We thus synthesized

Table 1. Stabilities of the Conjugates[a]

oligonucleotide	C_{50} (μM)
TFO	0.18 ± 0.02
TFO-10	0.16 ± 0.03
TFO-11	0.29 ± 0.05
TFO-12	0.30 ± 0.07
TFO-13	0.39 ± 0.05
TFO-14	0.30 ± 0.04
TFO-15	0.26 ± 0.03

[a] C_{50} is the concentration at which 50% of the triplex is formed, after incubation for 24 h at 37 °C in 50 mM HEPES (pH 7.2), 10 mM $MgCl_2$, 50 mM NaCl, 10% sucrose, and 0.5 μg/μL tRNA.

three modified nucleobases containing an aminophenyl-thiazole moiety (**1**, **4**, and **7**), conjugated them to a 16-mer TFO that binds to a specific oligopyrimidine·oligopurine DNA sequence, and studied their ability to inhibit topo I. Each modified nucleobase was linked to the TFO with two different spacers bearing four and six carbon atoms so that we could study the influence of linker length on topo I inhibition. The six conjugates thus obtained (**TFO-10**–**TFO-15**) resulted in efficient topo IB inhibitors, confirming our proposed hypothesis. For the efficacy of inhibition of the topo IB, it is important to position and keep the inhibitor in the cleavage complex; the compounds not conjugated are inactive. Clearly, the TFO plays the role of positioning the base pair mimic. The observed cleavage site is a TpT site (Figure 1), suggesting that the nucleobases intercalate between two TA base pairs as hypothesized. In fact, here we have chosen **S** analogues that are known to specifically interact with AT-rich sequences of the DNA[16,22] because the targeted topo IB cleavage site at the 3′ end of the triplex site used in this study contained a T at position −1 and a T at position +1. In agreement, the DNase I footprinting experiments showed an exaltation of the cleavage in the presence of the conjugates at the 3′ end where the nucleobases are attached (Figure 2, lanes 20–25). Furthermore, FID experiments (Figure 3A,B) showed that the compounds bind to DNA only when the triplex is formed, suggesting a preference of intercalation in the triplex or at the duplex–triplex junction. Importantly, other intercalators will not act in the same manner because they may have a certain and different sequence preference.

The three compounds are equally active once conjugated. This is in perfect agreement with the proposed hypothesis. First, the three nucleobases differ little chemically: in compounds **1** and **4**, the site of attachment to the TFO changes from the meta to the para position, while compound **7** presents an additional aromatic substituent. Indeed, conjugate **TFO-15** (from nucleobase **7**) showed the best activity probably because the presence of the additional aromatic cycle strengthens the interactions favoring the intercalation. Second, the TFO positions the three base pair mimics with the same efficacy in the topo IB-mediated DNA cleavage complex, and it is this physical positioning of the base pair mimic as DNA interacalator in the cleavage complex (as confirmed by the DNase I footprinting and FID experiments) that is responsible for blocking the religation reaction of the enzyme and thus the stabilization of DNA cleavage. The base pair mimics alone have no effect. Third, the cleavage specificity is dictated by the recognition of the DNA by the TFO, and all conjugates bear the same TFO.

The efficacy of cleavage of these new conjugates is comparable to that of the TFO–CPT conjugates developed so far.

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An advantage compared to CPT conjugates[8] is that compounds **1**, **4**, and **7** did not destabilize greatly the triple-helical structure once conjugated to the TFO (Table 1). Only a slight destabilization (maximum of 2-fold) was observed. This is in agreement with previous findings observing that conjugation of intercalators at the 5′ end increased triplex stability,[31−35] but not at the 3′ end as shown here for the nucleobase conjugates.

In conclusion, on the basis of the crystal structure of the ternary complex, we have designed and synthesized six new inhibitors of topo IB that are very efficient and, even more importantly, sequence-specific. As we had demonstrated in the past for campthotecin analogues conjugated to a TFO, these inhibitors should not present secondary effects because the unconjugated compounds are not active. In addition, the compounds do not destabilize triplex formation, a main difference compared to CPT conjugates. These important features represent a great advantage for the future development of these new compounds as topo I inhibitors.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H NMR and ^{13}C NMR spectra as well as HRMS spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Champoux, J. J. (2001) DNA Topoisomerases: Structure, Function and Mechanism. *Annu. Rev. Biochem. 70*, 369−413.

(2) Wang, J. C. (2002) Cellular roles of DNA topoisomerases: A molecular perspective. *Nat. Rev. Mol. Cell Biol. 3*, 430−440.

(3) Pommier, Y. (2006) Topoisomerase I inhibitors: Camptothecins and beyond. *Nat. Rev. Cancer 6*, 789−802.

(4) Hande, K. R. (2003) Topoisomerase II inhibitors. *Cancer Chemother. Biol. Response Modif. 21*, 103−125.

(5) Staker, B. L., Hjerrild, K., Feese, M. D., Behnke, C. A., Burgin, A. B. Jr., and Stewart, L. (2002) The mechanism of topoisomerase I poisoning by a camptothecin analog. *Proc. Natl. Acad. Sci. U.S.A. 99*, 15387−15392.

(6) Staker, B. L., Feese, M. D., Cushman, M., Pommier, Y., Zembower, D., Stewart, L., and Burgin, A. B. (2005) Structures of three classes of anticancer agents bound to the human topoisomerase I-DNA covalent complex. *J. Med. Chem. 48*, 2336−2345.

(7) Marchand, C., Antony, S., Kohn, K. W., Cushman, M., Ioanoviciu, A., Staker, B. L., Burgin, A. B., Stewart, L., and Pommier, Y. (2006) A novel norindenoisoquinoline structure reveals a common interfacial inhibitor paradigm for ternary trapping of the topoisomerase I-DNA covalent complex. *Mol. Cancer Ther. 5*, 287−295.

(8) Oussedik, K., Francois, J. C., Halby, L., Senamaud-Beaufort, C., Toutirais, G., Dallavalle, S., Pommier, Y., Pisano, C., and Arimondo, P. B. (2010) Sequence-specific targeting of IGF-I and IGF-IR genes by camptothecins. *FASEB J. 24*, 2235−2244.

(9) Arimondo, P. B., Bailly, C., Boutorine, A., Ryabinin, V., Syniakov, A., Sun, J. S., Garestier, T., and Hélène, C. (2001) Directing Topoisomerase I-mediated DNA Cleavage to Specific Sites by Camptothecin tethered to Minor and Major Groove Ligands. *Angew. Chem., Int. Ed. 40*, 3045−3048.

(10) Arimondo, P. B., Bailly, C., Boutorine, A., Sun, J. S., Garestier, T., and Hélène, C. (1999) Targeting topoisomerase I cleavage to specific sequences of DNA by triple helix-forming oligonucleotide conjugates. A comparison between a rebeccamycin derivative and camptothecin. *C. R. Acad. Sci., Ser. III 322*, 785−790.

(11) Arimondo, P. B., Boutorine, A., Baldeyrou, B., Bailly, C., Kuwahara, M., Hecht, S. M., Sun, J. S., Garestier, T., and Hélène, C. (2002) Design and optimization of camptothecin conjugates of triple helix- forming oligonucleotides for sequence-specific DNA cleavage by topoisomerase I. *J. Biol. Chem. 277*, 3132−3140.

(12) Arimondo, P. B., Thomas, C. J., Oussedik, K., Baldeyrou, B., Mahieu, C., Halby, L., Guianvarc'h, D., Lansiaux, A., Hecht, S. M., Bailly, C., and Giovannangeli, C. (2006) Exploring the cellular activity of camptothecin-triple-helix-forming oligonucleotide conjugates. *Mol. Cell. Biol. 26*, 324−333.

(13) Duca, M., Guianvarc'h, D., Oussedik, K., Halby, L., Garbesi, A., Dauzonne, D., Monneret, C., Osheroff, N., Giovannangeli, C., and Arimondo, P. B. (2006) Molecular basis of the targeting of topoisomerase II-mediated DNA cleavage by VP16 derivatives conjugated to triplex-forming oligonucleotides. *Nucleic Acids Res. 34*, 1900−1911.

(14) Arimondo, P. B., Laco, G. S., Thomas, C. J., Halby, L., Pez, D., Schmitt, P., Boutorine, A., Garestier, T., Pommier, Y., Hecht, S. M., Sun, J. S., and Bailly, C. (2005) Activation of camptothecin derivatives by conjugation to triple helix-forming oligonucleotides. *Biochemistry 44*, 4171−4180.

(15) Guianvarc'h, D., Fourrey, J. L., Maurisse, R., Sun, J. S., and Benhida, R. (2002) Synthesis, incorporation into triplex-forming oligonucleotide, and binding properties of a novel 2′-deoxy-C-nucleoside featuring a 6-(thiazolyl-5)benzimidazole nucleobase. *Org. Lett. 4*, 4209−4212.

(16) Guianvarc'h, D., Fourrey, J. L., Maurisse, R., Sun, J. S., and Benhida, R. (2003) Design of artificial nucleobases for the recognition of the AT inversion by triple-helix forming oligonucleotides: A structure-stability relationship study and neighbour bases effect. *Bioorg. Med. Chem. 11*, 2751−2759.

(17) Li, J. S., Chen, F. X., Shikiya, R., Marky, L. A., and Gold, B. (2005) Molecular recognition via triplex formation of mixed purine/pyrimidine DNA sequences using oligoTRIPs. *J. Am. Chem. Soc. 127*, 12657−12665.

(18) Mizuta, M., Banba, J., Kanamori, T., Tawarada, R., Ohkubo, A., Sekine, M., and Seio, K. (2008) New nucleotide pairs for stable DNA triplexes stabilized by stacking interaction. *J. Am. Chem. Soc. 130*, 9622−9623.

(19) Rahman, S. M., Seki, S., Obika, S., Haitani, S., Miyashita, K., and Imanishi, T. (2007) Highly stable pyrimidine-motif triplex formation at physiological pH values by a bridged nucleic acid analogue. *Angew. Chem., Int. Ed. 46*, 4306−4309.

(20) Rusling, D. A., Powers, V. E., Ranasinghe, R. T., Wang, Y., Osborne, S. D., Brown, T., and Fox, K. R. (2005) Four base recognition by triplex-forming oligonucleotides at physiological pH. *Nucleic Acids Res. 33*, 3025−3032.

(21) Sasaki, S., Taniguchi, Y., Takahashi, R., Senko, Y., Kodama, K., Nagatsugi, F., and Maeda, M. (2004) Selective formation of stable triplexes including a TA or a CG interrupting site with new bicyclic nucleoside analogues (WNA). *J. Am. Chem. Soc. 126*, 516−528.

(22) Guianvarc'h, D., Benhida, R., Fourrey, J. L., Maurisse, R., and Sun, J. S. (2001) Incorporation of a novel nucleobase allows stable oligonucleotide-directed triple helix formation at the target sequence containing a purine·pyrimidine interruption. *Chem. Commun.*, 1814−1815.

(23) Vekhoff, P., Halby, L., Oussedik, K., Dallavalle, S., Merlini, L., Mahieu, C., Lansiaux, A., Bailly, C., Boutorine, A., Pisano, C., Giannini, G., Alloatti, D., and Arimondo, P. B. (2009) Optimized synthesis and

enhanced efficacy of novel triplex-forming camptothecin derivatives based on gimatecan. *Bioconjugate Chem. 20*, 666−672.
(24) Shim, Y. H., Arimondo, P. B., Laigle, A., Garbesi, A., and Lavielle, S. (2004) Relative DNA binding affinity of helix 3 homeodomain analogues, major groove binders, can be rapidly screened by displacement of prebound ethidium bromide. A comparative study. *Org. Biomol. Chem. 2*, 915−921.
(25) Wang, Z. (2010) Hantzsch Thiazole Synthesis. In *Comprehensive Organic Name Reactions and Reagents*, pp 1330−1334, John Wiley & Sons, New York.
(26) Arimondo, P. B., Angenault, S., Halby, L., Boutorine, A., Schmidt, F., Monneret, C., Garestier, T., Sun, J. S., Bailly, C., and Hélène, C. (2003) Spatial organization of topoisomerase I-mediated DNA cleavage induced by camptothecin-oligonucleotide conjugates. *Nucleic Acids Res. 31*, 4031−4040.
(27) Lacroix, L., Lacoste, J., Reddoch, J. F., Mergny, J. L., Levy, D. D., Seidman, M. M., Matteucci, M. D., and Glazer, P. M. (1999) Triplex formation by oligonucleotides containing 5-(1-propynyl)-2′-deoxyuridine: Decreased magnesium dependence and improved intracellular gene targeting. *Biochemistry 38*, 1893−1901.
(28) Chomilier, J., Sun, J. S., Collier, D. A., Garestier, T., Hélène, C., and Lavery, R. (1992) A computational and experimental study of the bending induced at a double-triple helix junction. *Biophys. Chem. 45*, 143−152.
(29) Arimondo, P. B., Bailly, C., Boutorine, A., Moreau, P., Prudhomme, M., Sun, J. S., Garestier, T., and Hélène, C. (2001) Triple helix-forming oligonucleotides conjugated to indolocarbazole poisons direct topoisomerase I-mediated DNA cleavage to a specific site. *Bioconjugate Chem. 12*, 501−509.
(30) Arimondo, P. B., Bailly, C., Boutorine, A., Prudhomme, M., Sun, J.-S., Garestier, T., and Hélène, C. (2000) Recognition and Cleavage of DNA by Rebeccamycin- or Benzopyridoquinoxaline-Triplex-Forming Oligonucleotide Conjugates. *Bioorg. Med. Chem. 8*, 777−784.
(31) Capobianco, M. L., De Champdore, M., Francini, L., Lena, S., Garbesi, A., and Arcamone, F. (2001) New TFO conjugates containing a carminomycinone-derived chromophore. *Bioconjugate Chem. 12*, 523−528.
(32) Garbesi, A., Bonazzi, S., Zanella, S., Capobianco, M. L., Giannini, G., and Arcamone, F. (1997) Synthesis and binding properties of conjugates between oligodeoxynucleotides and daunorubicin derivatives. *Nucleic Acids Res. 25*, 2121−2128.
(33) Stierle, V., Duca, M., Halby, L., Senamaud-Beaufort, C., Capobianco, M. L., Laigle, A., Jolles, B., and Arimondo, P. B. (2008) Targeting MDR1 gene: Synthesis and cellular study of modified daunomycin-triplex-forming oligonucleotide conjugates able to inhibit gene expression in resistant cell lines. *Mol. Pharmacol. 73*, 1568−1577.
(34) Sun, J. S., François, J. C., Montenay-Garestier, T., Saison-Behmoaras, T., Roig, V., Chassignol, M., Thuong, N. T., and Hélène, C. (1989) Sequence-specific intercalating agents. Intercalation at specific sequences on duplex DNA via major-groove recognition by oligonucleotide intercalator conjugates. *Proc. Natl. Acad. Sci. U.S.A. 86*, 9198−9202.
(35) Sun, J. S., Giovannangeli, C., Francois, J. C., Kurfurst, R., Montenay-Garestier, T., Asseline, U., Saison-Behmoaras, T., Thuong, N. T., and Hélène, C. (1991) Triple-helix formation by α oligodeoxynucleotides and α oligodeoxynucleotide-intercalator conjugates. *Proc. Natl. Acad. Sci. U.S.A. 88*, 6023−6027.