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A novel achiral *seco*-cyclopropylpyrido[*e*]indolone (CPyI) analog of CC-1065 and the duocarmycins: Synthesis, DNA interactions, in vivo anticancer and anti-parasitic evaluation

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ABSTRACT

The synthesis of an achiral *seco*-hydroxy-aza-CBI-TMI analog (**8**) of the duocarmycins is reported. Its specificity for the DNA minor groove of AT-rich sequences and covalent bonding to adenine-N3 was ascertained by a thermal cleavage assay. Compound **8** was found to be cytotoxic in the nanomolar range against murine and human cancer cells. It was further demonstrated that compound **8** was active against murine melanoma (B16-F0) grown in C57BL/6 mice. Compound **8** was also shown to inhibit the growth of the protozoan parasites *Leishmania donovani*, *Leishmania mexicana*, *Trypanosoma brucei*, and *Plasmodium falciparum* in culture.

1. Introduction

The potent cytotoxic compounds (+)-CC-1065[1,2] (**1**) and (+)-duocarmycin SA (DUMSA, **2**)[2,3] as shown in Figure 1 are DNA minor groove and AT sequence selective alkylating agents isolated from the fermentation broth of *Streptomyces zelensis*[4] and *Streptomyces* sp.,[5] respectively. Both **1** and **2** exert biological activity by the covalent modification of the cyclopropylpyrrolo[*e*]indolone (CPI) moiety with the adenine-N3 of DNA. The mechanism of action was confirmed by the isolation of adenine-CC-1065[6] and adenine-duocarmycin SA adducts[7] from thermally cleaved DNA-substrate adducts. (+)-CC-1065 and (+)-DUMSA preferentially bind to the sequences 5′-PuNTTA-3′ and 5′-AAAAA-3′ reacting with the N3-position of the underlined adenine residue.[6–8] Most recently, (+)-yatakemycin **3**, isolated from *Streptomyces* sp., represents the latest and most potent member of this class of compounds.[9]

Although (+)-CC-1065 exhibits potent cytotoxicity, its potential as an anticancer drug is hampered by delayed lethal toxicity to mice.[4b] DUMSA was found to be devoid of this toxicity, however toxicity towards bone marrow was observed instead.[5] As a result, several analogs of CC-1065 and the duocarmycins have been developed for clinical evaluation, including adozelesin,[1b,4c,10] carzelesin,[11] bizelesin,[12] and KW-2189.[13] All of these compounds, aside from bizelesin and KW-2189, failed to progress beyond Phase I clinical evaluation due to severe toxicity towards the bone marrow.[10–12] On the other hand, bizelesin and KW-2189 have been investigated in Phase II clinical trials against several different types of cancer.[14] Over the past two decades, there has been a continued interest in the development of novel analogs of CC-1065 and the duocarmycins that show effective anticancer activity whilst maintaining limited toxicity towards host cells. Numerous analogs of

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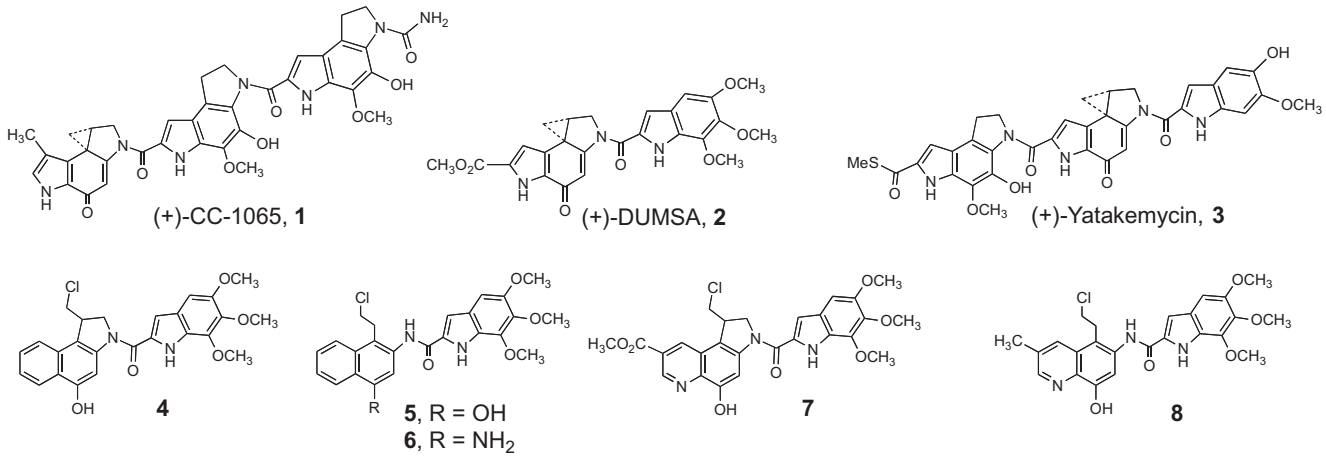


Figure 1. Structures of (+)-CC-1065 (**1**), (+)-duocarmycin SA (**2**), (+)-yatakemycin (**3**), racemic *seco*-hydroxy-CBI-TMI (**4**), which is a previously reported chiral variant of **8**.[16b] Also depicted are the structures of achiral molecules *seco*-hydroxy-CBI-TMI (**5**), *seco*-amino-CBI-TMI (**6**), and *seco*-hydroxy-aza-CBI-TMI (**8**).

CC-1065 and the duocarmycins with reduced toxicity towards bone marrow have been reported[1] according to the principle that the cytotoxic potency of the CPI moiety-containing compounds bears a direct relation to their solvolytic stability. DUMSA, one of the most cytotoxic analogs, is also the most solvolytically stable member of this class of compounds.[2,3,15] Examples of such analogs include cyclopropylbenzo[*e*]indolone (CBI),[16] cyclopropylpyrazolo[*e*]indolone (CPzI),[17] cyclopropylfurano[*e*]indolone (CFI),[18] and cyclopropylindolone (CI).[19] *seco*-Hydroxy-CBI-TMI **4** is an example of a CBI analog that has been widely studied by many groups including our research group.[16,20]

We have reported the design, synthesis and biological activity of the achiral analog represented by *seco*-hydroxy-CBI-TMI **5** as part of a study to establish whether the chiral center present in the natural products is required for DNA sequence recognition and biological activity. As a result, **5**, which lacks a stereocenter, was found to retain DNA sequence selectivity as well as having comparable in vitro cytotoxicity potency against B16 murine melanoma to its racemic counterpart **4** (IC_{50} 0.027 μM for **5** vs 0.078 μM for **4**).[20] Moreover, at the highest dosage, **5** and its achiral amino congener *seco*-amino CBI-TMI **6** (centanamycin) were found to exhibit activity against murine melanoma (B16-F0) grown in C57BL/6 mice as a solid tumor whilst maintaining minimal systemic toxicity.[20] This prompted us to refine the CBI pharmacophore present in achiral *seco*-hydroxy-CBI-TMI **5** and to further enhance its solubility in aqueous media, DNA binding and anticancer properties hence the development of *seco*-amino-CBI-TMI **6** within our laboratory.[20] Boger and co-workers synthesized 1,2,9,9a-tetrahydrocyclopropa[*c*]pyrido[3,2-*e*]indol-4-one-7-carboxylate **7** (CPyI) as a pharmacophore that was expected to enhance reactivity.[21] The presence of the fused pyridine functionality in **7** was shown to allow for the chelation of metal cations such as Zn^{2+} to the 8-ketoquinoline functionality on the CPyI pharmacophore which, in turn would provide a suitable means to selectively activate the agent upon the addition of a suitable Lewis acid. It has been reported that Zn is found in breast carcinoma at levels 700% higher than normal breast cells of the same type. It is hypothesized that analog **7** might exhibit an enhanced activity against breast carcinoma attributable to this difference in Zn levels.[22] For these reasons, it was logical to synthesize and study an achiral version of **7**, namely *seco*-cyclopropylpyrido[*e*]indolone (CPyI) analog **8** (*seco*-aza-CBI-TMI) of *seco*-hydroxy-CBI-TMI **5**. In addition to ascertaining the anticancer and DNA binding properties of **8**, it was interesting to investigate **8** for anti-parasitic activity as its amino congener **6** has been shown to have potent antimalarial and transmission-blocking activity.[23] Herein the DNA sequence specific alkylation along with in vitro and in vivo anticancer and anti-parasitic properties of compound **8** are reported.

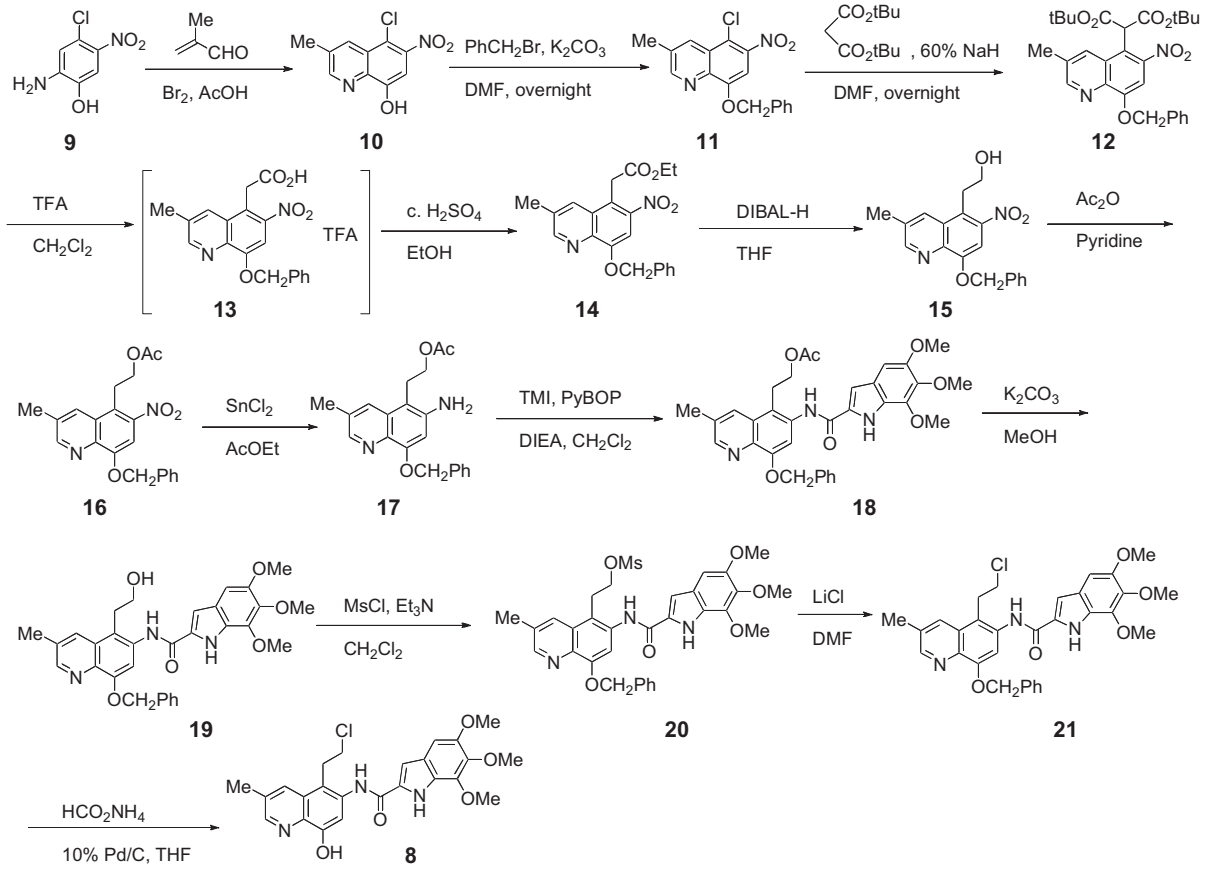
2. Results and discussion

2.1. Synthesis

The synthesis of the hydroxyl CPyI derivative **8** (Scheme 1) was based on a modified Skraup quinoline synthesis to provide the core structure **10**.[21a] Thus methacrolein was treated with **9** in the presence of bromine (1.0 equiv, AcOH, 100 °C, 1 h) to provide **10**.[24] Protection of phenol **10** (1.2 equiv of BnBr, 1.1 equiv of NaH, DMF 25 °C, overnight) gave **11** (19% yield over two steps based on **9**), which was followed by reaction of **11** with di-*tert*-butyl malonate (1.1 equiv), in DMF, in the presence of a stoichiometric amount of NaH (overnight, 60 °C) to furnish diester **12** in 67% yield. Subsequent acid-promoted removal of the *tert*-butyl groups (trifluoroacetic acid in CH_2Cl_2, 3 days) followed by decarboxylation of the diacid intermediate afforded acid **13** which was immediately esterified to give the compound **14** in 28% yield based on **12** over two steps. Reduction of ester **14** with DIBAL-H (2 equiv, 0 °C, 10 min) followed by treatment of the resultant alcohol **15** (formed in 47% yield) with acetic anhydride gave acetate **16** in 17% yield. Selective reduction of the aromatic nitro group ($SnCl_2{\cdot}2H_2O$, 5 equiv, 30 min) yielded the resulting amine **17** in 76% yield which was immediately coupled with 5,6,7-trimethoxyindole-2-carboxylic acid in the presence of benzotriazol-1-yloxy-tripyrrolidinophosphonium hexafluorophoshate (PyBop) and *N*,*N*-diisopropylethylamine (Hunig's base) to give amide **18** in 55% yield after 10 days. Methanolysis of the acetate group in **18** gave alcohol **19** in 67% yield. Mesylation of **19** (4.0 equiv methanesulfonyl chloride in presence of 4.0 equiv triethylamine, 30 min, 0 °C) followed by chlorination (55 equiv LiCl, dry DMF, 5 days, 25 °C), furnished **21** (via **20** which was used directly in its crude form). Removal of the protecting group on **21** by catalytic hydrogenation over 10% Pd/C provided the target achiral compound **8** in overall 50%. The compounds described in the paper were characterized by 500 MHz ^{1}H NMR, FT-IR, mass spectrometry, and accurate mass measurements. The >96% purity of compound **8** was ascertained by ^{1}H NMR and TLC analyses.

2.2. Thermal cleavage analysis

The sequence specificity of *seco*-hydroxy-aza-CBI-TMI **8** was assessed by a thermally induced DNA strand cleavage experiment, which is commonly used to probe the sequence specific covalent bonding with the purine-N3 in the minor groove.[20] The 208 base



Scheme 1. Synthesis of *seco*-hydroxy-aza-CBI-TMI (**8**).

pair DNA fragment used for these studies was obtained by PCR amplification from the pUC18 plasmid that was linearized with *Hin*d III. A 5′-^{32}P labeled primer was used as the forward primer so that each final probe copy was singly end labeled. A representative result from the thermally induced DNA strand break experiment on the previously reported *seco*-amino-CBI-TMI analog **6**[20b] is depicted in Figure 2 along with *seco*-hydroxy-aza-CBI-TMI **8**. The results indicate that both **6** and **8** have sequence selectivity for the 3′-A(865)AAAA cluster and in addition, display a dose-dependent response DNA alkylation effect. However, *seco*-hydroxy-aza-CBI-TMI analog **8** was about 10-fold less reactive than **6**. It is also worthy to note that **8** appeared to be more sequence discriminating than **6** which produced additional minor bands on the gel mainly at A(772) and A(843). However, in comparison to achiral *seco*-hydroxy-CBI-TMI, **5** (thermal cleavage gel not shown), **8** appears to be 10-fold more reactive.[20]

2.3. Cytotoxicity studies

An MTT-based growth inhibition assay was used to determine the cytotoxicity of achiral *seco*-hydroxy-aza-CBI-TMI **8**.[24,25] In addition to **8** the concentrations required for inhibiting the growth of murine L1210 lymphoma and B16-F0 melanoma cells by 50% (IC_{50} values) for compounds **4**, **5** and **6** were determined and reported previously following 3 days continuous exposure.[20,26] The IC_{50} values are given in Table 1. For comparison, the reported IC_{50} value CC-1065, **1**, against L1210 (3 days exposure) was reported as 30 pM.[27] Remarkably, **8** inhibited the growth of the tumor cells at sub-nanomolar concentrations, being approximately 10-fold more active against L1210 cells than against B16 cells (IC_{50} 0.72 nM for L1210 vs 2.3 nM for B16 cells; Table 1). In comparison to **8**, **6** exhibits decreased activity in both L1210 and B16 cell lines with both IC_{50} values (180 and 150 nM, respectively). In relation to the activity of achiral *seco*-hydroxy-CBI-TMI **5**, **8** is about 10-fold more active in B16 cells (IC_{50} 2.3 nM for **8** vs 27 nM for **5**) and roughly 75-fold (IC_{50} 0.72 nM for **8** vs 55 nM for **5**; Table 1) in L1210 cells. In addition, analog **8** possesses comparable cytotoxicity to CC-1065, **1** against L1210 cells, (IC_{50} 30 pM).[27] Furthermore, this is consistent with the observation that the cytotoxicity correlates with the length of the molecule, which, in turn, reflects its capacity for non-covalent interactions with the minor groove of DNA.[20b] In this case, the methyl group present on the aza-CBI moiety of **8** adds the extra length to the molecule over its non-methylated congeners **5** and **6**.

Compound **8** was subjected to further cytotoxicity testing at the National Cancer Institute against a panel of 54 human cancer cell lines.[28] Cell viability after 48 h of continuous exposure was ascertained using a sulforhodamine B assay. Compound **8** gave comparable cytotoxicity results against some cancers when compared to the NCI data for the previously reported *seco*-amino-CBI-TMI **6**.[16] Figure 3 shows the LC_{50} values (the concentration for killing 50% of the cells) for compound **8** against the panel of 54 different tumor cell lines. Bars extending to the right indicate cells that are more sensitive than average to **8**, whereas bars to the left indicate less sensitive cells. Several observations were made on this result. First, **8** exhibited activity in the nanomolar range for certain skin, central nervous system (CNS) and lung cancers. Second, **8** was selectively toxic towards a certain type of ovarian cancer over **6** whilst unlike **6**, **8** showed no toxicity against any cell lines associated with breast cancer (Fig. 3). Third, both **6** and **8** showed limited activity against leukemic cells. Fourth, both compounds showed activity against a number of solid tumors including melanoma, lung, colon and CNS. The specific reasons for the trends in cytotoxicity are not clear, and efforts are ongoing in our laboratories to address these issues.

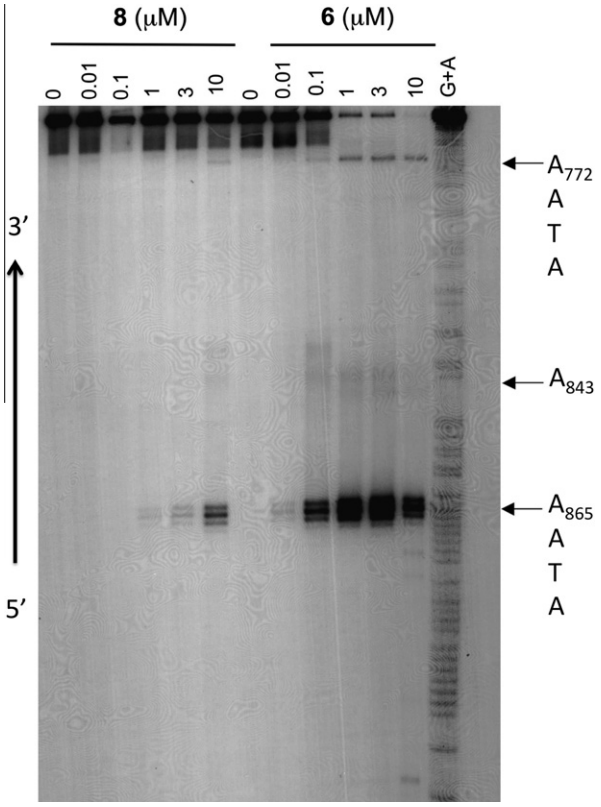


Figure 2. Thermal cleavage gel on compounds **6** and **8** showing adenine-N3 lesions on the bottom strand of a 5′-^{32}P labeled 208-bp fragment of pUC18 at the drug concentrations indicated. Arrows indicate the position and sequence context of the alkylated adenines (underlined). Drug/DNA incubations were for 5 h at 37 °C.

Table 1
Cytotoxicity of compounds **4**–**6** and **8**

Compd	Cytotoxicity (IC_{50} given in nM)	
	L1210	B16
4	8.4	78
5	180	150
6	55	27
8	0.72(±0.1)	2.3(±1)

2.4. In vivo anticancer studies of compound 8 on B16-F0 murine melanoma

This study was based according to a similar protocol disclosed by Li et al.[29] A total of eight animals within each group, 5–7 week old female C57/BL/6J mice were inoculated in the left flank with cultured B16-F0 murine melanoma cells on day 0. On days 1, 5, and 9 mice were treated with a 10 mg/kg dose of **8** in vehicle solution. Compound **8** was formulated in 1:2 PET/5% glucose solution and administered via an intraperitoneal route. The volume of the tumor was monitored every other day. These measurements were ceased when four of the animals in the test group (50%) had died. The in vivo anticancer effects for compound **8** were encouraging (data not shown). For the control (untreated) group, four of the mice had died by day 16, with the tumor volume steadily increasing from day 9 from a barely visible volume of ~100 mm^3, reaching ~2300 mm^3 by the time 50% of the mice had died (day 16). In comparison, in the treated group, the fourth mice had died on day 21, thus indicating an increase in lifespan within this group of mice. After the final 10 mg/kg injection (day 9), the average tumor size increased steadily from a volume of ~100 mm^3 to ~1700 mm^3 by day 16. At this point a decrease in tumor size followed until the death of the last mouse on day 21, with the average size of the tumor being ~2000 mm^3. As part of this study, the weights of the animals were also monitored at regular intervals. It was found that under the dose administered, compound **8** did not induce any significant weight loss following drug injection, compared to animals treated with the vehicle only, thus indicating no noticeable toxicity. This could be further corroborated by the fact that the mice in all groups had comparable red blood cell counts along with weights of the liver, kidney and spleen. The moderate reduction in tumor size induced by **8** along with minimal systemic toxicity encouraged us to consider exploring the anti-tumor activity at a higher dosage.

2.5. Anti-parasitic activity of compound 8

With the information in hand that compound **6**, an AT sequence specific-adenine-N3 alkylating agent, has potent antimalarial activity in vivo and in vitro against *Plasmodium falciparum*,[23] and attenuates *Plasmodium berghei*[30] sporozoites we assessed the ability of **8** to inhibit growth of the protozoan parasites, *Leishmania donovani*, *Leishmania mexicana*, *Trypanosoma brucei*, and *Plasmodium falciparum* in culture. The results provide evidence that compounds **6** and **8** show significant activity against *P. falciparum* with IC_{50} in low nanomolar range (Table 2). Overall, compound **6** was more active than **8** against *L. donovani* promastigotes and *T. brucei* procyclic life cycle stages. However, the aza-compound **8** gave comparable activity against *P. falciparum* indicating that this compound or the chemical scaffold possesses pharmacological potential for development as an antimalarial agent. Surprisingly, compounds **6** appeared to be far less potent against the kinetoplastid parasite *L. mexicana* whereas compound **8** is less potent against *L. donovani*, *L. mexicana* and *T. brucei* (Table 2). This difference may not be surprising since compounds **6** and **8** are postulated to exert their cytotoxic activity through their AT-sequence specific DNA lesions. The abundance of adenine-thymine nucleotides in the *Plasmodium* genome[31] is 80.6% making this parasite more susceptible to these drugs in comparison to the *Leishmania* and *Trypanosoma* genomes which have an adenine-thymine nucleotide content of 41%.[32] It should be emphasized that in contrast to the genomic DNA, the mitochondrial (kinetoplastid) DNA of *Leishmania* and *Trypanosoma* has a high AT codon bias which may be the target of compounds **6** and **8** in these parasites.[33]

3. Conclusion

In conclusion, the favorable DNA interaction properties, cytotoxicity against tumor cell lines, activity against murine melanoma grown in mice as a solid tumor and in vitro activity against *P. falciparum* strongly indicate that achiral *seco*-hydroxy-aza-CBI-TMI warrants further investigation as an anticancer agent and as a potential anti-parasitic agent. Efforts are ongoing within our laboratory to gain further insight into the selective activity against *P. falciparum* as well as the sensitivity profiles observed in the NCI cytotoxicity screen. The results from this study effectively demonstrate that analogs of CC-1065 and the duocarmycins, which lack a stereocenter would still exhibit DNA sequence specificity and antitumor activity.

4. Experimental

4.1. General

Solvents and organic reagents were purchased from Aldrich or Fisher and used without further purification. Dichloromethane

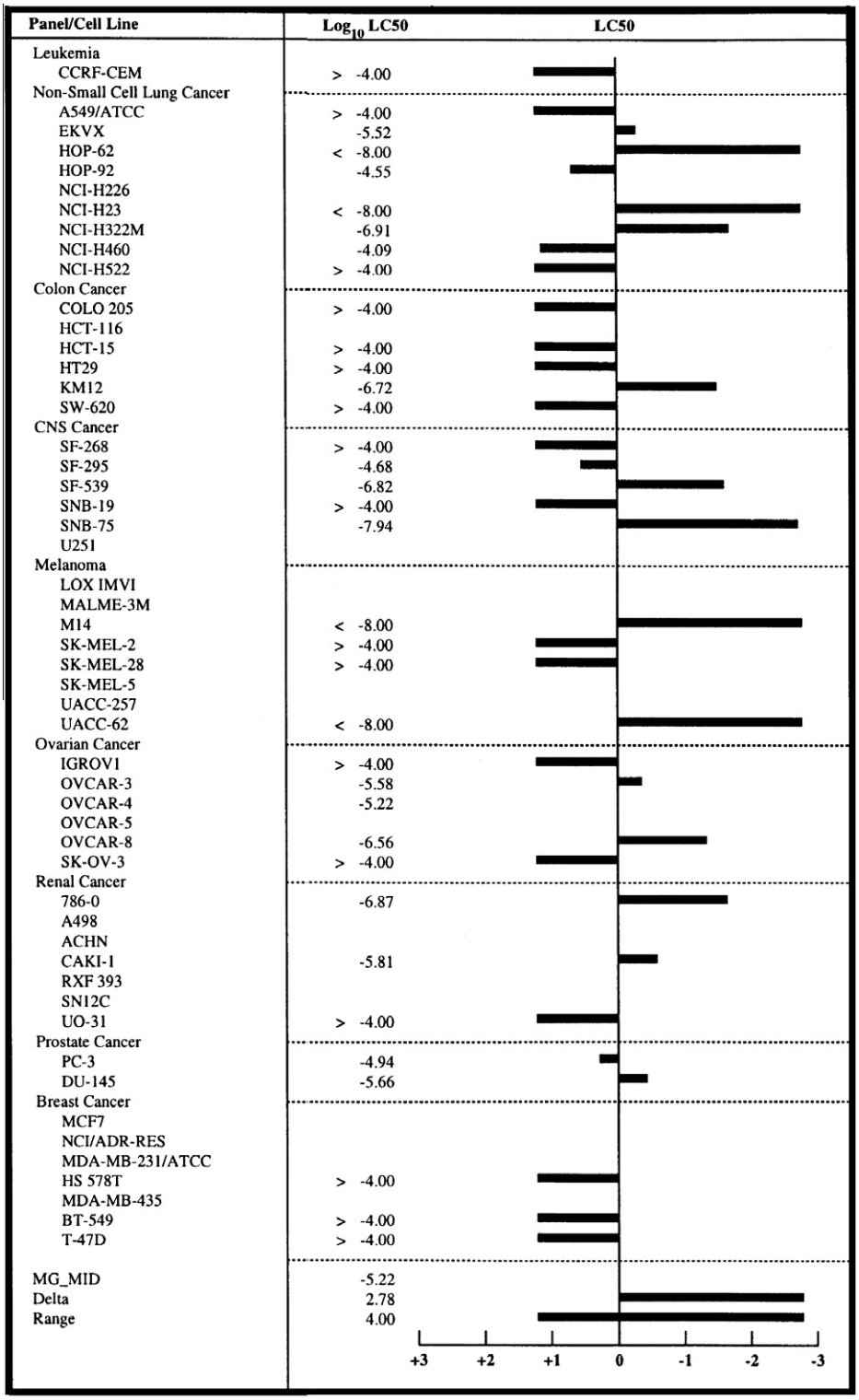


Figure 3. NCI cytotoxicity screen data (LC_{50} values) for compound **8** against a panel of 54 different human cancer cell lines.

(DCM) and dimethylformamide (DMF) were distilled over P_2O_5 and BaO, respectively, prior to use. Melting points (mp) were performed using a Mel-temp instrument, and the results were uncorrected. Infrared (IR) spectra were recorded using a Perkin Elmer Paragon 500 FT-IR spectrophotometer as films on KBr disks. ^1H NMR spectra were obtained using a Varian Unity INOVA 500 MHz instrument unless otherwise stated. Chemical shifts (δ) are reported at 20 °C in parts per million (ppm) downfield from internal tetramethylsilane. High-resolution mass spectra (HRMS) and low-resolution mass spectra (LRMS) were provided by the Mass Spectrometry Laboratory, University of South Carolina, Columbia, SC. Reactions were monitored using thin-layer chromatography (TLC) using commercially available precoated plates (Merck Kieselgel 60 F254 silica). Visualization was achieved with

Table 2
In vitro cytotoxicity data of compounds **6** and **8** against *Leishmania donovani*, *Trypanosoma brucei*, *Plasmodium falciparum* and *Leishmania mexicana*

Compd	Cytotoxicity[a] (nM)			
	L. donovani	*T. brucei*	*P. falciparum*	*L. mexicana*
6	13	1.9	1.8[b]	1024
8	340	56	1.8	119

[a] The error for these studies is ±10%.
[b] Previously reported within our research group.[23]

UV light at 254 nm or I_2 vapor staining. In addition to NMR and elemental analysis, HPLC analysis was used to determine the purity (>95%) of the compounds.

4.1.1. Synthesis of 11

To a solution of methacrolein (3.0 mL, 37.1 mmol) in AcOH (140 mL), bromine (1.9 mL, 37.1 mmol) was added and the resulting solution was stirred at room temperature until the appearance of a faint red color. Compound **9** was then added (7.0 g, 37.1 mmol) and the resulting mixture was heated at 100 °C for 1 h. The reaction mixture was filtered, and the solid was washed with water and dried in the hood to yield **10** (4.7 g, mixture with **9**) as a brown solid. To a solution of **10** (4.7 g, 19.7 mmol) in DMF (47 mL), K_2CO_3 (3.81 g, 27.6 mmol) and benzyl bromide (2.80 mL, 23.6 mmol) were added and the resulting mixture was stirred overnight at room temperature. The mixture was filtered, concentrated and extracted with ethyl acetate (50 ml). The solution was washed with water (50 mL) and saturated aqueous NaCl (50 mL), then dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column (AcOEt/Petroleum ether = 1:2) and washing with Et_2O to yield **11** (2.3 g, 19% from **9**) as a yellow solid. Mp 140–141 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) 8.97 (d, 2.0, 1H), 8.51 (d, 2.0, 1H), 7.52 (d, 7.5, 2H), 7.40 (t, 7.0, 2H), 7.38 (s, 1H), 7.34 (t, 7.0, 1H), 5.46 (s, 2H), 2.63 (s, 3H); EIMS *m*/*z* 238 (M^+, 100%); IR (neat) ν_{max} 3034, 1594, 1531, 1498, 1454, 1374, 1346, 1325, 1255, 1126, 1007, 970, 913, 884, 839, 784, 747, 697 cm^{-1}.

4.1.2. Compound 12

To a suspension of 60% NaH washed with petroleum ether (4.5 g, 0.11 mol) in DMF (200 mL), a solution of di-*tert*-butyl malonate (20.4 mL, 89.4 mmol) in DMF (50 mL) and **11** (24.5 g, 74.5 mmol) were added sequentially followed by heating at 60 °C overnight. The mixture was quenched slowly by adding saturated aqueous NH_4Cl until hydrogen gas evolution stopped. The mixture was diluted with water (300 mL). The resulting precipitate was filtered and dried in the hood to yield **12** (34.2 g, 67%) as a brown solid. Mp 150–152 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) 8.92 (d, 2.0, 1H), 8.32 (d, 2.0, 1H), 7.53 (d, 7.0, 2H), 7.40 (s, 1H), 7.40 (t, 8.0, 2H), 7.34 (t, 7.5, 1H), 5.47 (s, 2H), 5.25 (s, 1H), 2.56 (s, 3H), 1.45 (s, 18H); EIMS *m*/*z* 508 (M^+, 20%), EIHR *m*/*z* 508.2211 (M^+, $C_{28}H_{32}N_2O_7$ requires 508.2210); IR (neat) ν_{max} 2979, 2927, 1728, 1584, 1529, 1499, 1457, 1391, 1368, 1316, 1252, 1160, 1136, 847, 736, 699, 668 cm^{-1}.

4.1.3. Compound 14

To a solution of **12** (34.2 g, 67.3 mmol) in CH_2Cl_2 (680 mL), trifluoroacetic acid (68 mL) was added and the resulting solution stirred for 3 days at room temperature. The reaction mixture was concentrated under reduced pressure to give carboxylic acid **13**, which was immediately dissolved in ethanol containing 5% concentrated H_2SO_4 (34 mL) and refluxed overnight. The resulting mixture was concentrated and extracted with AcOEt (500 mL). The solution was washed with water (100 mL), saturated aqueous $NaHCO_3$ (100 mL), saturated aqueous NaCl (100 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was washed with Et_2O to yield **14** (7.2 g, 28%) as a brown solid. Mp 192–194 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) 8.95 (d, 2.0, 1H), 8.17 (d, 2.0, 1H), 7.54 (d, 7.5, 2H), 7.52 (s, 1H), 7.40 (t, 7.0, 2H), 7.34 (t, 7.5, 1H), 5.47 (s, 2H), 4.24 (s, 2H), 4.20 (q, 7.0, 2H), 2.60 (s, 3H), 1.26 (t, 7.5, 3H); EIMS *m*/*z* 380 (M^+, 45%), EIHR *m*/*z* 380.1376 (M^+, $C_{21}H_{20}N_2O_5$ requires 380.1372); IR (neat) ν_{max} 1724, 1584, 1519, 1501, 1457, 1426, 1382, 1373, 1357, 1324, 1243, 1207, 1118, 1018, 974, 916, 881, 845, 796, 754, 734, 699 cm^{-1}.

4.1.4. Compound 15

To a suspension of **14** (15.2 g, 40.0 mmol) in dry THF (300 mL) at 0 °C, DIBAL-H (1.5 M, 53.3 mL, 79.9 mmol) was added slowly and the solution was stirred at the same temperature for 10 min. The mixture was poured into water (0.6 L), then $CHCl_3$ (1 L) was added to the mixture and the gel was filtered over Celite. The organic layer was washed with saturated aqueous NaCl (50 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was triturated with $CHCl_3$ to yield **15** (6.2 g, 47%) as an orange solid. Mp 186–188 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) 8.93 (d, 2.0, 1H), 8.35 (s, 1H), 7.52 (d, 7.0, 2H), 7.39 (t, 7.0, 2H), 7.36 (s, 1H), 7.33 (t, 7.5, 1H), 5.44 (s, 2H), 4.03 (q, 7.0, 2H), 3.41 (t, 7.0, 2H), 2.60 (s, 3H), 1.85 (t, 5.5, 1H); EIMS *m*/*z* 338 (M^+, 40%), EIHR *m*/*z* 338.1271 (M^+, $C_{19}H_{18}N_2O_4$ requires 338.1267); IR (neat) ν_{max} 3232, 1521, 1504, 1454, 1374, 1363, 1335, 1312, 1241, 1202, 1123, 1052, 980, 913, 886, 838, 798, 782, 743, 720, 701 cm^{-1}.

4.1.5. Compound 16

To a solution of **15** (6.2 g, 18.9 mmol) in dry pyridine (16 mL), 98% acetic anhydride (2.2 mL, 22.7 mmol) was added and the resulting mixture stirred overnight at room temperature. The mixture was then concentrated and dissolved in AcOEt (200 mL). The organic layer was washed with H_2O (50 mL), saturated aqueous NaCl (10 mL), organic layer dried (over Na_2SO_4) and concentrated under reduced pressure to give a brown residue which was washed with *i*-Pr_2O to yield **16** (1.2 g, 17%) as a pale brown solid. Mp 130 °C (decomp.); ^{1}H NMR ($CDCl_3$, 500 MHz) 8.95 (s, 1H), 8.48 (s, 1H), 7.53 (d, 7.5, 2H), 7.40 (t, 7.0, 2H), 7.38 (s, 1H), 7.33 (t, 8.0, 1H), 5.46 (s, 2H), 4.42 (t, 8.0, 2H), 3.47 (t, 8.0, 2H), 2.63 (s, 3H), 2.05 (s, 3H); EIMS *m*/*z* 380 (M^+, 30%), EIHR *m*/*z* 380.1385 (M^+, $C_{21}H_{20}N_2O_5$ requires 380.1372); IR (neat) 3060, 2971, 2897, 1732, 1582, 1523, 1499, 1454, 1358, 1333, 1310, 1239, 1140, 1043, 898, 860, 838, 804, 778, 734, 694, 668 cm^{-1}.

4.1.6. Compound 17

To a solution of **16** (3.0 g, 7.89 mmol) in AcOEt (60 mL), $SnCl_2{\cdot}2H_2O$ (8.90 g, 39.4 mmol) was added and the resulting mixture stirred for 30 min at room temperature. The reaction was quenched with saturated aqueous $NaHCO_3$ (100 mL) and extracted with $CHCl_3$ (300 mL). The $CHCl_3$ layer was washed with saturated aqueous NaCl (10 mL), dried (over Na_2SO_4) and evaporated to dryness to yield **17** (2.1 g, 76%) as an orange foam. Mp 116 °C (decomp.); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.67 (s, 1H), 7.96 (s, 1H), 7.52 (d, 7.0, 2H), 7.35 (t, 7.0, 2H), 7.28 (t, 7.5, 1H), 7.20 (s, 1H), 5.42 (s, 2H), 4.21 (t, 7.5, 2H), 3.14 (t, 7.5, 2H), 2.52 (s, 3H), 2.07 (s, 3H), 1.62 (br s, 2H); EIMS *m*/*z* 350 (M^+, 80%); IR (neat) ν_{max} 3308, 3172, 1954, 2883, 1734, 1613, 1505, 1457, 1417, 1386, 1365, 1237, 1200, 1133, 1076, 1045, 1028, 912, 845, 734, 697, 668 cm^{-1}.

4.1.7. Compound 18

To a solution of **17** (1.0 g, 2.85 mmol) in CH_2Cl_2 (10 mL), 5,6,7-trimethoxyindole-2-carboxylic acid (TMI) (0.86 g, 3.42 mmol), *N*,*N*-diisopropylethylamine (1.21 mL, 6.85 mmol) and benzotriazol-1-yloxy-tripyroridinophosphonium hexafluorophosphate (PyBOP) (1.78 g, 3.42 mmol) were added under an N_2 atmosphere and stirred for 10 days at room temperature. The reaction mixture

was extracted with CH_2Cl_2 (100 mL) and washed with saturated aqueous $NaHCO_3$ (20 mL), saturated aqueous NaCl (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/Petroleum ether = 4:1) to yield **18** (0.91 g, 55%) as a brown foam. Mp 80–81 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) 9.35 (s, 1H), 8.79 (d, 1.5, 1H), 8.64 (s, 1H), 8.00 (s, 1H), 7.91 (s, 1H), 7.58 (d, 7.5, 2H), 7.37 (t, 7.5, 2H), 7.29 (t, 7.5, 1H), 7.19 (s, 1H), 6.87 (s, 1H), 5.45 (s, 2H), 4.35 (t, 7.0, 2H), 4.10 (s, 3H), 3.95 (s, 3H), 3.93 (s, 3H), 3.36 (t, 7.0, 2H), 2.56 (s, 3H), 2.17 (s, 3H); TOF MS ES+ *m*/*z* 584 (M^++H, 100%), TOFHR *m*/*z* 584.2401 (M^++H, $C_{33}H_{34}N_3O_7$ requires 584.2397); IR (neat) ν_{max} 3307, 2936, 1736, 1652, 1606, 1582, 1535, 1501, 1465, 1411, 1366, 1305, 1237, 1122, 1047, 998, 912, 833, 736, 698 cm^{-1}.

4.1.8. Compound 19

A solution of **18** (0.91 g, 1.56 mmol) in MeOH (18 mL) was treated with K_2CO_3 (0.26 g, 1.89 mmol) and stirred for 3 h at room temperature. The reaction mixture was extracted with $CHCl_3$ (100 mL) and washed with water (20 mL), saturated aqueous NaCl (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure to yield **19** (0.57 g, 67%) as a brown solid. Mp 110 °C (decomp.); ^{1}H NMR ($CDCl_3$, 500 MHz) 10.03 (s, 1H), 9.24 (s, 1H), 8.67 (d, 1.5, 1H), 7.85 (s, 1H), 7.80 (s, 1H), 7.56 (d, 7.5, 2H), 7.35 (t, 7.0, 2H), 7.28 (t, 8.0, 1H), 7.06 (d, 2.0, 1H), 6.82 (s, 1H), 5.35 (s, 2H), 4.13 (t, 5.5, 2H), 4.08 (s, 3H), 3.95 (s, 3H), 3.90 (s, 3H), 3.21 (t, 5.0, 2H), 2.49 (s, 3H); TOF MS ES+ *m*/*z* 542 (M^++H, 100%), TOF HRMS *m*/*z* 542.2307 (M^++H, $C_{31}H_{32}N_3O_6$ requires 542.2291); IR (neat) ν_{max} 3271, 1936, 1652, 1607, 1586, 1536, 1501, 1462, 1412, 1368, 1305, 1257, 1127, 1048, 996, 909, 831, 730, 699 cm^{-1}.

4.1.9. Compound 21

A solution of **19** (0.57 g, 1.05 mmol) in CH_2Cl_2 (8 mL) was treated with triethylamine (0.6 mL, 4.21 mmol) and methanesulfonyl chloride (0.33 mL, 4.21 mmol) at 0 °C and stirred for 0.5 h. The reaction mixture was extracted with $CHCl_3$ (50 mL) and washed with H_2O (10 mL), saturated aqueous $NaHCO_3$ (10 mL), saturated aqueous NaCl (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure to yield **20** (0.9 g, quant.) as a crude black oil which was immediately dissolved in dry DMF (20 mL). LiCl (2.46 g, 58.1 mmol) was added and the reaction mixture was stirred for 5 days at room temperature. The reaction was quenched with H_2O (100 mL). The resulting solid was filtered and dried in the hood to yield **21** (0.65 g, quant.) as a pale brown solid. Mp 150 °C (decomp.); ^{1}H NMR ($CDCl_3$, 500 MHz) 9.49 (br s, 1H), 8.89 (br s, 1H), 8.82 (s, 1H), 8.05 (s, 1H), 7.69 (s, 1H), 7.51 (d, 7.5, 2H), 7.32 (t, 6.5, 2H), 7.28 (m, 1H), 7.05 (s, 1H), 6.85 (s, 1H), 5.33 (s, 2H), 4.10 (s, 3H), 3.95 (t, 7.0, 2H), 3.95 (s, 3H), 3.92 (s, 3H), 3.55 (t, 6.0, 2H), 2.56 (s, 3H); TOFMS ES+ *m*/*z* 560 (M^++H, 100%), TOFHR *m*/*z* 560.1967 (M^++H, $C_{31}H_{31}ClN_3O_5$ requires 560.1952); IR (neat) ν_{max} 3244, 2936, 1654, 1577, 1542, 1506, 1465, 1410, 1369, 1305, 1238, 1200, 1118, 1049, 998, 910, 835, 752, 730, 699, 668 cm^{-1}.

4.1.10. Achiral *aza*-CBI-TMI 8

A solution of **21** (0.65 g, 1.16 mmol) in THF (13 mL) was added to 10% Pd/C (0.65 g) and cooled to −78 °C. The suspension was degassed under vacuum and under N_2 atmosphere. The mixture was warmed to room temperature and treated with a solution of ammonium formate (0.75 g, 11.6 mmol) in water (3.0 mL), and the reaction mixture was stirred for 3 days. The suspension was filtered over Celite and the filtrate was extracted with AcOEt (100 mL). The organic layer was washed with H_2O (10 mL), saturated aqueous NaCl (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure to yield **8** as a crude orange solid. The solid was washed with Et_2O to yield **15** (0.27 g, 50%) as a pure orange solid. Mp 220 °C (decomp.); ^{1}H NMR ($CDCl_3$, 500 MHz) 9.25 (s, 1H), 8.62 (s, 1H), 8.47 (s, 1H), 7.94 (s, 1H), 7.61 (s, 1H), 6.96 (s, 1H), 6.86 (s, 1H), 4.09 (s, 3H), 3.95 (s, 3H), 3.95 (t, 7.0, 2H), 3.92 (s, 3H), 3.52 (t, 6.0, 2H), 2.58 (s, 3H), 1.60 (br s, 1H); TOFMS ES+ *m*/*z* 470 (M^++H, 100%), TOFHR *m*/*z* 470.1485 (M^++H, $C_{24}H_{25}ClN_3O_5$ requires 470.1483); IR (neat) ν_{max} 3310, 2936, 2833, 2361, 1636, 1583, 1536, 1500, 1464, 1408, 1383, 1365, 1303, 1241, 1113, 1048, 997, 909, 833, 731, 669 cm^{-1}.

4.2. Cytotoxicity studies on murine leukemia and melanoma cell lines

The P815, B16-F0, and L1210 cell lines were obtained from American Type Tissue Culture Collection (ATCC) and the studies were conducted exactly according to a recently published procedure.[20a]

4.3. NCI in vitro cytotoxicity screen

Compound **8** was also tested against a panel of 60 human cancer cell lines at the National Cancer Institute, Bethesda, MD.[28] The cytotoxicity studies were conducted using a 48 h exposure protocol and a sulforhodamine B assay. Dose–response curves were used to generate the GI_{50} (concentration of drug needed to inhibit the growth by 50%), TGI (total growth inhibition), and LC_{50} values (concentration needed to kill 50% of cells).

4.4. Thermal cleavage assay

The thermally-induced cleavage assay was used to probe purine-N3 alkylations on the same region of the pUC18 plasmid and under the same experimental conditions as the ones previously reported for compound **6**.[20b] Briefly, the 208-bp probe subjected to drug modification was PCR amplified and defined by the primers 5′-CTCACTCAAAGGCGGTAATAC-3′ and 5′-TGGTATCTTTATAGTCCTGTCG-3′, the latter being 5′-end radiolabeled with [γ-^{32}P] ATP (5000 Ci/mmol, Amersham) using T4 polynucleotide kinase (New England Biolabs). Oligonucleotides were synthesised by Eurofins MWG Operon. 4 pmol of each primer were used for the exponential amplification of the 749–956 region of 0.4 ng of the pUC18 plasmid template DNA. The resulting PCR product was detected by agarose gel electrophoresis, isolated and purified using the Geneclean III kit (MP Biomedicals) following the manufacturer's standard protocol. Drug–DNA incubations were carried out in TEOA buffer [25 mmol/L triethanolamine, 1 mmol/L EDTA (pH, 7.2)] at 37 °C for 5 h, in a total volume of 50 μL, using 10 μL of the purified probe per reaction, enough to yield an activity of at least 200 counts/s. Following precipitation and lyophilisation, dried DNA pellets were heated at 90 °C for 30 min in a total volume of 100 μL of sodium citrate buffer [1.5 mmol/L sodium citrate, 15 mmol/L NaCl (pH, 7.2)]. Samples were finally resuspended in 5 μL formamide loading buffer and subjected to electrophoresis.

4.5. In vivo anticancer study of compound 8 on the growth of B16-F0 murine melanoma in C57/BL/6 mice

Female C57/BL/6J mice were obtained from the Jackson Laboratory and were 5–7 weeks old upon arrival. Groups of eight mice were inoculated with B16 cells that came directly from a culture flask without first being passaged through a mouse. The cultured cells were washed three times with PBS and resuspended in PBS at a concentration of 4.0×10^6 cells/mL. The cell suspensions (50 μL) were delivered subcutaneously (sc) to the left flank at day 0. At this concentration, each mouse received 200,000 tumor cells. The length and width of the tumors were measured almost every other day using calipers (Bel-Art Products, Vernier Type H

13415) and the tumor volume was calculated as: tumor volume (mm^3) = length (mm) × [width (mm)]^2.

The drug solutions were formulated by mixing the appropriate amounts for each drug with 1.6 mL of PET (poly(ethylene glycol) 400, absolute ethanol, and Tween 80 in 6:3:1 portions) and 3.2 mL of 5% glucose in water. In each case clear, colorless solutions were obtained. These solutions were stored at 20 °C and the compound's stability was monitored by RP-HPLC analysis. Each mouse received 100 μL of the drug solution via an intraperitoneal (ip) administration route on days 1, 5, and 9. Each injection delivered 10 mg/kg of compound **8**. Animal weights were taken every other day. The control group of mice was treated on a similar schedule with 100 μL of the vehicle.

4.6. *Plasmodium* growth inhibition studies on compound 8

Plasmodium falciparum was cultured in human erythrocytes at 3–5% hematocrit in complete medium, and in vitro growth assays were performed essentially as described elsewhere.[23] Centanamycin was tested at six concentrations, in triplicate, in three independent experiments.

4.7. Growth inhibition of *Leishmania* and *Trypanosoma brucei* by compound 8

Leishmania donovani and *Leishmania mexicana* promastigotes (5×10^4 cells/well) were cultured in 200 μL of DME-L media supplemented with 10% heat inactivated fetal bovine serum, hemin, xanthine, and penicillin-streptomycin.[34] *Trypanosoma brucei* 427 procyclic forms were grown in SDM 79 media. The parasites were cultured for 72 h at 26 °C in media containing in the presence of 0.03 nM to 64 μM compound **8**. Cell proliferation was assessed by adding 20 μL of Alamar blue dye solution (Trek Diagnositics, Cleveland, OH) and measuring the levels of dye reduction by fluorescence (excitation 530 nm and emission 590 nm) after a 15 h incubation. All experiments were performed at least twice using triplicate samples. Data was analyzed using the MicroCal Origins 7.0 software package.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.05.078.

References and notes

1. (a)*Advances in DNA Sequence Specific Agents*; Jones, G. B., Palumbo, M., Eds.; JAI Press Inc.: Greenwich, CT, 1998; Vol. 3, (b) Arcamone, F. M.; Animati, F.; Barbieri, B.; Confligliacchi, E.; D'Alessio, R.; Geroni, C.; Giuliani, F. C.; Lazzari, E.; Menozzi, M.; Mongelli, N.; Penco, S.; Verini, M. A. *J. Med. Chem.* **1989**, *32*, 774; (c) Li, L. H.; Wallace, T. L.; DeKoning, T. F.; Warpehoski, M. A.; Kelly, R. C.; Prairie, M. D.; Drueger, W. C. *Invest. New Drugs* **1987**, *5*, 329; (d) Li, L. H.; Kelly, R. C.; Warpehoski, M. A.; McGovern, J. P.; Gebhard, I.; DeKoning, T. F. *Invest. New Drugs* **1991**, *9*, 137; (e) Warpehoski, M.. In *Advances in DNA Sequence Specific Agents*; Hurley, L. H., Ed.; JAI Press, Inc.: Greenwich, CT, 1992; Vol. 1, pp 217–245.
2. (a) Boger, D. L.; Johnson, D. S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1438; (b) Boger, D. L.; Johnson, D. S. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 3642.
3. (a) Boger, D. L. *Acc. Chem. Res.* **1995**, *28*, 20; (b) Boger, D. L. *Chemtracts: Org. Chem.* **1991**, *4*, 329.
4. (a) Hanka, L. J.; Dietz, A.; Gerpheide, S. A.; Kuentzel, S. L.; Martin, D. G. *J. Antibiot.* **1978**, *31*, 1211; (b) McGovern, J. P.; Clarke, G. L.; Pratte, E. A.; DeKoning, T. F. *J. Antibiot.* **1983**, *37*, 63; (c) Warpehoski, M. A.; Gebhard, J.; Kelly, R. C.; Krueger, W. C.; Li, L. H.; McGovern, J. P. *J. Med. Chem.* **1988**, *31*, 590.
5. (a) Ichimura, H.; Ogawa, T.; Takahashi, K.; Kobayashi, E.; Kawamoto, I.; Yasuzawa, T.; Takahashi, I.; Nakano, H. *J. Antibiot.* **1990**, *43*, 1037; (b) Yaszawa, T.; Saitoh, Y.; Ichimura, M.; Takahashi, I.; Sano, H. *J. Antibiot.* **1991**, *44*, 445; (c) Yasuzawa, T.; Muroi, K.; Ichimura, M.; Takahashi, I.; Ogawa, T.; Takahashi, K.; Sano, H.; Saitoh, Y. *Chem. Pharm. Bull.* **1995**, *43*, 378; (d) Ichimura, M.; Ogawa, T.; Takahashi, K.; Mihara, A.; Takahashi, I.; Nakano, H. *Oncol. Res.* **1993**, *5*, 165; (e) Gomi, K.; Kobayashi, E.; Miyoshi, K.; Ashizawa, T.; Okamoto, A.; Ogawa, T.; Katsumata, S.; Mihara, A.; Okabe, M.; Hirata, T. *Jpn. J. Cancer Res.* **1992**, *83*, 113.
6. Hurley, L. H.; Reynolds, V. S.; Swenson, D. H.; Petzold, G. L.; Scahill, T. A. *Science* **1984**, *226*, 843.
7. Boger, D. L.; Johnson, D. S.; Yun, W. *J. Am. Chem. Soc.* **1994**, *116*, 1635.
8. Reynolds, V. L.; Molineux, I. J.; Kaplan, D. J.; Swenson, D. H.; Hurley, L. H. *Biochemistry* **1985**, *24*, 6228.
9. (a) Tichenor, M. S.; Boger, D. L. *Nat. Prod. Rep.* **2008**, *25*, 220; (b) Ghosh, N.; Sheldrake, H. M.; Searcey, M.; Pors, K. *Curr. Top. Med. Chem.* **2009**, *9*, 1494.
10. (a) Hightower, R. D.; Sevin, B. U.; Pevras, J. P.; Untch, M.; Angioli, R.; Averette, H. *Gynecol. Oncol.* **1992**, *42*, 186; (b) Cote, S.; Momparler, R. L. *Anti-Cancer Drugs* **1993**, *4*, 327; (c) Fleming, G. F.; Ratain, S. M.; O'Brien, R. L.; Schilsky, P. C.; Ramos, R.; Richards, J. M.; Vogelzang, N. J.; Kasunic, D. A.; Earhart, R. H. *J. Natl. Cancer Inst.* **1994**, *86*, 368; (d) Foster, B. J.; Larusso, P. M.; Poplin, E.; Zalupski, M.; Valdivieso, M.; Wozniak, A.; Flaherty, L.; Kasunic, D. A.; Earhart, R. H.; Baker, L. H. *Invest. New Drugs* **1996**, *13*, 321; (e) Burris, H.; Earhart, R.; Kuhn, J.; Shaffer, D.; Smith, L.; Weiss, G.; Kusanic, D.; Radbury, G.; Campbell, L.; Von Hoff, D. *Proc. Am. Assoc. Cancer Res.* **1992**, *33*, 265; (f) Burris, H. A.; Dleras, V. C.; Tunca, M.; Earhart, R. H.; Eckardt, J. R.; Rodriguez, G. I.; Shaffer, D. S.; Fields, S. M.; Campbell, E.; Scaaf, L.; Kasunic, D.; Von Hoff, D. D. *Anti-Cancer Drugs* **1997**, *8*, 588; (g) Foster, B. J.; LoRusso, P. M.; Poplin, E.; Zalupski, M.; Valdivieso, M.; Wozniak, A.; Flaherty, L.; Kasunic, D. A.; Earhart, R. H.; Baker, L. H. *Invest. New Drugs* **1996**, *13*, 321.
11. (a) Li, L. H.; Dekoning, J. F.; Kelly, R. C.; Krueger, W. C.; McGovern, J. P.; Padbury, G. E.; Petzold, G. L.; Wallace, T. L.; Ouding, R. J.; Praire, M. D.; Gebhard, I. *Cancer Res.* **1992**, *52*, 4904; (b) van Telligen, O.; Punt, C. J. A.; Awada, A.; Wagener, D. J. T.; Piccart, M. J.; Groot, Y.; Scaaf, L. J.; Henrar, R. E. C.; Nooijen, W. J.; Beijnen, J. H. *Cancer Chemomther. Pharmacol.* **1998**, *41*, 377.
12. (a) Walker, D. L.; Reid, F. M.; Ames, M. M. *Cancer Chemother. Pharmacol.* **1994**, *34*, 317; (b) Volpe, D. A.; Tomaszewski, J. E.; Parchment, R. E.; Garg, A.; Flora, K. P.; Murphy, M. J.; Grieshaber, C. K. *Cancer. Chemother. Pharmacol.* **1996**, *39*, 143; (c) Pitot, H. C., IV; Erlichman, C.; Reid, J. M.; Sloan, J. A.; Ames, M. M.; Bagniewski, P. G.; Atherton-Skaff, P.; Adjei, A. A.; Rubin, J.; Rayson, D.; Goldberg, R. M. *Proc. Am. Assoc. Cancer Res.* **1999**, *40*, 91.
13. (a) Nagamura, S.; Kobayashi, E.; Gomi, K.; Saito, H. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2147. and references therein; (b) Alberts, S. R.; Erlichman, C.; Reid, J. M.; Ames, M. M.; Sloan, J. A.; Richardson, R. L. *Proc. Am. Assoc. Cancer Res.* **1996**, *37*, 393; (c) Abbruzzese, J. L.; Madden, T.; Newman, R. A. *Proc. Am. Assoc. Cancer Res.* **1996**, *37*, 165; (d) Niitani, H.; Horikoshi, N.; Hasegawa, K.; Fukuoka, M.; Kudoh, S.; Hino, M. *Proc. Am. Assoc. Cancer Res.* **1995**, *36*, 243; (e) Amishiro, N.; Nagamura, S.; Murakata, C.; Okamoto, A.; Kobayashi, E.; Asada, M.; Gomi, K.; Tamaoki, T.; Okabe, M.; Yamaguchi, N.; Yamaguchi, K.; Saito, H. *Bioorg. Med. Chem.* **2000**, *8*, 381; (f) Asai, A.; Nagamura, S.; Kobayashi, E.; Gomi, K.; Saito, H. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2995.
14. (a) Cristofanilli, M.; Bryan, W. J.; Miller, L. L.; Chang, A. Y.; Gradishar, W. J.; Kufe, D. W.; Hortobagyi, G. N. *Anticancer Drugs* **1998**, *9*, 779; (b) Markovic, S. N.; Suman, V. J.; Vukov, A. M.; Fitch, T. R.; Hillman, D. W.; Adjei, A. A.; Alberts, S. R.; Kaur, J. S.; Braich, T. A.; Leitch, J. M.; Creagan, E. T. *Am. J. Clin. Oncol.* **2002**, *25*, 308; (c) Small, E. J.; Figlin, R.; Petrylak, D.; Vaughn, D. J.; Sartor, O.; Horak, I.; Pincus, R.; Kremer, A.; Bowden, C. *Invest. New Drugs* **2000**, *18*, 193; (d) Alberts, S. R.; Suman, V. J.; Pitot, H. C.; Camoriano, J. K.; Rubin, J. *J. Gastrointest. Cancer* **2008**, *38*, 10.
15. Boger, D. L.; McKie, J. A.; Han, N.; Tarbie, C. M.; Riggs, H. W.; Kitos, P. A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 659. and references therin.
16. (a) Boger, D. L.; McKie, J. A. *J. Org. Chem.* **1995**, *60*, 1271; (b) Boger, D. L.; Yun, W.; Han, N. *Bioorg. Med. Chem.* **1995**, *3*, 1429; (c) Aristoff, P. A.; Johnson, P. D. *J. Org. Chem.* **1992**, *57*, 6234.
17. Baraldi, P. G.; Cacciari, B.; Pinedo de las Infantas, M. J.; Romagnoli, R.; Spatullo, G.; Cozzi, P.; Mongelli, N. *Anti-Cancer Drug Des.* **1997**, *12*, 67.
18. Mohamadi, F.; Spees, M. M.; Staten, G. S.; Marder, P.; Kipka, J. K.; Johnson, D. A. *J. Med. Chem.* **1994**, *37*, 232.
19. (a) Boger, D. L.; Munk, S. A.; Zarrinmajeh, H.; Ishizaki, T.; Haught, J.; Bina, M. *Tetrahedron* **1991**, *47*, 2661; (b) Boger, D. L.; Munk, S. A.; Kitos, P. A.; Suttorwart, O. *J. Am. Chem. Soc.* **1990**, *112*, 8961.
20. (a) Sato, A.; McNulty, L.; Cox, K.; Kim, S.; Scott, A.; Daniell, K.; Summerville, K.; Price, C.; Hudson, S.; Kiakos, K.; Hartley, J. A.; Asao, T.; Lee, M. *J. Med. Chem.* **2005**, *48*, 3903. and references therein; (b) Kiakos, K.; Sato, A.; Asao, T.; McHugh, P. J.; Lee, M.; Hartley, J. A. *Mol. Cancer Ther.* **2007**, *6*, 2708.

21. (a) Boger, D. L.; Boyce, C. W. *J. Org. Chem.* **2000**, *65*, 4088; (b) Elis, D. A.; Wolkenberg, S. E.; Boger, D. L. *J. Am. Chem. Soc.* **2001**, *123*, 9299.
22. Comparative trace metal analyses of human cancerous tissues have shown for significant differences which may be exploited from a drug design perspective; see: Mulay, I. L.; Roy, R.; Knox, B. E.; Suhr, N. H.; Delaney, W. E. *J. Natl. Cancer Inst.* **1971**, *47*, 1.
23. Yanow, S. K.; Purcell, L. A.; Pradel, G.; Sato, A.; Rodriguez, A.; Lee, M.; Spithill, T. W. *J. Infect Dis.* **2008**, *197*, 527.
24. (a) Baker, R. H.; Tinsley, S. W. *J. Am. Chem. Soc.* **1950**, *72*, 393; (b) Tinsley, S. W. *J. Am. Chem. Soc.* **1955**, *77*, 4175.
25. Carmichael, J.; Degraff, W. G.; Gadzar, A. F.; Mina, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936.
26. Howard, T. T.; Lingerfelt, B. M.; Purnell, B. L.; Scott, A. E.; Price, C. A.; Townes, H. M.; McNulty, L.; Handl, H. L.; Summerville, K.; Hudson, S. J.; Bowen, P. J.; Kiakos, K.; Hartley, J. A.; Lee, M. *Bioorg. Med. Chem.* **2002**, *10*, 2941.
27. (a) Boger, D. L.; Yun, W. *J. Am. Chem. Soc.* **1994**, *116*, 7996; (b) Atwell, G. J.; Wilson, W. R.; Denny, W. A. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1493.
28. Boyd, R. B. *Princ. Pract. Oncol.* **1989**, *3*, 1.
29. Li, L. H.; Kelley, R. C.; Warpehoski, M. A.; McGovern, J. P.; Gebhard, I.; Dekonig, T. F. *Invest. New Drugs* **1991**, *9*, 137.
30. Purcell, L. A.; Yanow, S. K.; Lee, M.; Spithill, T. W.; Rodriguez, A. *Infect. Immun.* **2008**, *76*, 1193.
31. (a) Gardner, M. J.; Hall, N.; Fung, E. *Nature* **2002**, *419*, 498; (b) Carlton, J. M.; Anguioli, S. V.; Suh, B. B.; Kooij, T. W.; Pertea, M.; Silva, J. C. *Nature* **2002**, *419*, 512; (c) Hall, N.; Karras, M.; Raine, J. D. *Science* **2005**, *307*, 82.
32. Peacock, C. S.; Seeger, K.; Harris, D.; Murphy, L.; Ruiz, J. C.; Quail, M. A.; Peters, N.; Adlem, E.; Tivey, A.; Aslett, M.; Kerhornou, A.; Ivens, A.; Fraser, A.; Rajandream, M. A.; Carver, T.; Norbertczak, H.; Chillingworth, T.; Hance, Z.; Jagels, K.; Moule, S.; Ormond, D.; Rutter, S.; Squares, R.; Whitehead, S.; Rabbinowitsch, E.; Arrowsmith, C.; White, B.; Thurston, S.; Bringaud, F.; Baldauf, S. L.; Faulconbridge, A.; Jeffares, D.; Depledge, D. P.; Oyola, S. O.; Hilley, J. D.; Brito, L. O.; Tosi, L. R.; Barrell, B.; Cruz, A. K.; Mottram, J. C.; Smith, D. F.; Berriman, M. *Nat. Genet.* **2007**, *39*, 839.
33. Simpson, L.; Neckelmann, N.; de la Cruz, V. F.; Simpson, A. M.; Feagin, J. E.; Jasmer, D. P.; Stuart, J. E. *J. Biol. Chem.* **1987**, *262*, 6182.
34. Jardim, A.; Hanson, S.; Ullman, B.; McCubbin, W. D.; Kay, C. M.; Olafson, R. W. *Biochem. J.* **1995**, *305*, 315.