Water Soluble Heliomycin Derivatives to Target i-Motif DNA

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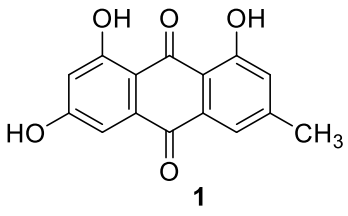
Heliomycin (also known as resistomycin) is an antibiotic with a broad spectrum of biological activities. However, low aqueous solubility and poor knowledge of its chemical properties have limited the development of this natural product. Here, we present an original scheme for the introduction of aminoalkylamine residues at positions 3, 5 and 7 of heliomycin and, using this, have prepared a series of novel water-soluble derivatives. The addition of side-chains to the heliomycin scaffold significantly improves their interaction with different DNA secondary structures. One derivative, 7-deoxy-7-(2-aminoethyl)amino-10-*O*-methylheliomycin (**8e**) demonstrated affinity, stabilization potential and good selectivity towards i-motif-forming DNA sequences over duplex and G-quadruplex. Heliomycin derivatives therefore represent promising molecular scaffolds for further development as DNA-i-motif interacting ligands and potential chemotherapeutic agents.

Introduction

Annelated polyketides are a potent class of secondary metabolites found in various microorganisms and embryophytes.[1] Representative examples include emodin (**1**), doxorubicin (**2**), chartreusin (**3**) and hypericin (**4**). These and many others have been of interest for decades by virtue of their broad spectrum of biological effects.[2] Heliomycin (also known as resistomycin, **5**) is a polyketide antibiotic that was previously isolated independently by both German (1951) and Russian (1958) scientists[3] and has since demonstrated various biological activities. Depending on the concentration, heliomycin has been shown to block either RNA or protein synthesis in bacterial cells, exhibiting both bacteriostatic and bactericidal effects.[4] Taking advantage of these properties, heliomycin was approved for the treatment of upper respiratory tract infections and skin lesions in the USSR and has been in use for several decades.[5] Heliomycin has also demonstrated high antiviral activity against influenza, herpes, smallpox and tick-borne encephalitis viruses.[6] The antiproliferative potency[7] of heliomycin is also comparable to the gold standard of cancer chemotherapy, doxorubicin, making this natural product attractive for lead optimization for the development of new antitumor agents.



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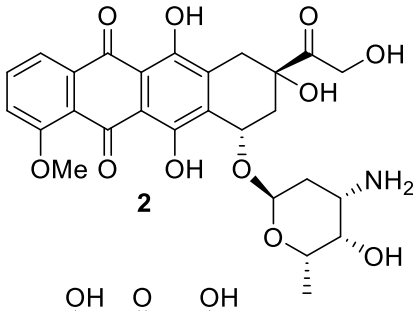

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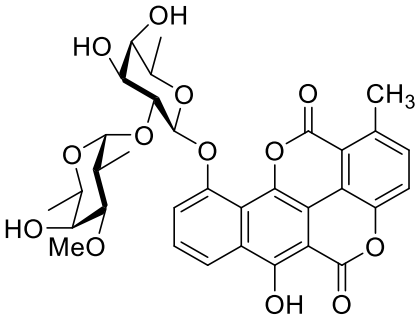

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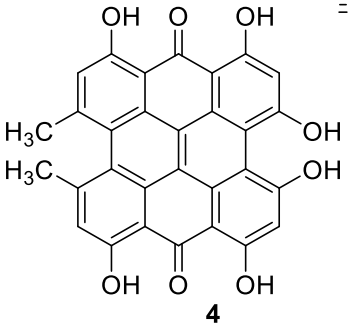

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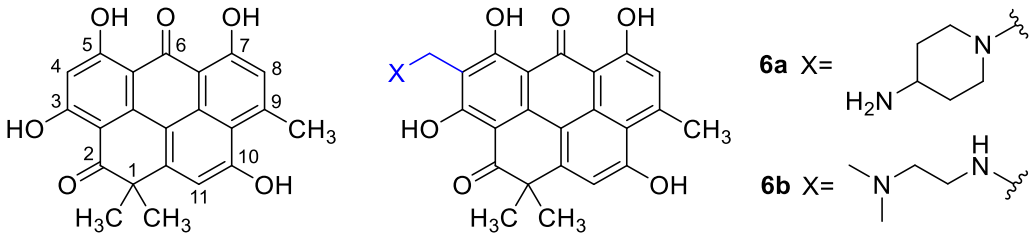
Despite the fact that heliomycin was first described in the middle of the last century, there are relatively few publications on chemical modifications of the scaffold. These cover methylation, acetylation, halogenation, and mild methods of oxidation and reduction.[8] Moreover, the biological activity of heliomycin derivatives is almost entirely unexplored. Recently we demonstrated that heliomycin (**5**) can undergo a Mannich reaction to produce 4-aminomethyl derivatives (e.g. compounds **6a**,**b**).[9] The use of functionalized amines allows a wide variety of heliomycin derivatives to be obtained and the introduction of these functionalities significantly affects both their physicochemical and biological properties. In particular, the introduction of diamines (e.g., 4-aminopiperidine) led to a water-soluble 4-aminomethyl derivative of heliomycin (**6a**) which inhibits topoisomerase I and suppresses the growth of tumor cells, including cell-lines presenting with activated mechanisms of multidrug resistance (MDR).[9] With such biological potential for these compounds, study of new heliomycin derivatives and enhancing their physico-chemical properties for biological studies is worthwhile.



Heliomycin (**5**) **6a,b**

A number of fused polyketides are capable of binding to nucleic acids this is implicated in their respective biological activity.[10] Although heliomycin itself binds only weakly to DNA, the introduction of a 4-aminomethyl fragment, especially those containing diamine residues (e.g., 4-aminopiperidine, 3-aminopyrrolidine, and ethylenediamine) significantly increases their affinity to DNA.[9] The poly-fused planar structure of heliomycin seems to be a promising scaffold for the

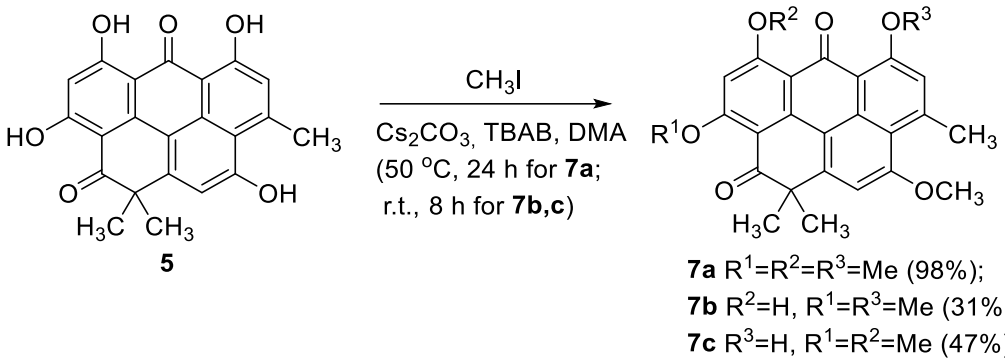
development of new ligands for nucleic acids yet their limited solubility in aqueous solutions limits their use. Introduction of diaminoalkanes to a central DNA-binding scaffold has previously been shown to modulate the affinity for different folded topologies of DNA and RNA sequences.[11] GC-rich DNA sequences can form alternative secondary structures called G-quadruplexes and i-motifs,[12] which are prevalent in genomic DNA,[13] exist in cells[14] and play key roles in different biological functions including the regulation of gene expression.[15] Although there are now thousands of compounds that have been shown to target G-quadruplexes,[16] there are only a few classes of compounds that have been shown to bind i-motif structures.[17] To create heliomycin derivatives capable of potential DNA secondary-structure binding, introduction of side chains to the heliomycin core is necessary. Methods to achieve this are relatively unexplored, thus, to develop heliomycin derivatives, new synthetic routes to introduce side chains are required. Herein, we describe the synthesis of novel water soluble heliomycin derivatives and their structure activity relationships, including characterization of their DNA-interacting properties with different DNA secondary structures (double helical, G-quadruplex and i-motif), and results of preliminary screening of their anticancer properties.

Results and Discussion

To develop a scheme for the transformation of the hydroxy groups of heliomycin into aminoalkylamino moieties, we initially attempted to apply direct substitution by treatment with amines. Despite the presence of electron-withdrawing carbonyl groups, the hydroxy groups of heliomycin were not replaced when heated with ethylenediamine. This is likely caused by ionization of the hydroxy groups under basic conditions, leading to a decrease in their reactivity in the nucleophilic substitution reactions.

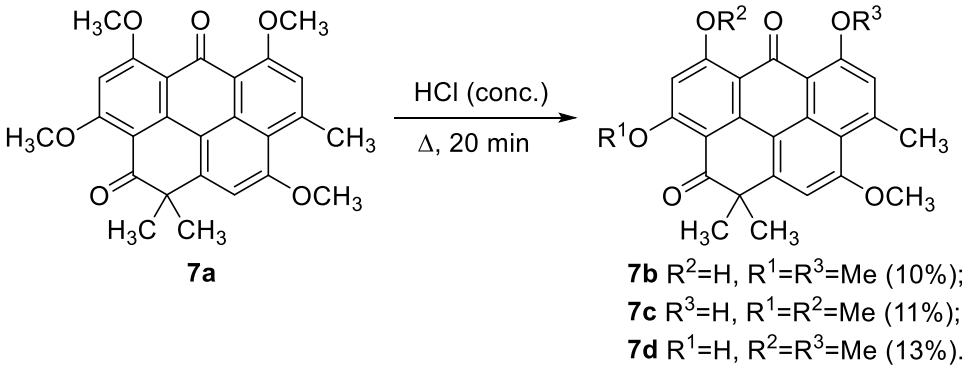
We previously established that alkoxy groups at the *peri*-positions to carbonyls of poly-fused anthraquinone derivatives are easily replaced by various amines.[18] This method was successfully

applied to the preparation of amino-derivatives of heliomycin. First, methylation of the hydroxy groups of heliomycin was investigated and conditions were optimized. In accordance with described procedure[8c] the alkylation of heliomycin by treatment with an excess of methyl iodide in the presence of potassium carbonate led a challenging-to-separate mixture of di-, tri-, and tetra-*O*-methyl derivatives. It was found that the use of Cs_2CO_3 as the base and a phase transfer catalyst (e.g., TBAB) significantly accelerated the alkylation of heliomycin. Addition of 8-fold excess both of methyl iodide and dry Cs_2CO_3 in the presence of catalytic amounts of TBAB at 50 °C provides an exhaustive methylation of heliomycin, giving the tetra-*O*-methyl derivative **7a** in quantitative yield (Scheme 1). Decreasing the reaction temperature and amount of the base led to different mixtures of di-, tri- and tetra-*O*-methyl ethers. It was found that treatment of heliomycin **5** with 3.5 equivalents of Cs_2CO_3 and 6-fold excess of methyl iodide at room temperature resulted in a mixture with two major components. Chromatographic separation of the formed mixture yielded two products (red and yellow) which have the exact mass corresponding to tri-*O*-methyl ethers of heliomycin. Brockmann and co-workers[8c] previously identified the red isomer as 3-hydroxy derivative (5,7,10-tri-*O*-methylheliomycin) and the yellow as the 5-hydroxy- or the 7-hydroxy analogue.[8c] However, we identified the red isomer from our experiments to be 3,7,10-tri-*O*-methylheliomycin (**7b**) and the yellow one as 3,5,10-tri-*O*-methyl ether **7c** (Scheme 1). Without the third isomer, which was not detected in our alkylation conditions, we cannot precisely postulate that assignment of isomers in the paper of 1969[8c] is incorrect.



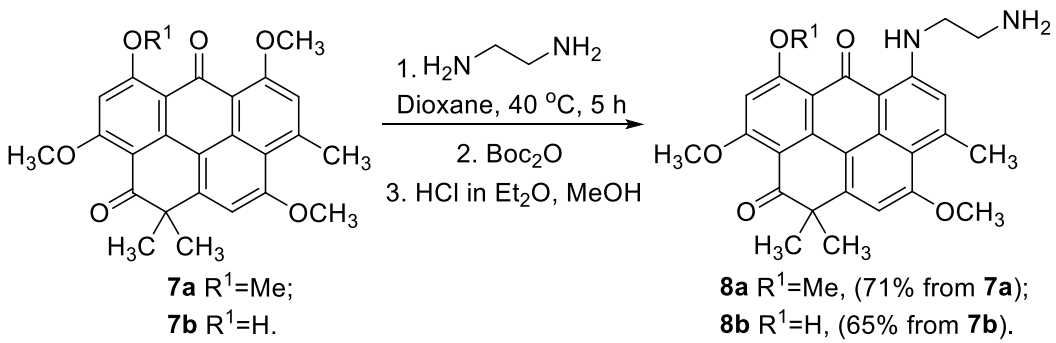
Scheme 1. Synthesis of tetra-*O*-methyl **7a** and tri-*O*-methyl derivatives **7b**, **7c** from heliomycin (**5**).

Previously we found that treatment of alkoxy derivatives of heteroarene-fused anthraquinones with HCl or HBr in acetic acid is an effective method for cleavage of corresponding alkoxy groups in *peri*-positions to carbonyls.[18] Thus, a partial cleavage of *O*-methyl ether groups by boiling **7a** in conc. hydrochloric acid led to a mixture of tri-*O*-methyl derivatives **7b-d** (Scheme 2). The new yellow isomer isolated from this reaction was found to be 5,7,10-tri-*O*-methylheliomycin **7d**. The assignment of ^{1}H and ^{13}C resonances of **7a-d** was done by simultaneous analysis of $^{1}H\{^{13}C\}$ HSQC, $^{1}H\{^{13}C\}$ HMBC, COSY, 1D-NOESY and 2D ROESY NMR experimental data (Supporting Information, Figure S1). These results confirm the structures of compounds **7b-d** are correctly assigned.



Scheme 2. Partial cleavage of methoxy groups of the tetra-*O*-methyl derivative **7a**.

Next, we studied the substitution of the methoxy groups of tetra-*O*-methylheliomycin (**7a**) with amines (Scheme 2). The treatment with diaminoethane at 40 °C led to replacement of the methoxy group to form a mixture of substitution products with a predominance of a mono(aminoethylamino)derivative. The crude mixture was acylated with Boc_2O that simplified isolation and purification of the product. After column chromatography, the main Boc-protected intermediate was treated with HCl to cleave the protecting group to give compound **8a** as the hydrochloride salt. Tri-*O*-methylheliomycin (**7b**) reacts with diaminoethane in the same manner as the tetra-*O*-methyl analogue (**7a**) and gave the 7-(2-aminoethyl)amino derivative **8b** in good yield (Scheme 3). The position of (2-aminoethyl)amine side chain of derivatives **8a**,**b** was proven by 2D NMR methods; characteristic H(CH_2)-H(7) cross-peaks were observed in ROESY spectra of the both compounds (Supporting Information, Table S1, S2, Figure S1).



7a R^1=Me;
7b R^1=H.

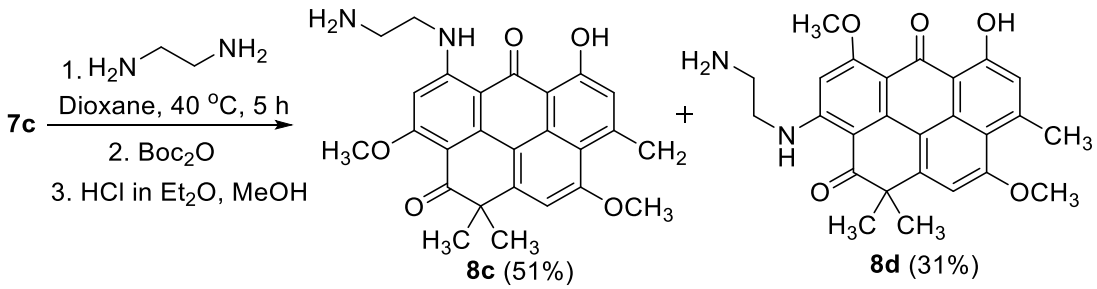
8a R^1=Me, (71% from **7a**);
8b R^1=H, (65% from **7b**).

Scheme 3. Synthesis of 7-aminoalkylamino derivatives of heliomycin **8a**,**b**.

DFT calculations (B3LYP/6-31+G(d)) provided the rationale for observed selectivity of substitution of 7-methoxy group of tetra-*O*-methylheliomycin (**7a)**. Calculated values of total energy (ΔE_{total}, Table S3) of Meisenheimer's complexes (MC) formed by attack of diaminoethane onto C-3, C-5, C-7 and C-10 (MC_3, MC_5, MC_7, MC_{10}, respectively, Figure S2) of **7a** specified that the more

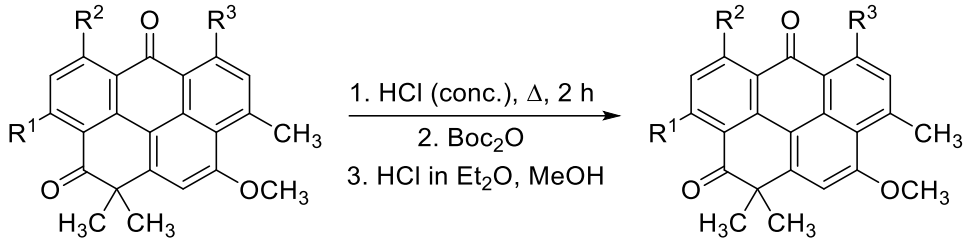
favorable site is the C-7 atom of the benzo[cd]pyrene-2,6-dione scaffold. In particular, MC_7 is more stable than MC_3 and MC_5 by 9.5 kcal/mol (Table S3). The length of the C-N bond between the side chain and aromatic ring in MC_7 is shorter (R_{C-N} = 1.493 Å, Table S3) than that of MC_3 (R_{C-N} = 1.553 Å) and MC_5 (R_{C-N} = 1.577 Å). The higher stability of MC_7 is additionally associated with the facilitated migration of the H-atom from the NH_2-group of the attacking end of the diaminoethane by coordination to the C-6 carbonyl oxygen (R_{N-H} = 1.731 Å, R_{O-H} = 1.000 Å, Figure S2). When simulating the substitution of the methoxy group at 10-position of **7a** the geometric parameters of the structure with the minimum on the potential energy surface does not correspond to the non-anionic four-coordinated Meisenheimer complex (where R_{CN} is 1.49-1.58 Å). Instead of MC complex, an anionic associate of tetra-O-methylheliomycin (**7a**) and diaminoethane and with a weak "long-range" interaction (R_{CN} = 4.521 Å) without the formation of a covalent or coordination bond was detected. Apparently, the formation of MC_{10} is hindered by the simultaneous shielding of the C-10 atom by the neighboring methyl and methoxy groups. Moreover, the partial charge correlates with the selectivity of substitution: the highest partial charge was calculated for C-7, while the lowest value observed at the 10-carbon (Table S3).

Similarly, the treatment of 3,5,10-tri-*O*-methylheliomycin (**7c**) with diaminoethane resulted in a mixture of isomeric 3- and 5-amino substituted derivatives **8c** and **8d**, respectively (Scheme 4). The isomers **8c** and **8d** were separated by column chromatography; the structure of individual derivatives is supported by 2D NMR data.



Scheme 4. Synthesis of 5- and 3-(2-aminoethyl)amino derivatives of heliomycin **8c** and **8d**.

Dealkylation of the methoxy groups of aminoalkylamino derivatives of heliomycin **8a-d** was investigated. When 3,5,10-tri-*O*-methyl-7-(2-aminoethyl)amino derivative **8a** was boiled for 2 h in the concentrated hydrochloric acid methoxy groups were cleaved exclusively at positions 3 and 5 and gave derivative **8e** in a high yield (Scheme 5). Again, for simplification of isolation and purification, the terminal amino group in **8e** was acylated with Boc_2O, and the obtained Boc-derivative purified by chromatography and then converted into the hydrochloride salt of pure **8e**. Similarly, demethylation of 3- and 5-(aminoalkylamino) isomers **8c**,**d** led to the derivatives **8f**,**g**.

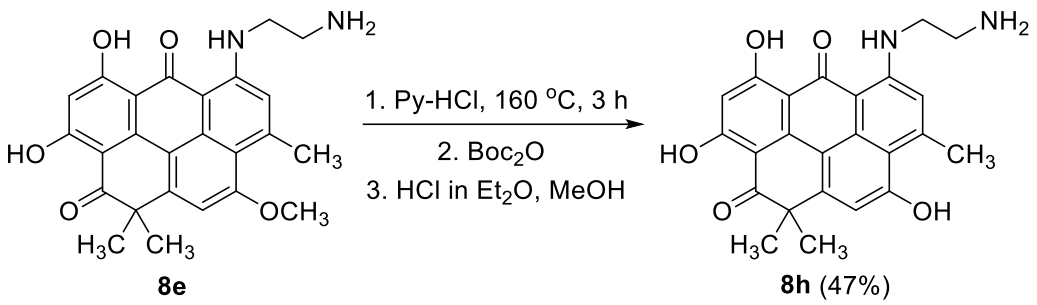


8a R^1=R^2=OMe, R^3=$NH(CH_2)_2NH_2$; **8e** R^1=R^2=OH, R^3=$NH(CH_2)_2NH_2$ (81%);
8c R^1=OMe, R^3=OH, R^2=$NH(CH_2)_2NH_2$; **8f** R^1=R^3=OH, R^2=$NH(CH_2)_2NH_2$ (86%);
8d R^2=OMe, R^3=OH, R^1=$NH(CH_2)_2NH_2$. **8g** R^2=R^3=OH, R^1=$NH(CH_2)_2NH_2$ (83%).

Scheme 5. Cleavage of the methoxy groups of derivatives **8a**,**c**,**d**.

The methoxy group at the 10-position of **8a-g** has marginal stability to cleavage, even by hydrobromic acid or hydrogen bromide solution (33%) in glacial acetic acid. However, the 10-methoxy derivative **8e** was demethylated to into 7-deoxy-7-(2-aminoethyl)amino heliomycin (**8h**) by heating in pyridine hydrochloride at 160 °C (Scheme 6).[19]

The developed synthetic procedures above led to a new class of heliomycin derivatives with improved solubility (Table S4), acceptable for further biological evaluation.



8e **8h** (47%)

Scheme 6. Cleavage of methoxy group at the position 10 of heliomycin derivative **8e**.

DNA can adopt different types of structures depending on the sequence and environmental conditions.[20] The best studied of these are G-quadruplexes and i-motifs. Guanine-rich sequences can form 4-stranded structures called G-quadruplexes, formed from Hoogsteen hydrogen bonding between guanines to form tetrads and further stabilized by stacking interactions and coordination with cations.[21] Cytosine-rich sequences can fold into i-motifs, four-stranded structures composed of two hairpins held together by intercalated, hemi-protonated C-C base pairs.[22] The nature of the formation of C-C base pairs means these structures are stabilized in acidic conditions and their structure is highly responsive to pH.[23] This partially limited widespread interest in studying these structures and targeting them with ligands, but now i-motifs, similar to G-quadruplexes, have been shown to exist in cells[14b] and affect a number of biological processes including modulation of gene expression.[15b] Although there are thousands of ligands described to target G-quadruplex,[24] there are far fewer described to target the i-motif.[17] Given the complementary nature of the sequences that may form G-quadruplexes and i-motifs, it is prudent to assess interaction with both structures[25] as well as double helical DNA.

Assessment of the relative binding to different DNA targets was performed using a fluorescent indicator displacement (FID) assay. This assay is based on displacement of a generic DNA-binding ligand, thiazole orange (TO) and were performed in a manner similar to that previously described.[26] We assessed the relative binding of ligands against i-motif forming

sequences from the promoter regions of HIF1A,[27] DAP,[13a] and c-MYC[28] (c-MYCC) as well as the tandem repeat sequences found in the insulin linked polymorphic region (ILPR)[29] and the human telomere (hTeloC).[30] This range of DNA sequences allows assessment of well-established i-motif targets that are stable at acidic pH (cMYC, ILPR and hTeloC) alongside i-motifs that are stable at neutral pH (HIF1A and DAP). To compare against other types of DNA structures we also examined the ligands against double stranded DNA as well as G-quadruplex sequences from the promoter regions of c-MYC (c-MYCG)[13a,28] and the human telomere (hTeloG).[31] We also examined a known G-quadruplex from bacteria (NASG).[32] These structures and sequences represent a range of lengths and topologies to enable understanding of ligand selectivity for particular types of DNA structure.

The results from the TO displacement assays indicate that introduction of aminoalkylamino side chains generally enhances the ability of the parent antibiotic to interact with all tested secondary structures of DNA (Table S5, S6). Heliomycin (**5**) was found to have moderate interaction with DNA with between 13% and 43% displacement of TO, depending on the DNA structure. The introduction of 4-aminomethyl side chains to this scaffold (derivatives **6a**,**b**) was shown to increase TO displacement across all types of DNA with displacement values between 45 and 85%. Introduction of this type of side chain not only improves water solubility but also allows additional interactions between the basic amino groups and the negatively charged sugar phosphate backbone in the DNA structures. It was found that 7-(2-aminoethyl)amino derivative **8e** showed a significant TO displacement from all the tested i-motif-forming DNA sequences, with displacements between 64% and 76%, whereas displacement from duplex and G-quadruplex DNA was substantially weaker. For example, TO displacement from double stranded was only 9.7% and the displacement from G-quadruplexes DNA was found to be between 15 and 44%. This data clearly shown that **8e** has a preference for i-motif DNA. We initially considered that some of our results may have arisen from different protonation states, by virtue of using an acidic pH for some of the i-motif forming

sequences. However, there was no correlation between the displacement and the pH at which the experiment was performed; in fact, the best displacement was observed for DAP at pH 7.0 (76%).

Replacement of (2-aminoethyl)amino group from position 7 to 3 or 5 (derivatives **8e**, **8f** and **8g**, respectively) attenuates relative i-motif binding capability, which is especially evident for the 3-(2-aminoethyl)amino derivative **8g** with TO displacement values of between 38 and 54%. Methylation of the 3 and 5-hydroxy groups in **8e** to give derivative **8a** resulted in no differences to relative i-motif binding capabilities, however **8a** was found to bind double stranded and G-quadruplex DNAs better than **8e**. Similarly, dealkylation of the 10-methoxy group of derivative **8e** to give **8h** demonstrated similar TO displacement from i-motifs but also leads to a dramatic decrease of selectivity over duplex- and G-quadruplex-forming DNA sequences.

To further investigate the DNA-interacting properties of **8e** we used Förster Resonance Energy Transfer (FRET) based DNA melting experiments to measure any ligand-induced changes in melting temperature of the different DNA structures.[33] The FRET-based melting experiments show that the compound **8e** has a relatively low stabilization of double stranded and G-quadruplex forming sequences (ΔT_m = 6, 5 and 4 °C for DS_{FRET}, $hTeloG_{FRET}$ and $NASG_{FRET}$ respectively, Figure S3, Table S7). No stabilization was observed for the i-motif forming sequences from DAP_{FRET} and $hTeloC_{FRET}$. The i-motif forming sequences from $HIF1A_{FRET}$, $cMYC_{FRET}$ and the $ILPR_{FRET}$ displayed stabilization of a second transition on the addition of **8e** which gave rise to significant stabilization temperatures (ΔT_m = 10 °C for $HIF1A_{FRET}$, ΔT_m = 25 °C for $cMYC_{FRET}$ and 17 °C for the $ILPR_{FRET}$). This indicates slightly different stabilization properties compared to the relative affinity measured by the FID assay. These techniques are measuring different DNA-ligand interaction properties, so this is not unexpected. The i-motif forming sequences from both cMYC and the ILPR are known to have multiple conformations so it is likely that the ligand preferentially stabilizes one of these. Less is known about HIF1A, but **8e** also stabilizes a second transition for this sequence under the conditions of the experiment. This observation is similar to that previously

observed for the ligand NCI-71795, where a second transition is observed for ligand-induced stabilization of the i-motif forming sequence from cMYC.[25c] The FRET melting experiments and FID experiments indicate that ligand **8e** shows both strong relative binding as well as high stabilization for the i-motifs from $cMYC_{FRET}$ and the $ILPR_{FRET}$ whereas the comparative interactions for G-quadruplex and double stranded are much less. Together this indicates that ligand **8e** has some preference for interacting with i-motif DNA.

Given the biophysical properties of the ligands, we were interested in the effects the ligands had on tumor cells. Antiproliferative potency against mammalian tumor cells, such as murine leukemia L1210, human T-cell leukemia CEM, cervical carcinoma HeLa and non-cancerous human dermal microvascular endothelial cells HMEC-1 was measured using MTT assays. 7-Deoxy-7-(2-aminoethyl)amino-10-*O*-methylheliomycin **8e** and its 10-dealkylated analogue **8h** were found to suppress proliferation of different cancer cell lines from low micromolar to sub-micromolar IC_{50} values, similar to the parent molecule heliomycin (**5**, Table 1). Dealkylation of the 10-O-methoxy group of **8e** lead to a slight decrease in activity (**8h**, Table 1). These results indicate that modification of the hydroxy groups in heliomycin to aminoalkylamino groups increases the overall solubility of this molecular scaffold, yet does not decrease their cytotoxicity.

Table 1. Antiproliferative potencies (MTT assay after 72 h exposure; IC_{50}, μM) of heliomycin derivatives **8e**, **h** and reference compounds heliomycin (**5**) and doxorubicin (**2**).

Compound	IC_{50}, μM			
	L1210	CEM	HeLa	HMEC-1
8e	0.8 ± 0.1	0.8 ± 0.0	0.8 ± 0.0	0.7 ± 0.1
8h	0.2 ± 0.0	2.1 ± 0.7	1.6 ± 0.4	1.2 ± 0.1
Heliomycin (**5**)	0.7 ± 0.0	0.5 ± 0.2	0.1 ± 0.0	0.5 ± 0.1
Dox (**2**)	0.4 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0

Conclusion

In summary, we have developed a methodology for the transformations of the 3-, 5- and 7-hydroxy groups in the antibiotic heliomycin into amino groups via methylation, subsequent nucleophilic substitution of methoxy groups with amines, and demethylation of the residual methoxy groups. The optimized procedures of the alkylation and dealkylation provided three isomeric tri-*O*-methyl ethers of heliomycin and facilitated the precise assignment of the correct structures, clarifying Brockman's previous assumption.[8e] The developed modification introduces possibilities for the transformation of hydroxy groups into amines and significantly diversify parental antibiotic to produce a series of new derivatives with improved solubility in aqueous media, preferable for biological characterization and preclinical studies. Unexpectedly, one derivative, 7-deoxy-7-(2-aminoalkyl)amino-10-*O*-methylheliomycin (**8e**) has promising affinity and good selectivity towards i-motif-forming DNA sequences over duplex and G-quadruplexes, indicating the potential of heliomycin derivatives for targeting i-motif DNA structures.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Varian Mercury 400 Plus instrument operated at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR) or Bruker AVANCE III 500 (Bruker Biospin, Rheinstetten Germany) NMR spectrometer equipped with a broadband Z-gradient probehead with direct observe BB coil (PABBO) at 500.18 MHz for ^{1}H and 125.77 MHz for ^{13}C respectively. 1D and 2D NMR spectra were processed using TopSpin 3.2 (Bruker) or ACD Labs Spectra Processor Academic Edition. The ^{1}H and ^{13}C signal assignment was done by using of ^{1}H{^{13}C} HSQC, ^{1}H{^{13}C} HMBC, COSY, 1D-NOE and 2D ROESY NMR experiments data. For 1D NOE and 2D ROESY experiments mixing times of 600 ms and 400 ms were used correspondingly. For selective excitation an 80 ms Gaussian shaped pulse was used. Chemical shifts were measured in DMSO-d_6 or $CDCl_3$ using tetramethylsilane as an internal standard. Analytical TLC was performed on Silica Gel F_{254} plates (Merck). Column chromatography was performed using SilicaGel Merck 60. Melting points were determined using a Buchi SMP-20 apparatus and are uncorrected. High resolution mass spectra were recorded with electron spray ionization on a Bruker Daltonics microOTOF-QII instrument. UV spectra were recorded on a Hitachi-U2000 spectrophotometer. The IR-spectra were obtained on a Nicolet-iS10 Fourier transform IR spectrometer (Thermo scientific, USA) with DTGS detector, splitter KBr and a Smart Performer module equipped with a ZnSe-crystal (ATR). Spectra were run at 3000-650 cm^{-1} with a resolution 4 cm^{-1} and proceeded using the OMNIC-7.0 program package. HPLC was performed using a Shimadzu Class-VP V6.12SP1 system, A: 0.01 M H_3PO_4 pH = 2.6; B: MeCN. All solutions were dried over Na_2SO_4 and evaporated at a reduced pressure using IKA RV 10 rotary evaporator at < 45 ºC. All products were vacuum dried at room temperature. All solvents, chemicals and reagents were obtained from Sigma-Aldrich, St. Louis, MO unless specified otherwise, and used without purification. The purity of final compounds **8a-h** was >95% as determined by HPLC analysis.

Preparation of compounds and Spectral Data.

*3,5,7,10-Tetramethoxy-1,1,9-trimethyl-1H-benzo[cd]pyrene-2,6-dione (**7a**).* To a mixture of heliomycin **5** (0.40 g, 1.1 mmol), anhydrous Cs_2CO_3 (2.86 g, 8.8 mmol) and tetra-*n*-butylammonium bromide (35 mg, 0.1 mmol) in dry *N,N*-dimethylacetamide (40 mL) iodomethane (0.55 mL, 8.8 mmol) was added. The suspension was stirred for 24 h at 50 °C in sealed flask, cooled and poured into cold water (200 mL). The yellow precipitate was filtered off, washed with water (3×30 mL) and dried. The yield of compound **7a** was 0.47 g (98%) as a yellow solid, mp >250 °C (decomp). UV $\lambda_{max.}$ (nm), DMSO (lgε): 264 (4.5), 282 (4.5), 309 (4.2), 412 (4.1), 426 (4.1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (1H, s, H-8); 6.89 (1H, s, H-11); 6.55 (1H, s, H-4); 4.10 (3H, s, OCH_3); 4.06 (3H, s, OCH_3); 4.02 (3H, s, OCH_3); 4.00 (3H, s, OCH_3); 2.87 (3H, s, CH_3); 1.60 (6H, s, $2CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.3; 182.0; 165.8; 164.5; 161.4; 160.6; 149.4; 144.8; 140.4; 132.0; 117.0; 115.9; 115.1; 114.0; 112.4; 108.4; 101.6; 94.2; 56.4; 56.1; 56.0; 55.2; 49.0; 27.0 (2C); 26.6. HRMS (ESI) calculated for $C_{26}H_{25}O_6$ $[M+H]^+$: 433.1646, found 433.1664.

*5-Hydroxy-3,7,10-trimethoxy-1,1,9-trimethyl-1H-benzo[cd]pyrene-2,6-dione (**7b**); 7-Hydroxy-3,5,10-trimethoxy-1,1,9-trimethyl-1H-benzo[cd]pyrene-2,6-dione (**7c**).* To a mixture of heliomycin **5** (0.40 g, 1.1 mmol), anhydrous Cs_2CO_3 (1.25 g, 3.9 mmol) and tetra-*n*-butylammonium bromide (35 mg, 0.1 mmol) in dry *N,N*-dimethylacetamide (40 mL) iodomethane (0.41 mL, 6.6 mmol) was added. The suspension was stirred for 8 h at room temperature in sealed flask and poured into cold water (200 mL). The yellow precipitate was filtered off, washed with water (3×30 mL) and dried. The residue was purified by a silica gel column chromatography using chloroform-methanol (19:1) as the eluent.

*5-Hydroxy-3,7,10-trimethoxy-1,1,9-trimethyl-1H-benzo[cd]pyrene-2,6-dione (**7b**)*. The yield of compound **7b** was 0.14 g (31%) as a red solid, mp >250 °C (decomp). TLC: chloroform-ethyl

acetate (9:1), R_f = 0.44. UV $\lambda_{max.}$ (nm), DMSO (lgε): 264 (4.4), 279 (4.4), 290 (4.3), 363 (4.0), 450 (4.1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.74 (1H, s, OH); 7.15 (1H, s, H-8); 7.01 (1H, s, H-11); 6.54 (1H, s, H-4); 4.22 (3H, s, OCH_3); 4.08 (3H, s, OCH_3); 4.03 (3H, s, OCH_3); 2.96 (3H, s, CH_3); 1.62 (6H, s, $2CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.2; 186.1; 170.6; 166.7; 163.8; 161.4; 152.6; 148.8; 140.1; 131.9; 117.2; 115.4; 111.8; 111.4; 108.5; 108.2; 102.9; 98.3; 56.6; 56.3; 55.5; 49.2; 27.5 (2C); 27.4. HRMS (ESI) calculated for $C_{25}H_{23}O_6$ $[M+H]^+$: 419.1489, found 419.1476.

*7-Hydroxy-3,5,10-trimethoxy-1,1,9-trimethyl-1H-benzo[cd]pyrene-2,6-dione (**7c**)*. The yield of compound **7c** was 0.21 g (47%) as a yellow solid, mp >250 °C (decomp). TLC: chloroform-ethyl acetate (9:1), R_f = 0.33. UV $\lambda_{max.}$ (nm), DMSO (lgε): 263 (4.4), 274 (4.4), 314 (4.2), 364 (4.0), 424 (4.1), 444 (4.3). ^{1}H NMR (400 MHz, $CDCl_3$) δ 16.92 (1H, s, OH); 6.94 (1H, s, H-8); 6.91 (1H, s, H-11); 6.61 (1H, s, H-4); 4.17 (3H, s, OCH_3); 4.10 (3H, s, OCH_3); 4.05 (3H, s, OCH_3); 2.81 (3H, s, CH_3); 1.56 (6H, s, $2CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.0; 184.3; 169.6; 166.7; 165.5; 161.3; 150.8; 148.5; 141.4; 130.0; 121.5; 115.0; 110.9; 110.1; 109.1; 108.5; 102.4; 93.9; 56.3; 56.2; 55.4; 49.1; 26.7 (2C); 26.5. HRMS (ESI) calculated for $C_{25}H_{22}O_6$ $[M+H]^+$: 419.1489, found 419.1470.

*5-Hydroxy-3,7,10-trimethoxy-1,1,9-trimethyl-1H-benzo[cd]pyrene-2,6-dione (**7b**)*; *7-Hydroxy-3,5,10-trimethoxy-1,1,9-trimethyl-1H-benzo[cd]pyrene-2,6-dione (**7c**); 3-Hydroxy-3,7,10-trimethoxy-1,1,9-trimethyl-1H-benzo[cd]pyrene-2,6-dione (**7d**).* A mixture of compound **7a** (0.20 g, 0.5 mmol) and conc. hydrochloric acid (30 mL) was refluxed with stirring for 20 min, cooled to room temperature and evaporated *in vacuo*. The crude solid was diluted with chloroform (60 mL), washed with water (3×30 mL), dried and the solvent was evaporated. The resulted mixture of the products was separated by a silica gel column chromatography using chloroform-methanol (19:1) as the eluent.

*5-Hydroxy-3,7,10-trimethoxy-1,1,9-trimethyl-1H-benzo[cd]pyrene-2,6-dione (**7b**)*. The yield of compound **7b** was 22 mg (10%) as a red solid, mp >250 °C (decomp). TLC: chloroform-ethyl acetate (9:1), R_f = 0.44.

*7-Hydroxy-3,5,10-trimethoxy-1,1,9-trimethyl-1H-benzo[cd]pyrene-2,6-dione (**7c**).* The yield of compound **7c** was 24 mg (11%) as a yellow solid, mp >250 °C (decomp). TLC: chloroform-ethyl acetate (9:1), R_f = 0.33.

*3-Hydroxy-3,7,10-trimethoxy-1,1,9-trimethyl-1H-benzo[cd]pyrene-2,6-dione (**7d**).* The yield of compound **7d** was 29 mg (13%) as a yellow solid, mp >250 °C (decomp). TLC: chloroform-ethyl acetate (9:1), R_f = 0.21. UV $\lambda_{max.}$ (nm), DMSO (lgε): 268 (4.5), 283 (4.5), 313 (4.2), 412 (4.1), 427 (4.1). ^{1}H NMR (100 MHz, $CDCl_3$) δ 14.39 (1H, s, OH); 7.18 (1H, s, H-8); 6.94 (1H, s, H-11); 6.52 (1H, s, H-4); 4.13 (3H, s, OCH_3); 4.07 (3H, s, OCH_3); 4.06 (3H, s, OCH_3); 2.96 (3H, s, CH_3); 1.69 (6H, s, $2CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.7; 181.6; 169.2; 168.4; 161.9; 161.1; 149.1; 145.0; 139.8; 131.9; 117.2; 116.3; 115.4; 113.9; 111.5; 103.6; 102.3; 98.4; 56.5 (2C); 55.4; 46.5; 28.9 (2C); 26.8. HRMS (ESI) calculated for $C_{25}H_{23}O_6$ $[M+H]^+$: 419.1489, found 419.1481.

*7-(2-Aminoethyl)amino-3,5,10-trimethoxy-1,1,9-trimethyl-1H-benzo[cd]pyrene-2,6-dione hydrochloride (**8a**)*. To a solution of tetra-*O*-methylheliomycin **7a** (0.20 g, 0.46 mmol) in dioxane (15 mL) was added diaminoethane (0.2 mL, 3.0 mmol) and stirred for 5 h at 40 °C. The reaction mixture was cooled to room temperature and diluted with an aqueous solution of HCl (0.1 N, 20 mL). The product was extracted with hot *n*-butanol (3×20 mL), washed with water (2×10 mL) and concentrated under a reduced pressure. The residue was dissolved in a mixture of water (10 mL), isopropanol (15 mL) and THF (15 mL) then NEt_3 (0.2 mL) and Boc_2O (0.30 g, 1.4 mmol) were added. The reaction mixture was stirred 1 h at 40 °C then the N-Boc-protected intermediates were extracted with butanol (3×10 mL), washed with an aqueous solution of HCl (1 N, 10 mL) and water

(2×20 mL) and concentrated under a reduced pressure. The crude product was purified by a silica gel column chromatography using chloroform-methanol (5:1) as the eluent. The residue was dissolved in hot chloroform (10 mL) and a solution of HCl in methanol (5 wt. %, 1 mL) was added and stirred 24 h at room temperature. The solvent was evaporated in vacuum and the solid was dissolved in hot water (1 mL) and re-precipitated with acetone. The precipitate was filtered off, washed with acetone, diethyl ether and dried. The yield of compound **8a** was 0.16 g (71%) as a yellow solid, mp >250 °C (decomp). HPLC (LW=260 nm, gradient B 20→70% (30 min)), t_R=17.5 min, purity 95%. UV $\lambda_{max.}$ (nm), DMSO (lgε): 265 (4.4), 301 (4.3), 381 (3.9), 431 (4.0), 454 (4.1). ^{1}H NMR (400 MHz, DMSO-d_6), δ 11.88 (1H, br s, NH); 8.14 (3H, br s, NH_3); 7.01 (1H, s, H-8); 6.91 (1H, s, H-11); 6.66 (1H, s, H-4); 4.02 (3H, s, OCH_3); 4.00 (3H, s, OCH_3); 3.97 (3H, s, OCH_3); 3.84-3.81 (2H, m, CH_2); 3.23-3.19 (2H, m, CH_2); 2.78 (3H, s, CH_3); 1.41 (6H, s, $2CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 199.0; 181.0; 165.5; 163.4; 160.1; 153.6; 148.9; 145.0; 139.1; 130.9; 114.8; 112.7; 111.3; 109.9; 107.9; 106.0; 101.9; 94.4; 56.1; 55.9; 55.4; 48.6; 39.9; 38.4; 26.4; 26.2 (2C). HRMS m/z (ESI): calculated for $C_{27}H_{29}N_2O_5$ $[M+H]^+$: 461.2071, found: 461.2095.

*7-(2-Aminoethyl)amino-5-hydroxy-3,10-dimethoxy-1,1,9-trimethyl-1H-benzo[cd]pyrene-2,6-dione hydrochloride (**8b**)*. This compound was prepared from derivative **7b** as described for **8a**. The yield of compound **8b** was 0.15 g (65%) as a yellow solid, mp >250 °C (decomp). HPLC (LW=345 nm, gradient B 20→90% (30 min)), t_R=16.9 min, purity 95%. UV $\lambda_{max.}$ (nm), DMSO (lgε): 262 (4.5), 300 (4.2), 398 (4.1), 440 (4.0), 465 (4.2). ^{1}H NMR (400 MHz, DMSO-d_6), δ 11.12 (1H, br s, NH); 7.21 (1H, s, H-8); 7.19 (1H, s, H-11); 6.63 (1H, s, H-4); 4.07 (3H, s, OCH_3); 3.92 (3H, s, OCH_3); 3.87-3.84 (2H, m, CH_2); 3.13-3.10 (2H, m, CH_2); 2.86 (3H, s, CH_3); 1.50 (6H, s, $2CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 197.7; 183.2; 168.7; 165.5; 160.9; 154.7; 141.4; 140.0;

138.3; 130.4; 114.9; 113.1; 109.7; 107.0; 106.5; 103.6; 103.4; 97.6; 56.1; 55.9; 48.8; 39.8; 38.0; 26.9 (2C); 26.7. HRMS m/z (ESI): calculated for $C_{26}H_{27}N_2O_5$ $[M+H]^+$: 447.1914, found: 447.1903.

*5-(2-Aminoethyl)amino-7-hydroxy-3,10-dimethoxy-1,1,9-trimethyl-1H-benzo[cd]pyrene-2,6-dione hydrochloride (**8c**); 3-(2-aminoethyl)amino-7-hydroxy-5,10-dimethoxy-1,1,9-trimethyl-1H-benzo[cd]pyrene-2,6-dione hydrochloride (**8d**).* To a solution of derivative **7c** (0.19 g, 0.46 mmol) in dioxane (15 mL) was added diaminoethane (0.6 mL, 9 mmol) and stirred for 5 h at 40 °C. Reaction mixture was cooled to room temperature and diluted with an aqueous solution of HCl (0.1 N, 50 mL). The product was extracted with hot *n*-butanol (3×20 mL), washed with water (2×10 mL) and concentrated under a reduced pressure. The residue was dissolved in a mixture of water (10 mL), isopropanol (15 mL) and THF (15 mL) then NEt_3 (0.2 mL) and Boc_2O (0.30 g, 1.4 mmol) were added. The reaction mixture was stirred 1 h at 40 °C then the N-Boc-protected intermediates were extracted with butanol (3×10 mL), washed with an aqueous solution of HCl (1 N, 10 mL) and water (2×20 mL) and concentrated under a reduced pressure. The mixture of N-Boc-protected products **8c** and **8d** was separated by a silica gel column chromatography using chloroform-methanol (5:1) as the eluent. Individual products were dissolved in hot chloroform (10 mL) and a solution of HCl in methanol (5 wt. %, 1 mL) was added and stirred 24 h at room temperature. The solvent was evaporated and the solid was dissolved in hot water (1mL) and re-precipitated with acetone. The precipitate was filtered off, washed with acetone, diethyl ether and dried.

*5-(2-Aminoethyl)amino-7-hydroxy-3,10-dimethoxy-1,1,9-trimethyl-1H-benzo[cd]pyrene-2,6-dione hydrochloride (**8c**).* The yield of compound **8c** was 0.11 g (51%) as a yellow solid, mp >250 °C (decomp). HPLC (LW=345 nm, gradient B 20→90% (30 min)), t_R=16.9 min, purity 95%. UV $\lambda_{max.}$ (nm), DMSO (lgε): 262 (4.4), 287 (4.4), 346 (4.3), 441 (4.3). ^{1}H NMR (500 MHz, DMSO-d_6), δ 15.85 (1H, s, OH); 10.45 (1H, t, J = 6.2 Hz, NH); 8.30 (3H, br s, NH_3); 7.10 (1H, s, H-8); 6.91

(1H, s, H-11); 6.37 (1H, s, H-4); 4.06 (3H, s, OCH_3); 4.01 (3H, s, OCH_3); 3.83 (2H, q, J = 6.4 Hz, CH_2); 3.17-3.14 (2H, m, CH_2); 2.79 (3H, s, CH_3); 1.46 (6H, s, $2CH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ 197.4; 184.9; 166.8; 165.8; 160.9; 156.2; 151.9; 147.9; 141.3; 128.8; 120.4; 114.7; 110.6; 107.2; 105.3; 104.3; 104.0; 91.1; 56.3; 55.9; 48.2; 39.2; 37.2; 26.6 ($2CH_3$); 26.0 (C). HRMS m/z (ESI): calculated for $C_{26}H_{27}N_2O_5$ $[M+H]^+$: 447.1914, found: 447.1896.

*3-(2-Aminoethyl)amino-7-hydroxy-5,10-dimethoxy-1,1,9-trimethyl-1H-benzo[cd]pyrene-2,6-dione hydrochloride (**8d**).* The yield of compound **8d** was 69 mg (31%) as an orange solid, mp >250 °C (decomp). HPLC (LW=290 nm, gradient B 20→70% (30 min)), t_R=20.6 min, purity 96%. UV $\lambda_{max.}$ (nm), DMSO (lgε): 268 (4.3), 292 (4.4), 348 (4.1), 477 (4.1). ^{1}H NMR (500 MHz, DMSO-d_6), δ 17.66 (1H, s, OH); 10.93 (1H, t, J = 6.2 Hz, NH); 8.24 (3H, br s, NH_3); 7.24 (1H, s, H-8); 6.97 (1H, s, H-11); 6.47 (1H, s, H-4); 4.09 (3H, s, OCH_3); 4.06 (3H, s, OCH_3); 3.83 (2H, q, J = 6.5 Hz, CH_2); 3.13 (2H, q, J = 5.7 Hz, CH_2); 2.81 (3H, s, CH_3); 1.59 (6H, s, $2CH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ 201.3; 182.2; 169.4; 166.5; 161.2; 156.8; 151.5; 147.6; 142.4; 129.0; 121.3; 114.2; 109.8; 107.8; 106.6; 104.5; 101.5; 92.2; 56.6; 56.1; 46.8; 38.7; 37.5; 29.1 (2C); 25.9. HRMS m/z (ESI): calculated for $C_{26}H_{27}N_2O_5$ $[M+H]^+$: 447.1914, found: 447.1913.

*7-(2-Aminoethyl)amino-3,5-dihydroxy-10-methoxy-1,1,9-trimethyl-1H-benzo[cd]pyrene-2,6-dione hydrochloride (**8e**).* Compound **8a** (90 mg, 0.2 mmol) was boiled in conc. hydrochloric acid (3 mL) for 2 h, cooled to room temperature and evaporated. The crude residue was dissolved in a mixture of water (5 mL), isopropanol (5 mL) and THF (5 mL) then triethylamine (0.3 mL) and Boc_2O (0.2 g, 1.9 mmol) were added and reaction mixture was stirred for 1 h at 40 °C. N-Boc-protected derivative of **8e** was extracted with hot *n*-butanol (3×20 mL), the extract was washed with an aqueous solution of HCl (1 N, 10 mL) and water (2×20 mL) and concentrated under a reduced pressure. The crude was purified by a silica gel column chromatography using chloroform-methanol

(5:1) as the eluent. The residue was dissolved in a mixture of hot chloroform-methanol (15 mL, 1:1) and a solution of HCl in methanol (5 wt. %, 1 mL) was added and stirred 24 h at room temperature. The solvent was evaporated and the solid was dissolved in hot water (2 mL) and re-precipitated with acetone. The precipitate was filtered off, washed with acetone, diethyl ether and dried. The yield of compound **8e** was 76 mg (81%) as an orange solid, mp >250 °C (decomp). HPLC (LW=272 nm, gradient B 30→60% (30 min)), t_R=14.1 min, purity 98%. UV $\lambda_{max.}$ (nm), DMSO (lgε): 264 (4.5), 296 (4.2), 331 (4.2), 399 (4.1), 440 (4.0), 464 (4.3). ^{1}H NMR (400 MHz, DMSO-d_6), δ 16.05 (2H, s, OH); 11.00 (1H, t, J = 6.2 Hz, NH); 8.41 (3H, br s, NH_3); 7.23 (1H, s, H-8); 7.19 (1H, s, H-11); 6.23 (1H, s, H-4); 4.09 (3H, s, OCH_3); 3.88-3.84 (2H, m, CH_2); 3.10-3.07 (2H, m, CH_2); 2.84 (3H, s, CH_3); 1.58 (6H, s, $2CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 204.9; 182.7; 171.0; 169.2; 161.7; 155.0; 151.3; 148.3; 138.0; 130.1; 115.3; 113.1; 108.6; 107.2; 104.7; 104.0; 102.1; 100.3; 56.2; 46.6; 39.6; 37.76; 28.7 (2C); 26.6. HRMS m/z (ESI): calculated for $C_{25}H_{25}N_2O_5$ $[M+H]^+$: 433.1756, found: 433.1779.

*5-(2-Aminoethyl)amino-3,7-dihydroxy-10-methoxy-1,1,9-trimethyl-1H-benzo[cd]pyrene-2,6-dione hydrochloride (**8f**).* This compound was prepared from compound **8c** as described for **8e**. The yield of compound **8f** was 80 mg (86%) as an orange solid, mp >250 °C (decomp). HPLC (LW=254 nm, gradient B 20→90% (30 min)), t_R=23.0 min, purity 99%. UV $\lambda_{max.}$ (nm), DMSO (lgε): 262 (4.2), 288 (4.3), 345 (4.2), 457 (4.2). ^{1}H NMR (400 MHz, DMSO-d_6), δ 10.41 (1H, t, J = 6.2 Hz, NH); 7.89 (1H, s, OH); 7.22 (1H, s, H-8); 6.89 (1H, s, H-11); 6.22 (1H, s, H-4); 4.09 (3H, s, OCH_3); 3.68-3.63 (2H, m, CH_2); 3.10-3.05 (2H, m, CH_2); 2.77 (3H, s, CH_3); 1.58 (6H, s, $2CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 203.8; 184.6; 168.9; 167.4; 161.9; 157.7; 152.3; 148.6; 141.5; 128.4; 120.7; 114.6; 109.4; 107.5; 105.2; 105.1; 100.9; 93.7; 56.3; 46.0; 39.5; 37.2; 28.7 (2C); 26.0. HRMS m/z (ESI): calculated for $C_{25}H_{25}N_2O_5$ $[M+H]^+$: 433.1756, found: 433.1736.

*3-(2-Aminoethyl)amino-5,7-dihydroxy-10-methoxy-1,1,9-trimethyl-1H-benzo[cd]pyrene-2,6-dione hydrochloride (**8g**).* This compound was prepared from compound **8d** as described for **8e**. The yield of compound **8g** was 78 mg (83%) as an orange solid, mp >250 °C (decomp). HPLC (LW=268 nm, gradient B 20→90% (30 min)), t_R=21.3 min, purity 99%. UV $\lambda_{max.}$ (nm), DMSO (lgε): 270 (4.4), 292 (4.4), 314 (4.3), 351 (4.4), 467 (4.2). ^{1}H NMR (400 MHz, DMSO-d_6), δ 13.75 (2H, s, 2OH); 10.63 (1H, t, J = 5.9 Hz, NH); 8.47 (3H, br s, NH_3); 7.09 (1H, s, H-8); 6.64 (1H, s, H-11); 6.26 (1H, s, H-4); 4.03 (3H, s, OCH_3); 3.74-3.69 (2H, m, CH_2); 3.10-3.07 (2H, m, CH_2); 2.64 (3H, s, CH_3); 1.47 (6H, s, $2CH_3$).^{13}C NMR (100 MHz, DMSO-d_6) δ 200.9; 184.1; 167.3; 163.3; 161.6; 157.4; 152.9; 149.2; 141.3; 128.3; 119.9; 114.9; 109.5; 106.0; 104.8; 103.6; 101.5; 94.5; 56.1; 47.2; 39.5; 37.3; 28.9 (2C); 26.1. HRMS m/z (ESI): calculated for $C_{25}H_{25}N_2O_5$ $[M+H]^+$: 433.1756, found: 433.1739.

*7-(2-Aminoethyl)amino-3,5,10-trihydroxy-1,1,9-trimethyl-1H-benzo[cd]pyrene-2,6-dione methanesulfonate (**8h**).* A mixture of compound **8e** (0.10 g, 0.2 mmol) and pyridinium chloride (10.0 g, 0.08 mol) was heated in inert atmosphere for 3 h at 160 ºC then cooled to room temperature. The residue was diluted with water (20 mL), the product was extracted with hot *n*-butanol (3×20 mL), the extract was washed with water (2×20 mL) and concentrated under a reduced pressure. The crude product was dissolved in a mixture of water (5 mL), isopropanol (5 mL) and THF (5 mL) then triethylamine (0.3 mL) and Boc_2O (0.2 g, 1.9 mmol) were added and reaction mixture was stirred for 1 h at 40 °C. N-Boc-protected derivative of **8h** was extracted with hot *n*-butanol (3×20 mL), the extract was washed with an aqueous solution of HCl (1 N, 10 mL) and water (2×20 mL) and concentrated under a reduced pressure. The crude was purified by a silica gel column chromatography using chloroform-methanol (9:1) as the eluent. The residue was dissolved in a

mixture of hot chloroform (15 mL,) and methanesulfonic acid (0.1 mL, 1.5 mmol) was added and stirred 24 h at room temperature. The solvent was evaporated and the solid was dissolved in hot water (2 mL) and re-precipitated with acetone. The precipitate was filtered off, washed with acetone, diethyl ether and dried. The yield of compound **8h** was 43 mg (47%) as an orange solid, mp >250 °C (decomp). HPLC (LW=272 nm, gradient B 20→60% (30 min)), t_R=21.5 min, purity 97%. UV $\lambda_{max.}$ (nm), DMSO (lgε): 264 (4.4), 299 (4.2), 320 (4.0), 398 (4.0), 438 (4.0), 463 (4.2). ^{1}H NMR (400 MHz, DMSO-d_6), δ 16.23 (1H, s, OH); 14.42 (1H, s, OH); 14.29 (1H, s, OH); 11.12 (1H, t, J = 6.0 Hz, NH); 8.65 (3H, br s, NH_3); 7.18 (1H, s, H-8); 7.05 (1H, s, H-11); 6.20 (1H, s, H-4); 3.85-3.81 (2H, m, CH_2); 3.15-3.12 (2H, m, CH_2); 2.88 (3H, s, CH_3); 2.38 (3H, s, SCH_3); 1.53 (6H, s, $2CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 204.6; 182.3; 171.3; 169.4; 161.6; 155.5; 151.2; 149.3; 138.1; 130.6; 114.2; 112.2; 109.3; 107.3; 107.1; 104.3; 101.9; 99.8; 46.0; 40.1; 38.0; 29.1 (2C); 26.4. HRMS m/z (ESI): calculated for $C_{24}H_{23}N_2O_5$ $[M+H]^+$: 419.1601, found: 419.1618.

Quantum chemical calculations. Calculations were carried out with complete optimization of geometric parameters using the hybrid density functional method B3LYP/6-31G (d)on the Spartan-10 software package.[32] Optimization was carried out according to the standard Pulay DIIS (direct inversion of the iterative subspace) procedure.[33] An analysis of the vibrational frequencies of the structures under study showed that they belong to the region of minima of the potential energy surface (there are no imaginary frequencies). The visualization of the calculation results was carried out using the Spartan-10[34] functions or using the Chemcraft package.[35]

Fluorescent Intercalator Displacement (FID) Assay. Oligonucleotides were purchased from Eurogentec and dissolved at approximately 1 mM in ultrapure water and quantified using a Nanodrop. DNA samples for experiments were prepared at the desired concentration in 10 mM

sodium cacodylate buffer at pH 7.0 (DS, hTeloG, NASG, DAP, c-mycG), pH 7.2 (HIF1A), pH 6.6 (c-mycC, ILPR) or pH 5.5 (hTeloC). G-quadruplex sample (hTeloG, NASG, c-mycG) buffers also contained 100 mM KCl. Samples were thermally annealed in an Applied Biosystems Veriti 96 well thermal cycler by holding at 95 °C for 5 min and cooling at a rate of 1 °C/min to 20 °C. 10 mM stock solutions of the candidate ligands were prepared in DMSO. Further dilutions were carried out in buffer per the experiment's requirements.

DNA Sequences

Name	**Sequence (5′ → 3′)**
HIF1A	CGC-GCT-CCC-GCC-CCC-TCT-CCC-CTC-CCC-GCG-C
ATXN2L	CCC-CCC-CCC-CCC-CCC-CCC-CCC-CCC
DAP	CCC-CCG-CCC-CCG-CCC-CCG-CCC-CCG-CCC-CC
c-mycC	CCT-TCC-CCA-CCC-TCC-CCA-CCC-TCC-CCA
ILPR	TGT-CCC-CAC-ACC-CCT-GTC-CCC-ACA-CCC-CTG-T
hTeloC	TAA-CCC-TAA-CCC-TAA-CCC-TAA-CCC
DS	GGC-ATA-GTG-CGT-GGG-CGT-TAG-C
DScomp	GCT-AAC-GCC-CAC-GCA-CTA-TGC-C
hTeloG	GGG-TTA-GGG-TTA-GGG-TTA-GGG
c-mycG	TGG-GGA-GGG-TGG-GGA-GGG-TGG-GGA-AGG-TGG-GGA
NASG	GGG-AGC-GGG-ACG-GGG-GCC-GGG

FID experiments were performed on a BMG CLARIOstar plate reader using 96-well solid black flat bottom plates. A 10 mM stock solution of thiazole orange (TO) was prepared in DMSO and diluted to 2 μM in buffer. Ninety microliters of the 2 μM TO solution were added to each well and fluorescence emission at 450 nm measured with excitation at 430 nm; this was normalized to 0%

representing background fluorescence. One microliter of the respective DNA (90 μM) was then added to give 1 μM DNA concentration, shaken using double orbital shaking at 700 rpm in the plate reader for 15 s, and allowed to equilibrate for 15 min. After equilibration, fluorescence emission was measured as before, and normalized to 100% representing maximal fluorescence enhancement from the TO probe binding to the DNA secondary structure. Aliquots of ligand (0.9 μL of 200 μM) were then titrated into each well (in triplicate) and measured as before. Fluorescence measurements after ligand addition were normalized between the 0 and 100% levels determined per the respective well. Percentage TO displacement was calculated as the difference between the normalized 100% fluorescence level and the normalized fluorescence measured after each ligand addition.

FRET Melting assay. Assessment of the ligand-induced change in melting temperature was performed using a fluorescence resonance energy transfer (FRET) DNA melting based assay.[25c] Labelled oligonucleotides were purchased from Eurogentec and were HPLC purified. The oligonucleotides were labelled with a donor fluorophore FAM (6-carboxyfluorescein) and acceptor fluorophore TAMRA (6-carboxytetramethyl-rhodamine). Solid DNA samples were initially dissolved as a stock solution in purified water (100 μM); further dilutions were carried out in the respective sodium cacodylate buffer: 0.2 μM DNA in 10 mM sodium cacodylate at pH 7.0 (DS_{FRET}, $hTeloG_{FRET}$, $NASG_{FRET}$, DAP_{FRET}), pH 7.2 ($HIF1A_{FRET}$), 6.6 ($cMYC_{FRET}$, $ILPR_{FRET}$) and pH 5.5 ($hTeloC_{FRET}$). G-quadruplex sample ($hTeloG_{FRET}$, $NASG_{FRET}$) buffers also contained 100 mM KCl. Samples were thermally annealed in a heat block at 95 °C for 5 minutes and cooled slowly to room temperature overnight. Strip-tubes (QIAgen) were prepared with DNA solution and added ligand at 1 μM (5 equivalents). Control samples for each run were prepared with the same quantity of DMSO with the DNA in buffer. Fluorescence melting curves were determined in a QIAgen Rotor-Gene Q-series PCR machine, using a total volume of 20 μL. Samples were held at 25 °C for 5 minutes then ramped to 95 °C at increments of 1 °C, holding the temperature at each step for 1 minute.

Measurements were made with excitation at 470 nm and detection at 510 nm. DNA melting points were determined using the first derivative of the melting curve. Final analysis of the data was carried out using QIAgen Rotor-Gene Q-series software and Origin or Excel.

FRET DNA Sequences

Name	**Sequence (5′ → 3′)**
$HIF1A_{FRET}$	FAM-[CGC-GCT-CCC-GCC-CCC-TCT-CCC-CTC-CCC-GCG-C]-TAMRA
DAP_{FRET}	FAM-[CCC-CCG-CCC-CCG-CCC-CCG-CCC-CCG-CCC-CC]-TAMRA
$c\text{-}mycC_{FRET}$	FAM-[TCC-CCA-CCT-TCC-CCA-CCC-TCC-CCA-CCC-TCC-CCA]-TAMRA
$ILPR_{FRET}$	FAM-[TGT-CCC-CAC-ACC-CCT-GTC-CCC-ACA-CCC-CTG-T]-TAMRA
$hTeloC_{FRET}$	FAM-[TAA-CCC-TAA-CCC-TAA-CCC-TAA-CCC]-TAMRA
DS_{FRET}	FAM-[TAT-AGC-TAT-A-HEG(18)-TAT-AGC-TAT-A]-TAMRA
$hTeloG_{FRET}$	FAM-[GGG-TTA-GGG-TTA-GGG-TTA-GGG]-TAMRA
$NASG_{FRET}$	FAM-[GGG-AGC-GGG-ACG-GGG-GCC-GGG]-TAMRA

Cell lines, drug treatment and cytotoxicity assays. The reagents were from Sigma-Aldrich, St. Louis, MO. The murine leukemia L1210, human cervical cancer HeLa, human lymphocyte CEM and non-cancerous human dermal microvascular endothelial HMEC-1 cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA). Cells were supplemented with 5% fetal calf serum (HyClone, Logan, UT), 2 mM *L*-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C under 5% CO_2 in a humidified atmosphere. The L1210, HeLa, CEM

and HMEC-1 cell lines were propagated in RPMI-1640 supplemented with 5-10% fetal calf serum, 0.075% $NaHCO_3$ and 2 mM *L*-glutamine. Cells in a logarithmic growth phase were used in all experiments. Tested compounds were dissolved in DMSO as 10 mM stock solutions followed by serial dilutions in water immediately before experiments. The cytotoxicity was determined in a formazan conversion assay (MTT-test). Briefly, cells (5×10^3 in 190 mL of culture medium) were plated into a 96-well plate and treated with 0.1% DMSO (vehicle control) or with increasing concentrations of compounds (0.1-50 μM; each concentration in duplicate) for 48 h (L1210 cells) or 72 h (other cell lines). After the completion of drug exposure, 0.5 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added into each well for an additional 2 h. Formazan was dissolved in DMSO, and the absorbance at 540 nm was measured. The cytotoxicity at a given drug concentration was calculated as the percentage of absorbance in wells with drug-treated cells to that of vehicle control cells (100%). The IC_{50} (50% growth inhibitory concentration) was defined as the concentration of the compound that inhibited MTT conversion by 50%.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at …

NMR data, copies of spectra, details of DFT calculation, parameters of solubility, FID data and FRET melting curves.

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Notes

The authors declare no conflict of interest.

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REFERENCES

(1) (a) Katz, L.; Donadio, S. *Annu. Rev. Microbiol*., **1993**, *47*, 875–912, https://doi.org/10.1146/annurev.mi.47.100193.004303, (b) Crawford, J.; Townsend, C. *Nat. Rev. Microbiol.* **2010**, *8*, 879–889, https://doi.org/10.1038/nrmicro2465.

(2) (a) Anderson, J. A.; Lin, B. K.; Williams, H. J.; Scott, A. Ian. *J. Am. Chem. Soc.* **1988**, *110*, 1623–1624, DOI: 10.1021/ja00213a046; (b) Hertweck, C. *Angew. Chem. Int. Ed.* **2009**, *48*, 4688–4716, https://doi.org/10.1002/anie.200806121; (c) Niraula, N. P.; Kim, S.; Sohng, J. K.; Kim, E. S. *Appl. Microbiol. Biotechnol.* **2010**, *87*, 1187–1194, https://doi.org/10.1007/s00253-010-2675-3; (d) Ueberschaar, N.; Xu, Z.; Scherlach, K.; Metsä-Ketelä, M.; Bretschneider, T.; Dahse, H.; Görls, H.; Hertweck, C. *J. Am. Chem. Soc.* **2013**, *135*, 17408–17416, https://doi.org/10.1021/ja4080024; (e) Huang, L. F.; Wang, Z. H.; Chen, S. L. *Chin. J. Nat. Med.* **2014**, *12*, 81–88, https://doi.org/10.1016/S1875-5364(14)60014-5.

(3) (a) Brockmann, H.; Schmidt-Kastner, G. *Naturwissenschaften* **1951**, *38*, 479–480; b) Brazhnikova, M. G.; Uspenskaia, T. A.; Sokolova, I. B.; Preobrazhenskaia, T. P.; Gauze, G. F.; Ukholina, R. S.; Shorin, V. A.; Rossolimo, O. K.; Vertogradova, T. P. *Antibiotiki (Mosc.)*, **1958**, *3*, 29–34 (Russian).

(4) (a) Vijayabharathi, R.; Bruheim, P.; Andreassen, T.; Raja, D. S.; Devi, P. B.; Sathyabama, S.; Priyadarisini, V.B. *J. Microbiol.* **2011**, *49*, 920–926, https://doi.org/10.1007/s12275-011-1260-5; (b) Zhang, Y.-L.; Li, S.; Jiang, D.-H.; Kong, L.-C.; Zhang, P.-H.; Xu, J.-D. *J. Agric. Food Chem.* **2013**, *61*, 1521−1524. https://doi.org/10.1021/jf305210u; (c) Zhu, H.; Swierstra, J.; Wu, C.; Girard, G.; Choi, Y. H.; Wamel, W. V.; Sandiford, S. K.; Wezel, G. P. V. *Microbiol.* **2014**, *160*, 1714–1726, https://doi.org/10.1099/mic.0.078295-0; (d) Liu, X.; Arai, M. A.; Toume, K.; Ishibashi, M. *Nat. Prod. Commun*. **2018**, *13*, 65–66, https://doi.org/10.1177/1934578X1801300119.

(5) (a) Smelov, N. S.; Mizonova, T .P. *Antibiot. Chemotherapy* **1974**, *19*, 369–371. (Russian); (b) Myasnikova, L. G.; Polyak, M. S. *Antibiotiki (Mosc.)* **1989**, *34*, 386–390 (Russian).

(6) (a) Roggo, B.E.; Petersen, F.; Delmendo, R.; Jenny, H.-B.; Peter, H. H.; Roesel, J. *J. Antibiot.* **1994**, *47*, 136–142, https://doi.org/10.7164/antibiotics.47.136; (b) Podol'skaya, S. V.; Naryshkina, N. A.; Sorokoumova, G. M.; Kaplun, A. P.; Fedorova, N. E.; Medzhidova, A. A.; Kuts, A. A.; Shvets, V. I. *B. Exp. Biol. Med.* **2005**, *139*, 349–351; (c) Slesarchuk, N. A.; Khvatov, E. V.; Chistov, A. A.; Proskurin, G. V.; Nikitin, T. D.; Lazarevich, A. I.; Ulanovskaya, A. A.; Ulashchik, E. A.; Orlov, A. A.; Jegorov, A. V.; Ustinov, A. V.; Tyurin, A. P.; Shmanai, V. V.; Ishmukhametov, A.; Korshun, V. A.; Osolodkin, D. I.; Kozlovskaya, L. I.; Aralov, A. V. *Bioorg. Med. Chem. Lett.* **2020**, *30*, 127100, https://doi.org/10.1016/j.bmcl.2020.127100.

(7) (a) Adinarayana, G.; Venkateshan, M. R.; Bapiraju, V. V. S. N. K.; Sujatha, P.; Premkumar, J.; Ellaiah, P.; Zeeck, A. *Russ. J. Bioorg. Chem.* **2006**, *32*, 295–230; (b) Vijayabharathi, R.; Bruheim, P.; Andreassen, T.; Raja, D. S.; Devi, P. B.; Sathyabama, S.; Priyadarisini, V. B.; *J. Microbiol.* **2011**, *49*, 920–926.

(8) (a) Rosenbrook, W. *J. Org. Chem.* **1967**, *32*, 2924–2925, https://doi.org/10.1021/jo01284a072; (b) Brockmann, H.; Reschke, T. Die konstitution des resistomycins, *Tetrahedron Lett.* **1968**, *27*, 3167–3170, https://doi.org/10.1016/S0040-4039(00)89579-9; (c) Brockmann, H.; Meyer, E.; Schrempp, K.; Reiners, F.; Reschke, T. *Chem. Ber.* **1969**, *102*, 1224–1246, https://doi.org/10.1002/cber.19691020415; (d) Ishida, K.; Maksimenka, K.; Fritzsche, K.; Scherlach, K.; Bringmann, G.; Hertweck, C. *J. Am. Chem. Soc.* **2006**, *128*, 14619–14624, https://doi.org/10.1021/ja064550u; (e) Ueberschaar, N.; Meyer, F.; Dahse, H.; Hertweck, C. *Chem. Commun*. **2016**, *52*, 4894–4897, DOI:10.1039/C6CC00890A.

(9) Nadysev, G.; Tikhomirov, A.; Lin, M.; Yang, Y.; Dezhenkova, L.; Chen, H.; Kaluzhny, D.; Schols, D.; Shtil, A.; Shchekotikhin, A.; Chueh, P. *Eur. J. Med. Chem.* **2018**, *143*, 1553–1562, https://doi.org/10.1016/j.ejmech.2017.10.055.

(10) (a) Barceló, F.; Capó, D.; Portugal, J. *Nucleic Acids Res.* **2002**, *30*, 4567–4573, https://doi.org/10.1093/nar/gkf558; (b) Bi, S.; Zhang, H.; Qiao, C.; Sun, Y.; Liu, C. *Spectrochim.*

Acta A **2008**, *69*, 123–129, https://doi.org/10.1016/j.saa.2007.03.017; (c) Manet, I.; Manoli, F.; Zambelli, B.; Andreano, G.; Masi, A.; Cellai, L.; Monti, S. *Phys. Chem. Chem. Phys.* **2011**, *13*, 540–551, DOI:10.1039/C0CP00898B.

(11) (a) Liu, H. Y.; Chen, A. C.; Yin, Q. K.; Li, Z.; Huang, S. M.; Du, G.; He, J. H.; Zan, L. P.; Wang, S. K.; Xu, Y. H.; Tan, J. H.; Ou, T. M.; Li, D.; Gu, L. Q.; Huang, Z. S. *J. Med. Chem.* **2017**, *60*, 5438–5454, https://doi.org/10.1021/acs.jmedchem.7b00099; (b) Miglietta, G.; Cogoi, S.; Marinello, J.; Capranico, G.; Tikhomirov, A. S.; Shchekotikhin, A.; Xodo, L. E. *J. Med. Chem.* **2017**, *60*, 9448–9461. https://doi.org/10.1021/acs.jmedchem.7b00622; (c) Tikhomirov, A. S.; Tsvetkov, V. B.; Kaluzhny, D. N.; Volodina, Y. L.; Zatonsky, G. V.; Schols, D.; Shchekotikhin, A. E. *Eur. J. Med. Chem.* **2018**, *159*, 59–73. https://doi.org/10.1016/j.ejmech.2018.09.054; (d) Pirota, V.; Nadai, M.; Doria, F.; Richter, S. *Molecules* **2019**, *24*, *426*, https://doi.org/10.3390/molecules24030426; (e) Pont, I.; Martínez-Camarena, Á.; Galiana-Roselló, C.; Tejero, R.; Albelda, M. T.; González-García, J.; Vilar, R.; García-España, E. *ChemBioChem.* **2020**, *21*, 1167–1177, https://doi.org/10.1002/cbic.201900678.

(12) Balasubramanian, S.; Hurley, L. H.; Neidle, S. *Nat. Rev. Drug Discov.* **2011**, *10*, 261–275, https://doi.org/10.1038/nrd3428.

(13) (a) Wright, E.P.; Huppert, J.L.; Waller, Z. A. E. *Nucleic Acids Res.* **2017**, *45*, 2951–2959, https://doi.org/10.1093/nar/gkx090; (b) Hänsel-Hertsch, R.; Spiegel, J.; Marsico, G.; Tannahill, D.; Balasubramanian, S. *Nat. Protoc.* **2018**, *13*, 551–564, https://doi.org/10.1038/nprot.2017.150.

(14) (a) Biffi, G.; Tannahill, D.; McCafferty, J.; Balasubramanian, S. *Nat. Chem.* **2013**, *5*, 182–186, https://doi.org/10.1038/nchem.1548; (b) Zeraati, M.; Langley, D. B.; Schofield, P.; Moye, A. L.; Rouet, R.; Hughes, W. E.; Bryan, T. M.; Dinger, M. E.; Christ, D. *Nat. Chem.* **2018**, *10*, 631–637, https://doi.org/10.1038; (c) Dzatko, S.; Krafcikova, M.; Hänsel-Hertsch, R.; Fessl, T.; Fiala, R.; Loja, T.; Krafcik, D.; Mergny, J.-L.; Foldynova-Trantirkova, S.; Trantirek, L. *Angew. Chem. Int. Ed.* **2018**, *57*, 2165–2169, DOI:10.1002/anie.201712284; (d) Di Antonio, M.; Ponjavic, A.; Radzevičius,

A.; Ranasinghe, R. T.; Catalano, M.; Zhang, X.; Shen, J.; Needham, L. -M.; Lee, S. F.; Klenerman, D.; Balasubramanian, S. *Nat. Chem.* **2020**, *12*, 832–837, https://doi.org/10.1038/s41557-020-0506-4.

(15) (a) Siddiqui-Jain, A.; Grand, C. L.; Bearss, D. J.; Hurley, L. H. *PNAS* **2002**, *99*, 11593-11598, https://doi.org/10.1073/pnas.182256799; (b) Kendrick, S.; Kang, H. J.; Alam, M. P.; Madathil, M. M.; Agrawal, P.; Gokhale, V.; Yang, D.; Hecht, S. M.; Hurley, L. H. *J. Am. Chem. Soc.* **2014**, *136*, 11, 4161–4171, https://doi.org/10.1021/ja410934b.

(16) Li, Q.; Xiang, J.-F.; Yang, Q.-F.; Sun, H.-X.; Guan, A-J.; Tang, Y.-L. *Nucleic Acids Res.* **2013**, *41*, 1115–1123, https://doi.org/10.1093/nar/gks1101.

(17) Assi, H. A.; Garavís, M.; González, C.; Damha, M. J. *Nucleic Acids Res.* **2018**, *46*, 8038–8056, https://doi.org/10.1093/nar/gky735.

(18) Tikhomirov, A. S.; Lin, C.-Y.; Volodina, Y. L.; Dezhenkova, L.G.; Tatarskiy, V. V.; Schols, D.; Shtil, A. A.; Kaur, P.; Chueh, P. J.; Shchekotikhin, A. E. *Eur. J. Med. Chem.* **2018**, *148*, 128–139, https://doi.org/10.1016/j.ejmech.2018.02.027.

(19) McDonagh, A. M.; Ward, M. D.; McCleverty, J. A. *New J. Chem.*, **2001**, *25*, 1236-1243, https://doi.org/10.1039/B104575J.

(20) Choia, J.; Majima, T. *Chem. Soc. Rev.* **2011**, *40*, 5893-5909, https://doi.org/10.1039/C1CS15153C.

(21) Sen, D.; Gibert, W. *Nature* **1988**, *334*, 364-366, https://doi.org/10.1038/334364a0.

(22) Gehring, K.; Leroy, J. -L.; Guéron M. *Nature* **1993**, *363*, 561–565, https://doi.org/10.1038/363561a0.

(23) Day, H. A.; Pavlou, P.; Waller Z. A. E. *Bioorg. Med. Chem.* **2014**, *22*, 4407–4418, https://doi.org/10.1016/j.bmc.2014.05.047.

(24) Andreeva, D. V.; Tikhomirov, A. S.; Shchekotikhin, A. E. *Russ. Chem. Rev.* **2021**, *90*, 1–38, https://doi.org/10.1070/RCR4968.

(25) (a) Pagano, A.; Laccarino, N.; Abdelhamid, M. A. S.; Brancaccio, D.; Garzarella, E. U.; Di Porzio, A.; Novellino, E.; Waller, Z. A. E.; Pagano, B.; Amato, J.; Randazzo, A. *Front. Chem.* **2018**, *6*, 281, https://doi.org/10.3389/fchem.2018.00281; (b) Abdelhamid, M. A. S.; Gates, A. J.; Waller, Z. A. E. *Biochemistry* **2019**, *58*, 4, 245–249, https://doi.org/10.1021/acs.biochem.8b00968; (c) King, J. J.; Irving, K. L.; Evans, C. W.; Chikhale, R. V.; Becker, R.; Morris, C. J.; Peña Martinez, C. D.; Schofield, P.; Christ, D.; Hurley, L. H.; Waller, Z. A. E.; Swaminathan Iyer, K.; Smith N. M. *J. Am. Chem. Soc.* **2020**, *142*, 20600–20604, https://doi.org/10.1021/jacs.0c11708.

(26) Sheng, Q.; Neaverson, J. C.; Mahmoud, T.; Stevenson, C. E. M.; Matthews, S. E.; Waller, Z. A. E. *Org. Biomol. Chem.* **2017**, *15*, 5669–5673, DOI: 10.1039/c7ob00710h.

(27) Brazier, J. A.; Shah, A.; Brown, G. D. *Chem. Commun.* **2012**, *48*, 10739–10741, https://doi.org/10.1039/C2CC30863K.

(28) Dai, J.; Hatzakis, E.; Hurley, L. H.; Yang, D. *Plos One* **2010**, *5*, e11647, https://doi.org/10.1371/journal.pone.0011647.

(29) Dhakal, S.; Lafontaine, J. L.; Yu, Z.; Koirala, D.; Mao, H. *Plos One* **2012**, *7*, e39271, https://doi.org/10.1371/journal.pone.0039271.

(30) Day, H. A.; Huguin, C.; Waller, Z. A. E. *Chem. Commun.* **2013**, *49*, 7696–7698, https://doi.org/10.1039/C3CC43495H.

(31) Moyzis, R. K.; Buckingham, J. M.; Cram, L. S.; Dani, M.; Deaven, L. L.; Jones, M. D.; Meyne, J.; Ratliff, R. L.; Wu, J. R. *PNAS*, **1988**, *85*, 6622–6626, https://doi.org/10.1073/pnas.85.18.6622.

(32) Waller, Z. A. E.; Pinchbeck, B. J.; Buguth, B. S.; Meadows, T. G.; Richardson, D. J.; Gates, A.J. *Chem. Commun.* **2016**, *52*, 13511–13514, DOI:10.1039/C6C C06057A.

(33) Wright, E. P.; Day, H. A.; Ibrahim, A. M.; Kumar, J.; Boswell, L. J. E.; Huguin, C.; Stevenson, C. E. M.; Pors, K.; Waller, Z. A. E. *Sci. Rep.* **2016**, *6*, 39456, https://doi.org/10.1038/srep39456.

(34) Koch, W.; Holthausen, M.C. A chemist's guide to density functional theory. Second Edition. Wiley-VCH Verlag GmbH, 2001, pp. 78–79.

(35) Wavefunction, Inc. 18401 Von Karman Avenue, Suite 370 Irvine, CA 92612 U.S.A. www.wavefun.com

(36) Spartan Software https://www.wavefun.com/products/spartan.html (accessed June 11, 2020).

(37) Rohwedder, T.; Schneider, R. *J. Math. Chem.* **2011***, 49(9)*, 1889–1914, doi:10.1007/s10910-011-9863-y.

(38) www.chemcraftprog.com

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