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A Novel, Unusually Efficacious Duocarmycin Carbamate Prodrug That Releases No Residual Byproduct

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Abstract

A unique heterocyclic carbamate prodrug of *seco*-CBI-indole$_2$ that releases no residual byproduct is reported as a new member of a class of hydrolyzable prodrugs of the duocarmycin and CC-1065 family of natural products. The prodrug was designed to be activated by hydrolysis of a carbamate releasing the free drug without the cleavage release of a traceable extraneous group. Unlike prior carbamate prodrugs examined that are rapidly cleaved in vivo, the cyclic carbamate was found to be exceptionally stable to hydrolysis under both chemical and biological conditions providing a slow, sustained release of the exceptionally potent free drug. An in vivo evaluation of the prodrug found that its efficacy exceeded that of the parent drug, that its therapeutic window of efficacy versus toxicity is much larger than the parent drug, and that its slow free drug release permitted the safe and efficacious use of doses 150-fold higher than the parent compound.

Introduction

Duocarmycin SA (**1**)[1] and CC-1065 (**2**)[2] are two parent members of a class of highly potent naturally occurring antitumor agents that also include duocarmycin A[3] and yatakemycin[4] (Figure 1). This unique class of natural products derives its antitumor properties from their ability to alkylate DNA in a sequence selective manner.[5,6] Comprehensive studies of the natural products, their synthetic unnatural enantiomers,[7] and key analogues have defined many of the fundamental features that control the DNA alkylation selectivity, efficiency, and catalysis, resulting in a detailed understanding of the relationships between structure, reactivity, and biological activity.[6,7,8]

CBI (1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one) is one of the most extensively studied synthetic analogues of the family since we first introduced it in 1989.[9] The CBI alkylation subunit is not only more synthetically accessible and participates in the now characteristic DNA alkylation reaction effectively,[10] but it has also been found to be four times more stable and four times more potent than the naturally occurring alkylation subunit of **2**, approaching the stability and potency of the duocarmycin SA (**1**) alkylation subunit.

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Supporting Information Available: A gel figure examining the DNA alkylation properties of **6**, the synthesis and characterization of *N*-Boc-ACBI (**21**) and *N*-CO_2Me-ACBI (**24**), and reactivity (solvolysis) data for **21** and **24**.

Since analogues incorporating the CBI alkylation subunit have also been established to exhibit efficacious in vivo antitumor activity in animal models, it is an excellent synthetic replacement on which to examine the structure–function features of the natural products, including new prodrug design and evaluation.[11]

During the course of the total syntheses of the natural products and related analogues including CBI-indole$_2$ (**5**),[11c] it was established that the synthetic phenol precursors such as **4**, which have yet to undergo the Winstein Ar-3' spirocyclization, are equipotent to and indistinguishable from their cyclized cyclopropane containing counterparts within in vitro cytotoxic assays, DNA alkylation studies, and in vivo antitumor models. Due to this indistinguishable behavior both in vitro and in vivo and because their extraordinary potency creates special precautions for their handling, protection of the phenol precursors not only permits safe handling during their preparation, but it also provides an effective site on which to create prodrugs that can be designed for controlled release in vivo.[12] Such prodrugs incorporating phenol acylation have been developed to simultaneously improve solubility, pharmacokinetics, storage life, handling safety, and efficacy in vivo.[12,13,14] Two such carbamate-based drugs, a derivative of duocarmycin A[12c–d] ($t_{1/2}$ = 20 h, calf serum) and carzelesin (U-80,244, $t_{1/2}$ < 1 h, human plasma),[12a–b] which are rapidly cleaved in vivo (1–20 h), entered clinical trials but have ultimately not progressed. In related studies, we disclosed the carbamate prodrugs **3a–f** of (+)-CBI-indole$_2$ as shown in Figure 2, many of which were found to be essentially equipotent to (+)-CBI-indole$_2$ **(5)** in vitro.[12e] This work established that the free drug is rapidly released in a cellular assay and is able to spirocyclize, alkylate DNA, and express its biological activity efficiently in a manner essentially indistinguishable from the free drug itself. Herein, we describe a novel intramolecular heterocyclic carbamate (+)-CBI-indole$_2$ prodrug **(6**, Figure 3) that is subject to an analogous hydrolysis mechanism of activation,[15] but that is both substantially more stable and upon activation does not release any extraneous or traceable functionality into the surrounding cellular environment. Significantly, the resulting drug is accordingly less potent both in vitro and in vivo, but substantially safer to handle and more efficacious in vivo, effectively taming the extraordinary potency of this class of antitumor drugs.

Chemistry

Synthesis

Prodrug (+)-**6** was synthesized[16] in 11 steps from known intermediate **7**[17] as shown in Scheme 1. The phenol of **7** was protected as its benzyl ether and **8** was hydrolyzed using LiOH to provide the carboxylic acid **9** in good overall yield. Carboxylic acid **9** was subjected to a Curtius rearrangement using diphenylphosphoryl azide (DPPA) and Et_3N in freshly distilled *t*-BuOH providing the Boc protected aniline **10** in 79% yield. The use of non-distilled *t*-BuOH resulted in low yields due to competing hydrolytic release of the free aniline. Regioselective C1 iodination of **10** and subsequent N-alkylation of **11** with 1,3-dichloropropene proceeded effectively, providing the cyclization precursor **12**. Finally, a selective 5-*exo*-trig free radical cyclization[18] of **12** using sub stoichiometric quantities of Bu_3SnH (0.9 equiv) provided **13** in 83% yield with only trace amounts of further reduced (debrominated) material observed.

Compound **13**, which has served as a key precursor in the divergent synthesis[19] of a series of compounds,[20] was further elaborated to aniline **15** using triphenylsilylamine[21] as an ammonia surrogate for a Pd(0) catalyzed aryl amination[22] with LiHDMS in THF and ligand **14** (Scheme 2). Fortunately, a solution of LiHMDS could be used in place of solid LiHMDS, which alleviated the need for use of a glove box as reported.[22] Other amination reactions, including the use of benzophenone imine and copper-promoted couplings with acetamidine, yielded only trace amounts of the desired amination product. Bu_4NF deprotection of the

resulting amine and debenzylation of the phenol under hydrogenation conditions produced aniline **15**. Aniline **15** was converted to the cyclic carbamate **16** by a double acylation with triphosgene, which proceeded cleanly and in quantitative yield. At this point, compound **16** was resolved into its two enantiomers using chiral phase HPLC with 20% *i*-PrOH/hexanes as the eluent. We chose to resolve **16** instead of **6** itself in order to permit access to additional resolved analogues and to avoid the lower solubility of the full prodrug **6** in the chromatography solvents. Each enantiomer of **16** was subjected to Boc deprotection with 4 N HCl in EtOAc and immediate N-acylation with **17**, providing (+)-and *ent*-(−)-**6** in 52% yield.

The parent compound of **6** was prepared as shown in Scheme 3 through a four step sequence. The aniline of intermediate **15** was differentially protected as a Fmoc carbamate. Subsequent Boc deprotection and coupling with carboxylic acid **17** gave **19**, which was Fmoc deprotected and cyclized upon treatment with piperidine to provide the parent compound **20** as a racemic mixture.

Stability of the Cyclic Carbamate Prodrug

In order to determine the ability of the free drug to be released under physiological conditions, the chemical reactivity of *N*-Boc-prodrug **16** was assessed under a variety of acidic, basic, and nucleophilic conditions. The cyclic carbamate of **16** proved robust to hydrolysis under acidic conditions (1:1 TFA:CH_2Cl_2, 4 N HCl in EtOAc) and was stable over a period of 48 h at 23 °C, although the Boc protecting group was readily cleaved under such conditions. Similarly, compound **6** was also found to be stable to the above acidic conditions for up to 72 h at 23 °C. As shown in Figure 4, **16** was also stable to organic bases in aprotic solvents (entries 1–3), but the cyclic carbamate was slowly hydrolyzed in the presence of $NaHCO_3$ in protic solvents in a reaction that proceeded at a greater rate as the polarity of the solution increased (entries 3–6). Compound **16** was found to be completely stable in the presence of the nucleophiles BnSH and BnOH (100 equiv) in MeOH and THF at 23 °C for 48 h, and was stable to $BnNH_2$ in THF, but was rapidly cleaved with $BnNH_2$ (100 equiv) in MeOH in 24 h.

Finally, the stability of the full prodrug **6** was examined in pH 7.0 phosphate buffer ($t_{1/2}$ > 4 weeks, no cleavage observed) and in human plasma ($t_{1/2}$ > 48 h, 5% free drug release) indicating that the cyclic carbamate is remarkably stable under both conditions, but subject to slow release in human plasma. By contrast, the open chain carbamates explored in earlier studies, including those leading to carzelesin, were designed for much more rapid release (1–20 h). We also found that **6** is incapable of alkylating DNA in cell-free systems[23] (see Supporting Information), indicating that any in vitro cytotoxic activity or in vivo antitumor activity of **16** or **6** is due to release of the free drug and not the prodrug itself.

Biological Properties

In Vitro Cytotoxic Activity

Both (+)- and *ent*-(−)-**6** and their *N*-Boc precursors **16** were tested for cell growth inhibition in a cytotoxic assay with the L1210 murine leukemia cell line (Figure 5). The natural enantiomer of the prodrug (+)-**6** was found to be approximately 200-fold less potent (IC_{50} of 6.6 nM) than the free drug *seco*-CBI-indole$_2$ **4** (IC_{50} of 30 pM) and 6-fold more potent that its unnatural enantiomer. The racemic parent drug (±)-**20** was found to have an IC_{50} of 210 pM, suggesting that the active enantiomer is approximately 3–4 fold less active than **4**, and indicating that the prodrug (+)-**6** is 30–70 fold less potent than the parent drug **20**. Consistent with expectations, the full prodrug **6** proved to be 100 to 1000 times more potent than its *N*-Boc precursor **16**, which in turn is 50–100 fold less active than *N*-Boc-CBI

(natural enantiomer IC_{50} = 80 nM).[9] This data is consistent with the remarkable stability of the prodrug to chemical hydrolysis conditions, pH 7 phosphate buffer, and in human plasma, and its ineffective in vitro DNA alkylation reaction[21] (not shown), indicating that the release of free drug is similarly slow under the conditions of an in vitro cellular assay as well. Despite the lower potency relative to the free drug **4** and the racemic parent compound **20**, it is notable that the cyclic carbamate prodrug (+)-**6** now displays an in vitro cellular potency (IC_{50} = 1–10 nM) on par with most clinically used antitumor drugs.

In Vivo Antitumor Activity

Even though results of the in vitro cellular assay showed that (+)-**6** is substantially less potent than its parent drug, the slow release of the compound could prove to be advantageous in vivo due to the inherent potency and toxicity of the parent compound. Therefore, the in vivo antitumor activity of (+)-**6** was assessed alongside *seco*-CBI-indole$_2$ (**4**) in an antitumor model consisting of L1210 murine leukemia cells implanted ip into DBA/2J mice (Figure 6), which has been used traditionally as an initial antitumor model for comparisons in this class.[11,12,14,15] A dose range of 300 to 9000 μg/kg for prodrug (+)-**6** (scaled to its in vitro cytotoxic activity IC_{50}) and 60 to 500 μg/kg for *seco*-CBI-indole$_2$ (**4)** and a dosing schedule (administered three times ip on days 1, 5, and 9) for both compounds was employed. A subtle, but additional important empirical observation made in the studies is that the prodrug administration is tolerated at the injection sites of the animals much better than the free drug.

The optimal does range for **4** was previously established (60–100 μg/kg) and was extended for the study herein to highlight its narrow therapeutic window versus the potential behavior of prodrug (+)-**6**. As anticipated, (+)-CBI-indole$_2$ (**4**) proved toxic at doses of 100–500 μg/kg leading to premature death of the animals and productive antitumor activity was observed only at the dose of 60 μg/kg (T/C = 197), albeit producing only 1/10 long term (250 days) survivors in this extended study (Figure 6). By contrast, the prodrug (+)-**6** exhibited productive antitumor activity over the entire and much larger dose range examined (30-fold range). The most efficacious activity was observed at the highest dose of 9000 μg/kg, producing 5/10 long term cures (>250 days, T/C > 980) and indicating that even higher doses may be not only tolerable, but potentially even more efficacious. This highest dose represents one that is 150 times greater than the optimal dose observed with (+)-**4**, in line with the 100–200 fold differences in their cytotoxic potencies. In addition, the dose range of over which (+)-**6** exhibited productive activity was much larger, the in vivo antitumor activity was more efficacious (T/C > 980), and long term cures (5/10 > 250 day survivors) were observed even without an effort at dosing optimization.

Conclusions

A novel heterocyclic carbamate prodrug **6** of (+)-CBI-indole$_2$, which can be released via hydrolysis, was synthesized and evaluated for its in vitro cytotoxic activity and in vivo antitumor activity. Compared to its open chain counterparts explored in earlier studies, the cyclic carbamate prodrug was found to be remarkably stable to chemical hydrolysis conditions as well as in pH 7.0 phosphate buffer and human plasma. Accordingly, **6** was less potent in vitro and in vivo compared to the parent drug **4**, but was found to be substantially safer and more efficacious in vivo, being superior in extending life expectancy of tumor-bearing animals even at 150-fold higher doses. Notable elements of the cyclic carbamate prodrug behavior include not only its hydrolysis liberation of the free drug that releases no residual byproduct (CO_2), but also its remarkable stability relative to its acyclic counterparts explored in early studies. This results in an apparent slow, sustained release of free drug that

permits the safer and more efficacious use of larger doses of drug (as much as 150-fold), effectively taming the extraordinary potency of this class of antitumor drugs

Experimental Section

General

Reagents and solvents were purchased reagent-grade and used without further purification. Pooled human plasma, with sodium citrate as an anticoagulant, was purchased from Innovative Research and stored at −20 °C. THF was freshly distilled from sodium benzophenone ketyl. *t*-BuOH was freshly distilled from calcium hydride. All reactions were performed in oven-dried glassware under an Ar atmosphere. Evaporation and concentration in vacuo was performed at 20 °C. TLC was conducted using precoated SiO_2 60 F254 glass plates from EMD with visualization by UV light (254 or 366 nm). Chiral phase HPLC was performed using a Shimadzu HPLC on a semi-preparative Diacel ChiralCel OD column (0.46 cm × 25 cm) with a flow rate of 7 mL/min and with UV detection at λ = 254 nm. Optical rotations were determined on a Rudolf Research Analytical Autopol III Automatic Polarimeter (λ = 589 nm, 25 °C). NMR (^1H or ^{13}C) were recorded on Bruker DRX-500 and DRX-600 NMP spectrophotometers at 298K. Residual solvent peaks were used as an internal reference. Coupling constants (*J*) (H,H) are given in Hz. Coupling patterns are designated as singlet (s), doublet (d), triplet (t), quadruplet (q), multiplet (m), or broad singlet (br). IR spectra were recorded on a Thermo Scientific Nicolet 380 FT-IR spectrophotometer and measured neat. High resolution mass spectral data were acquired on an Agilent Technologies high resolution LC/MSD-TOF, and the detected masses are given as *m/z* with *m* representing the molecular ion. The purity of each tested compound (>95%) was determined on an Agilent 1100 LC/MS instrument using a ZORBAX SB-C18 column (3.5 mm, 4.6 mm × 50 mm, with a flow rate of 0.75 mL/min and detection at 220 and 254 nm) with a 10–98% acetonitrile/water/0.1% formic acid gradient.

Ethyl 5-Bromo-4-hydroxy-2-naphthoate (7)

A solution of potassium *tert*-butoxide (20.0 g, 0.78 mol) at 55 °C in *t*-BuOH (249 mL) was treated with a premixed solution of diethyl succinate (40.4 mL, 0.243 mol) and 3-bromobenzaldehyde (18.9 mL, 0.162 mol) dropwise. Upon completion of the addition, the reaction mixture was warmed to 85 °C and stirred for 2 h. After 2 h, the reaction mixutre was cooled to 25 °C. The reaction mixture was acidified to pH < 4 with 2 N aqueous HCl and concentrated. The aqueous suspension was then extracted with ethyl acetate (3×). The organic layers were combined and washed with saturated aqueous $NaHCO_3$ (5×). The basic aqueous washes were combined and reacidified with 2 N aqueous HCl to pH 1. Finally, the aqueous phase was extracted with ethyl acetate (3×). The organic layers were combined, dried over Na_2SO_4, and concentrated under reduced pressure, which afforded the desired half ester (39.1 g, 77%) as an orange oil. The half ester (39.1 g, 0.124 mol) was dissolved in acetic anhydride (178 mL) and NaOAc (18.7 g, 0.137 mol) was added. The reaction mixture was warmed to 140 °C and stirred for 6 h. Upon completion, the reaction mixture was cooled to 25 °C and poured into H_2O. The aqueous layer was extracted with ethyl acetate (3×). The organic layers were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was dissolved in anhydrous ethanol (620 mL). K_2CO_3 (104 g, 0.624 mol) was added, and the reaction mixture was warmed at 80 °C for 1 h. The reaction mixture was cooled and acidified to pH 1 with 2 N aqueous HCl. The ethanol was removed under reduced pressure and the aqueous suspension was extracted with ethyl acetate (3×). The organic extracts were combined, dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography (SiO_2, 16 × 30 cm, 0–15% EtOAc/hexanes gradient elution) provided **7** (5.4 g, 15% over 3 steps) as a yellow solid and its 7-bromo isomer (12.4 g, 34% over 3 steps). ^1H NMR ($CDCl_3$, 500 MHz) δ 8.16 (s, 1H), 8.07 (s, 1H), 7.89 (d, *J* = 6.5 Hz,

1H), 7.73 (d, J = 6.5 Hz, 1H), 7.63 (s, 1H), 7.29 (t, J = 10 Hz, 1H), 4.43 (q, J = 6.0 Hz, 2H) 1.44 (t, J = 6.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.9, 152.7, 136.3, 133.6, 130.6, 129.2, 126.7, 123.6, 122.7, 115.2, 112.3, 61.3, 14.3. IR (film) ν_{max} 3367, 2979, 1690, 1227 cm^{-1}. ESI-TOF HRMS *m/z* 294.9959 ($M+H^+$, $C_{13}H_{11}BrO_3$ requires 294.9964).

Ethyl 4-(Benzyloxy)-5-bromo-2-naphthoate (8)

Naphthol **7** (3.20 g, 11.0 mmol) was dissolved in anhydrous DMF (78 mL). K_2CO_3 (3.05 g, 22.0 mmol), benzyl bromide (1.59 mL, 13.2 mmol), and Bu_4NI (163 mg, 0.440 mmol) were added. The solution was stirred at 25 °C for 16 h. The reaction mixture was poured into H_2O and extracted with ethyl acetate (3×). The organic extracts were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The solid was recrystallized with 5% EtOAc/hexanes and the mother liquor was further purified by flash chromatography (SiO_2, 6 × 15 cm, 10–20% EtOAc/hexanes gradient elution) affording additional **8** (3.30 g combined, 77%) as a brown crystalline solid. ^{1}H NMR ($CDCl_3$ 500 MHz) δ 8.17 (s, 1H), 7.87 (d, J = 7.5 Hz, 1H), 7.85 (d, J = 8.0 Hz, 1H), 7.61 (d, J = 7.5 Hz, 2H), 7.57 (s, 1H), 7.40 (t, J = 7.5 Hz, 2H), 7.35–7.32 (m, 1H), 7.29 (t, J = 7.5 Hz, 1H), 5.30 (s, 2H), 4.43 (q, J = 7.0 Hz, 2H), 1.44 (t, J = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.0, 154.5, 136.1, 136.0, 135.0, 129.3, 128.3, 128.0 (2C), 127.8, 126.8, 125.8, 124.1, 116.7, 106.9, 71.2, 61,1. IR (film) ν_{max} 2980, 1712, 1413, 1236 cm^{-1}. ESI-TOF HRMS *m/z* 385.0433 ($M+H^+$, $C_{20}H_{17}BrO_3$ requires 385.0434).

4-(Benzyloxy)-5-bromo-2-naphthoic Acid (9)

Ester **8** (2.29 g, 5.94 mmol) was dissolved in a 3:1:1 mixture of THF:CH_3OH:H_2O (0.1 M). $LiOH•H_2O$ was added and the reaction mixture was stirred at 25 °C for 24 h. Upon completion, the reaction mixture was acidified to pH 1 with the addition of 10% aqueous HCl. A precipitate formed during the acidification and it was collected by vacuum filtration. The remaining aqueous layer was then extracted with ethyl acetate (3×). The organic extracts were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The filtered and extracted products were combined to give **9** (2.09 g, 100%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.23 (s, 1H), 8.10 (d, J = 6.0 Hz, 1H), 7.91 (d, J = 6.5 Hz, 1H), 7.61 (d, J = 7.0 Hz, 2H), 7.55 (s, 1H), 7.43–7.39 (m, 3H), 7.33 (t, J = 7.0 Hz, 1H), 5.35 (s, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 166.7, 153.8, 136.2, 135.9, 135.0, 129.8, 128.8, 128.2, 127.8, 127.7, 127.5, 124.7, 123.8, 115.5, 107.0, 70.4. IR (film) ν_{max} 3368, 2969, 1680 cm^{-1}. ESI-TOF HRMS *m/z* 357.0125 ($M+H^+$, $C_{18}H_{13}BrO_3$ requires 357.0121).

tert-Butyl-(4-(benzyloxy)-5-bromonaphthalen-2-yl)carbamate (10)

Carboxylic acid **9** (950 mg, 2.66 mmol) was dissolved in freshly distilled *t*-BuOH (0.01 M) over 4Å molecular sieves. Et_3N (467 μL, 3.35 mmol) and diphenylphosphoryl azide (602 μL, 2.79 mmol) were added. The reaction mixture was warmed to 85 °C under Ar and stirred for 14 h. Upon completion, the mixture was filtered through cotton to remove the molecular sieves and concentrated under reduced pressure. The residue was diluted with 10% aqueous HCl and extracted with EtOAc (3×). The organic extracts were combined and washed with H_2O (2×) and saturated aqueous NaCl. The organic phase was dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography (SiO_2, 5 × 12 cm, 5% EtOAc/hexanes elution) provided **10** (1.02 g, 89%) as a tan solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.62 (m, 2H), 7.58 (d, J = 7.8 Hz, 2H), 7.49 (s, 1H), 7.39 (t, J = 7.2 Hz, 2H), 7.33 (t, J = 6.0 Hz, 1H), 7.16 (t, J = 7.8 Hz, 1H), 7.03 (s, 1H), 6.58 (s, 1H), 5.22 (s, 2H), 1.54 (s, 9H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 155.2, 152.5, 137.6, 136.4, 136.3, 131.3, 130.0, 128.4, 127.9, 127.3, 126.9, 120.4, 120.2, 120.1, 116.6, 107.8, 101.8, 71.4, 28.3. IR (film) ν_{max}

3325, 2977, 1702, 1156 cm^{-1}. ESI-TOF HRMS *m/z* 428.0856 ($M+H^+$, $C_{22}H_{22}BrNO_3$ requires 428.0856).

tert-Butyl-(4-(benzyloxy)-5-bromo-1-iodonaphthalen-2-yl)carbamate (11)

Carbamate **10** (1.20 g, 2.80 mmol) was dissolved in freshly distilled THF (0.17 M) under Ar and in the absence of light, and $TsOH•H_2O$ (53 mg, 0.28 mmol) and *N*-iodosuccinamide (753 mg, 3.30 mmol) were added. The reaction mixture was allowed to stir at 25 °C for 2 h. After 2 h, the reaction was quenched with the addition saturated aqueous $NaHCO_3$ and diluted with ethyl acetate. The organic layer was washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography (SiO_2, 5 × 16 cm, 5% EtOAc/hexanes elution) provided **11** (1.47 g, 94%) as an orange solid. ^1H NMR ($CDCl_3$, 500 MHz) δ 8.16 (s, 1H), 8.09 (d, *J* = 8.0 Hz, 1H), 7.70 (d, *J* = 7.5 Hz, 1H), 7.62 (d, *J* = 7.0 Hz, 2H), 7.39 (t, *J* = 7.2 Hz, 2H), 7.34 (d, *J* = 7.0 Hz, 1H), 7.32 (s, 1H), 7.25–7.22 (m, 2H), 5.28 (s, 2H), 1.58 (s, 9H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 155.8, 152.4, 139.0, 136.7, 135.9, 132.2, 131.9, 130.0, 128.5, 128.3, 128.0, 127.9, 121.4, 120.2, 120.1, 117.0, 101.9, 81.3, 71.3, 28.3. IR (film) ν_{max} 3378, 2978, 1730, 1225, 1145 cm^{-1}. ESI-TOF HRMS *m/z* 553.9820 ($M+H^+$, $C_{22}H_{21}BrINO_3$ requires 553.9822).

tert-Butyl-(4-(benzyloxy)-5-bromo-1-iodonaphthalen-2-yl)-(3-chloroallyl)carbamate (12)

Compound **11** (1.65 g, 2.99 mmol) and Bu_4NI (55 mg, 0.15 mmol) were dissolved in anhydrous DMF (0.16 M) and the solution was cooled to 0 °C. Once cooled, 60% NaH in mineral oil (239 mg, 5.98 mmol) was added and the reaction mixture was allowed to stir at 0 °C for 30 min. 1,3-Dichloropropene (0.84 mL, 8.97 mmol) was added dropwise and the solution was warmed to room temperature. After 1 h, the reaction mixture was quenched with the addition of saturated aqueous NH_4Cl and diluted with ethyl acetate. The organic layer was washed with H_2O, saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography (SiO_2, 5 × 8 cm, 10% EtOAc/hexanes elution) provided an *E*/*Z* mixture of alkene **12** (1.818 g, 96%) as a yellow foam. ^1H NMR (acetone-d_6, 600 MHz) δ 8.35 (m, 2H), 7.92 (d, *J* = 7.2 Hz, 2H), 7.61 (br, 4H) 7.45 (t, *J* = 8.4 Hz, 2H), 7.42–7.40 (m, 4H), 7.35–7.33 (m, 2H), 7.18 (d, *J* = 18.0 Hz, 2H), 6.21–6.08 (m, 3H), 5.39 (s, 4H), 4.60 (dd, *J* = 18.9, 5.4 Hz, 1H), 4.42 (dd, *J* = 15.0, 7.2 Hz, 1H), 4.27 (dd, *J* = 15.6, 6.6 Hz, 1H), 3.99 (dd, *J* = 14.1, 6.6 Hz, 1H), 1.55 (br, 4H), 1.28 (br, 14H). ^{13}C NMR (acetone-d_6, 150 MHz) δ 157.28, 157.27, 154.8, 154.6, 145.9, 145.8, 139.2, 139.1, 138.2, 138.1, 136.13, 136.12, 135.4 (2C), 130.9, 130.2, 130.1, 129.9, 129.86, 129.81, 129.7, 129.4, 126.2, 125.4, 123.2, 122.2, 118.2, 112.4, 112.3, 98.0, 97.2, 82.2, 81.8, 72.8, 72.6, 50.6, 47.3, 29.3. IR (film) ν_{max} 2974, 2928, 1697, 1156, 749 cm^{-1}. ESI-TOF HRMS *m/z* 627.9750 ($M+H^+$, $C_{25}H_{24}BrClINO_3$ requires 627.9746).

tert-Butyl 1,2-Dihydro-5-(benzyloxy)-6-bromo-1-(chloromethyl)-1*H*-benzo[*e*]indole-3(2*H*)-carboxylate (13)

Alkene **12** (1.81 g, 2.89 mmol) and AIBN (140 mg, 0.86 mmol) were dissolved in benzene (0.05 M). Freshly prepared Bu_3SnH (701 μL, 2.60 mmol) was added and the system was purged of oxygen using Ar and the freeze/pump/thaw method. The reaction mixture was warmed to 80 °C for 12 h. Upon completion, the reaction mixture was concentrated under reduced pressure and purified by flash chromatography (10% w/w KF fused SiO_2, 5 × 16 cm, 0–10% EtOAc/hexanes gradient elution) to provide **13** (1.32 g, 90%) as a white solid. ^1H NMR (acetone-d_6, 600 MHz) δ 7.98 (br, 1H), 7.81 (d, *J* = 8.4 Hz, 1H), 7.65–7.63 (m, 3H), 7.41 (t, *J* = 7.2 Hz, 2H), 7.35–7.30 (m, 2H), 5.31 (s, 2H), 4.21–4.16 (m, 2H), 4.12–4.09 (m, 1H), 3.96 (dd, *J* = 11.1, 3.0 Hz, 1H), 3.71 (dd, *J* = 8.4, 11.4 Hz, 1H), 1.58 (s, 9H). ^{13}C NMR (acetone-d_6, 150 MHz) δ 157.8, 153.8, 144.3, 138.4, 135.0, 132.6, 130.1 129.7, 129.4, 124.9, 124.3, 121.6, 119.4, 117.2, 100.7, 82.4, 72.8, 54.4, 48.6, 43.1, 29.5. IR

(film) ν_{max} 2926, 1692, 1330, 1135, 752 cm^{-1}. ESI-TOF HRMS *m/z* 502.0772 ($M+H^+$, $C_{25}H_{25}BrClNO_3$ requires 502.0779).

tert-Butyl 1,2-Dihydro-6-amino-1-(chloromethyl)-5-hydroxy-1*H*-benzo[*e*]indole-3(2*H*)-carboxylate (15)

An oven-dried microwave vial was charged with $Pd_2(dba)_3$ (10.9 mg, 11 μmol), 2-dicyclohexylphosphinobiphenyl (**14**, 8.3 mg, 0.023 mmol), and $(C_6H_5)_3SiNH_2$ (72.1 mg, 0.261 mmol). The vial was evacuated and filled with Ar. Compound **13** (120 mg, 0.238 mmol) was added and the vial was evacuated again. Toluene (2.3 mL) was added and the vessel was purged with Ar. Finally, LiHMDS (0.29 mL, 1 M in THF) was added and the vessel was sealed. The reaction was submerged in a 100 °C oil bath for 24 h. After 24 h, the reaction mixture was cooled to room temperature, diluted with diethyl ether, filtered through a plug of Celite, and concentrated. The residue was dissolved in THF (15 mL) and cooled to 0 °C. Bu_4NF (0.36 mL, 1 M in THF) was added dropwise. The reaction mixture was allowed to stir for 30 min before being quenched with the addition of saturated aqueous NH_4Cl and diluted with ethyl acetate. The organic layer was washed with saturated aqueous NaCl, dried over Na_sSO_4, and concentrated. The residue was purified by flash chromatography (SiO_2, 4 × 8 cm, 5–10% EtOAc/hexanes gradient elution). The product was carried on to the next reaction mixture without characterization due to co-elution of triphenyl byproduct. The amine (104 mg theoretical, 0.238 mmol) was dissolved in anhydrous CH_3OH (6 mL) under Ar. 10% Pd/C (29 mg, 0.024 mmol) was added and the atmosphere was exchanged with H_2. The reaction mixture was allowed to stir at 25 °C for 5 h. The reaction mixture was diluted with diethyl ether, filtered through Celite, and concentrated under reduced pressure. Flash chromatography (SiO_2, 3 × 8 cm, 50–70% Et_2O/hexanes gradient elution) provided **15** (56 mg, 67% over 3 steps) as a tan solid. ^1H NMR (acetone-d_6, 600 MHz) δ 7.48 (br, 1H), 7.12 (t, *J* = 7.8 Hz, 1H), 6.84 (d, *J* = 7.8 Hz, 1H), 6.44 (d, *J* = 6.6 Hz, 1H), 4.13–4.05 (m, 2H), 3.92–3.87 (m, 2H), 3.55 (t, *J* = 10.8 Hz, 1H), 1.54 (s, 9H). ^{13}C NMR (acetone-d_6, 150 MHz) δ 158.6, 153.7, 148.8, 134.8, 130.0, 126.8, 115.3, 112.5, 111.4, 108.7, 99.6, 81.7, 54.1, 48.3, 43.4, 29.3. IR (film) ν_{max} 3391, 2974, 1706, 1583, 1406, 1142 cm^{-1}. ESI-TOF HRMS *m/z* 349.1323 ($M+H^+$, $C_{18}H_{21}ClN_2O_3$ requires 349.1313).

tert-Butyl 10-(Chloromethyl)-5-oxo-9,10-dihydro-4*H*-pyrrolo[3',2':5,6]naphtho[1,8-*de*][1,3]oxazine-8(5*H*)-carboxylate (16)

Naphthol **15** (56 mg, 0.160 mmol) and triphosgene (47 mg, 0.160 mmol) were dissolved in toluene (3.2 mL) at 25 °C. The reaction mixture was stirred for 1 h before being diluted with H_2O and ethyl acetate. The organic layer was washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography (SiO_2, 2 × 6 cm, 20–50% EtOAc/hexanes gradient elution) provided **16** (60 mg, 100%) as a yellow solid. ^1H NMR (acetone-d_6, 600 MHz) δ 9.86 (s, 1H), 7.66 (br, 1H), 7.37 (t, *J* = 8.4 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 6.66 (d, *J* = 7.8 Hz, 1H), 4.19–4.18 (m, 2H), 4.07–4.05 (m, 1H), 3.98 (dd, *J* = 11.1, 3.6 Hz, 1H), 3.77 (dd, *J* = 8.2, 11.4 Hz, 1H), 1.58 (s, 9H). ^{13}C NMR (acetone-d_6, 150 MHz) δ 178.5, 153.7, 152.8, 148.37, 148.31, 146.1, 137.0, 136.9, 131.8, 131.2, 118.8, 116.8, 110.0, 105.5, 100.3, 82.7, 54.5, 48.5, 42.5, 29.4. IR (film) ν_{max} 2924, 1701, 1606, 1405, 1332, 1140 cm^{-1}. ESI-TOF HRMS *m/z* 375.1105 ($M+H^+$, $C_{19}H_{19}ClN_2O_4$ requires 375.1106).

The enantiomers were resolved on a semi-preparative Diacel chiralcel OD column (0.46 cm × 25 cm) with 20% *i*-PrOH/hexanes elution; α = 1.38.

(1*S*)-**16**: $[\alpha]^{23}_D$ −31 (*c* 0.75, THF), natural enantiomer. (1*R*)-**16**: $[\alpha]^{23}_D$ +32 (*c* 0.80, THF), unnatural enantiomer.

N-(2-(10-(Chloromethyl)-5-oxo-5,8,9,10-tetrahydro-4*H*-pyrrolo[3',2':5,6]naphtho[1,8-*de*][1,3]oxazine-8-carbonyl)-1*H*-indol-5-yl)-1*H*-indole-2-carboxamide (6)

Compound **16** (7.5 mg, 0.020 mmol) was dissolved in 4 N HCl in EtOAc (0.5 mL) and the mixture was allowed to stir at room temperature for 25 min. The solvent was removed under a stream of nitrogen and the residue was redissolved in anhydrous DMF (0.4 mL). EDCI (11.4 mg, 0.06 mmol) and **17** (7.0 mg, 0.022 mmol) were added and the reaction mixture was allowed to stir at 25 °C for 24 h. The reaction mixture was quenched with the addition of H_2O and diluted with ethyl acetate. The organic phase was washed with 2 N aqueous HCl (3×), saturated aqueous $NaHCO_3$ (5×), and saturated aqueous NaCl. The organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by PTLC (SiO_2, 40% THF/toluene) to provide **6** (6.08 mg, 52%, typically 52–60%) as a tan solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 11.85 (s, 1H), 11.75 (s, 1H), 11.14 (br, 1H), 10.20 (s, 1H), 8.25 (s, 1H), 7.91 (s, 1H), 7.67 (d, J = 8.4 Hz, 1H), 7.59 (dd, J = 9.0, 1.8 Hz, 1H), 7.48 (t, J = 9.0 Hz, 2H), 7.43 (m, 4H), 7.27 (s, 1H), 7.21 (t, J = 7.8 Hz, 1H), 7.07 (t, J = 7.8 Hz, 1H), 6.66 (dd, J = 5.7, 3.0 Hz, 1H), 4.87 (t, J = 10.2 Hz, 1H), 4.61 (dd, J = 10.8, 2.4 Hz, 1H), 4.03–4.02 (m, 1H), 4.00–3.98 (m, 2H). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 160.2, 159.4, 149.8, 146.5, 143.4, 136.6, 134.8, 133.3, 131.8, 131.7, 130.7, 129.5, 129.1, 127.9, 127.03, 127.00, 126.9, 123.4, 121.5, 119.5, 119.4, 118.7, 115.3, 112.8, 112.29, 112.21, 108.7, 106.1, 104.4, 103.3, 99.8, 54.7, 47.2, 40.8. IR (film) ν_{max} 3255, 1731, 1603, 1514, 1400, 1232, 794, 733 cm^{-1}. ESI-TOF HRMS *m/z* 576.1431 ($M+H^+$, $C_{32}H_{22}ClN_5O_4$ requires 576.1433).

(1*S*)-**6**: $[\alpha]^{23}_D$ +18.4 (*c* 0.21, THF), natural enantiomer. (1*R*)-**6**: $[\alpha]^{23}_D$ −18.5 (*c* 0.24, THF), unnatural enantiomer.

N-(2-(5-Amino-4-oxo-1,2,9,9a-tetrahydrocyclopropa[*c*]benzo[*e*]indole-2-carbonyl)-1*H*-indol-5-yl)-1*H*-indole-2-carboxamide (20)

Intermediate **15** (10 mg, 0.028 mmol) was suspended in H_2O (0.4 mL) and cooled to 0 °C. Fmoc-Cl (9.6 mg, 0.037 mmol) in dioxane (0.2 mL) was added and the reaction mixture was allowed to slowly warm to room temperature over 17 h. The reaction mixture was diluted with H_2O and extracted with EtOAc (2×). The organic layers were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was dissolved in 4 N HCl in EtOAc (0.8 mL) and the mixture was allowed to stir at room temperature for 25 min. The solvent was removed under a stream of nitrogen and the residue was redissolved in anhydrous DMF (0.8 mL). EDCI (10.7 mg, 0.056 mmol) and **17** (10.7 mg, 0.34 mmol) were added and the reaction mixture was allowed to stir at 25 °C for 24 h. The reaction mixture was quenched with the addition of H_2O and diluted with EtOAc. The organic phase was washed with 2 N aqueous HCl (3×), saturated aqueous $NaHCO_3$ (5×), and saturated aqueous NaCl. The organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was dissolved in DMF (0.8 mL) and piperidine (160 μL) was added. The reaction mixture was allowed to stir at room temperature for 1 h after which the solvent was removed under reduced pressure. The residue was purified by PTLC (SiO_2, 60% THF/toluene) to provide **20** (4.1 mg, 29% over 4 steps) as a yellow solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 11.81 (s, 1H), 11.72 (s, 1H), 10.17 (s, 1H), 8.21 (s, 1H), 7.67 (d, J = 7.8 Hz, 1H), 7.60 (d, J = 9.0 Hz, 1H), 7.47 (d, J = 9.0 Hz, 2H), 7.42 (s, 1H), 7.25 (s, 1H), 7.21 (t, J = 7.8 Hz, 1H), 7.17 (t, J = 8.4 Hz, 1H) 7.07 (t, J = 7.8 Hz, 1H), 6.81 (s, 1H), 6.58 (d, J = 8.4 Hz, 1H), 6.20 (d, J = 7.2 Hz, 1H), 4.60–4.57 (m, 1H), 4.45 (d, J = 10.2 Hz, 1H), 3.07 (m, 1H), 1.61 (t, J = 4.8 Hz, 1H), 1.51–1.49 (m, 1H). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 188.8, 161.1, 159.2, 158.4, 150.5, 142.1, 136.4, 133.4, 132.4, 131.5, 131.4, 129.7, 126.7, 126.6, 123.2, 121.2, 119.6, 119.5, 113.6, 112.9, 112.6, 112.0, 110.5, 107.7, 106.8, 103.1, 63.1, 53.6, 32.4, 29.8, 24.1. ESI-TOF HRMS *m/z* 514.1872 ($M+H^+$, $C_{31}H_{23}N_5O_3$ requires 514.1874).

In Vivo Antitumor Activity

B6D2F1 mice were injected intraperitoneal (i.p.) with syngeneic L1210 cells (1×10^6) on day 0. Ten mice were randomly assigned to control vehicle or treatment groups for compounds (+)-**4** and (+)-**6** at doses of 60, 100, 250, and 500 g/kg/inj for (+)-**4** or 300, 1000, 3000, and 9000 μg/kg/inj for (+)-**6**. Compounds (+)-**4** and (+)-**6** were formulated in 100% DMSO, and injected i.p. on study days 1, 5, and 9. Following injection of tumor cells, animals were monitored daily and weighed two times per week. Percent survival (T/C) for treated and control groups were determined by dividing the total survival days for each treatment group by the total survival days for the control group and multiplying × 100. All animal studies were carried out in the animal facilities of The University of Kansas Medical Center with strict adherence to the guidelines of the IACUC Animal Welfare Committee of KUMC (IACUC approval # 2009–1837).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CBI	1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one
DPPA	diphenylphosphoryl azide
LiHMDS	lithium bis(trimethylsilyl)amide
THF	tetrahydrofuran
BnSH	benzyl thiol
BnOH	benzyl alcohol
$BnNH_2$	benzylamine
EtOAc	ethyl acetate
DMF	dimethylformamide
MeOH	methanol
Fmoc-Cl	fluorenylmethyloxycarbonyl chloride

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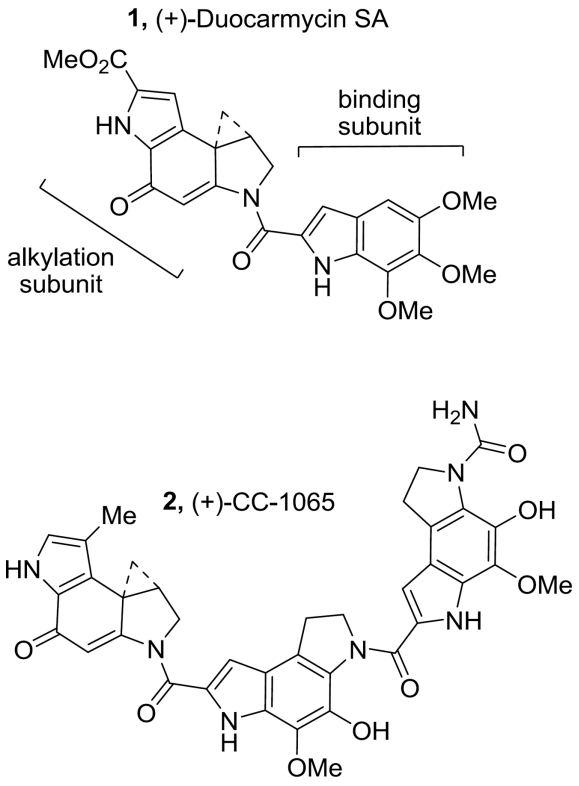


Figure 1.
Natural Products.

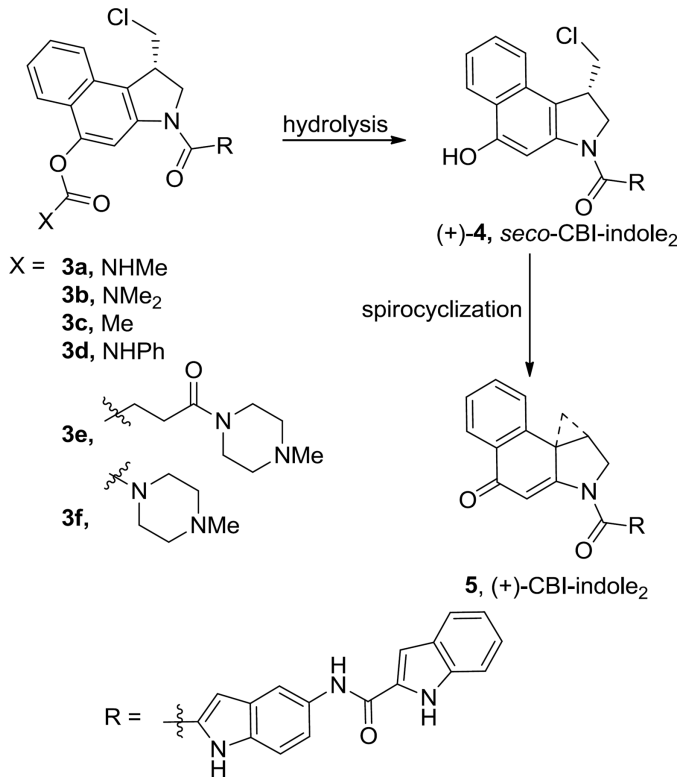
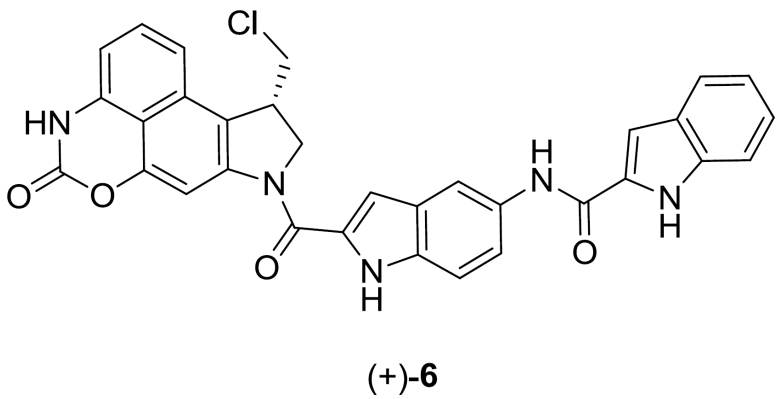


Figure 2.
Carbamate prodrug design.



(+)-**6**

Figure 3.
Cyclic carbamate prodrug **6**.

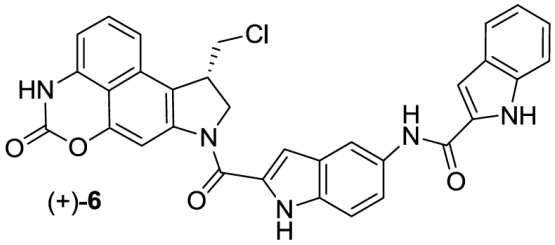
Entry	Solvent	Base[a]	2 h[b]	24 h[b]	48 h[b]
1	CH_2Cl_2	Et_3N	stable	stable	stable
2	CH_2Cl_2	DMAP	stable	stable	12%
3	THF	$NaHCO_3$	stable	stable	7%
4	THF:H_2O (1:1)	$NaHCO_3$	<4%	4%	9%
5	DMF:H_2O (1:1)	$NaHCO_3$	7%	12%	19%
6	MeOH	$NaHCO_3$	18%	74%	100%

[a]excess base (>100 equiv) used; [b]percent of **16** hydrolyzed as determined by LCMS analysis at 254 nm absorption; all reactions were run at 23 °C

Figure 4.
N-Boc prodrug **16** stability under basic conditions.

Compd	IC_{50} L1210 natural (nM)	unnatural (nM)
1, duocarmycin SA	0.010	0.100
2, CC-1065	0.020	0.020
4, CBI-indole$_2$	0.030	0.900
16	4900	5800
6	6.6	40
(±)**-20**	0.210	

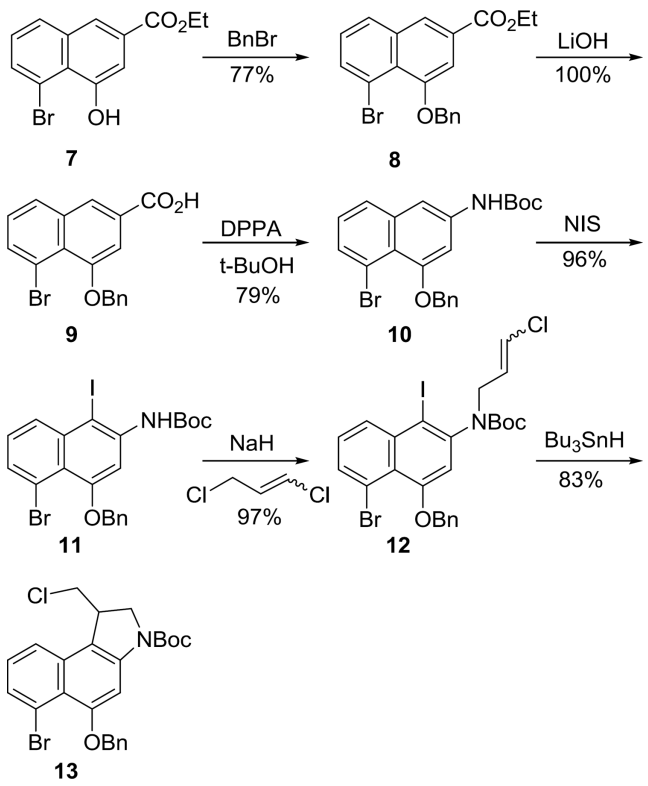
Figure 5.
In vitro cytotoxic activity.



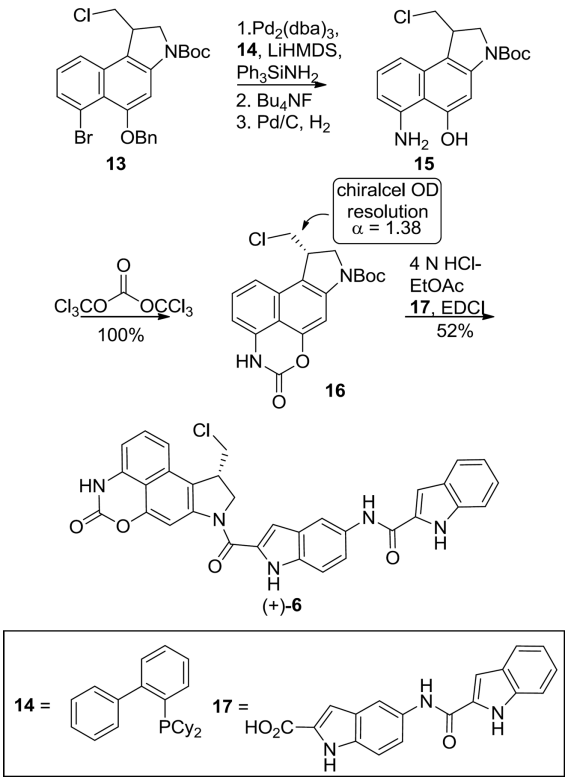
Compd	Dose μg/kg[a]	MSP days[b]	T/C[c]	Surviving Mice[d]
none	0	17.6	100	0/10
4	60	>34.7	>197	1/10
4	100	6.4	36	0/10
4	250	3.7	21	0/10
4	500	3.0	17	0/10
6	300	24.7	140	0/10
6	1000	>48.5	>275	1/10
6	3000	>55.3	>310	1/10
6	9000	>172.6	>980	5/10

[a]Dose (μg/kg wt. of animal) administered i.p. on days 1, 5, and 9. [b]MSP = Mean Survival Period (days). [c]T/C = Treated/Control (MSP) x 100. [d]No. of live animals after 250 days (terminated).

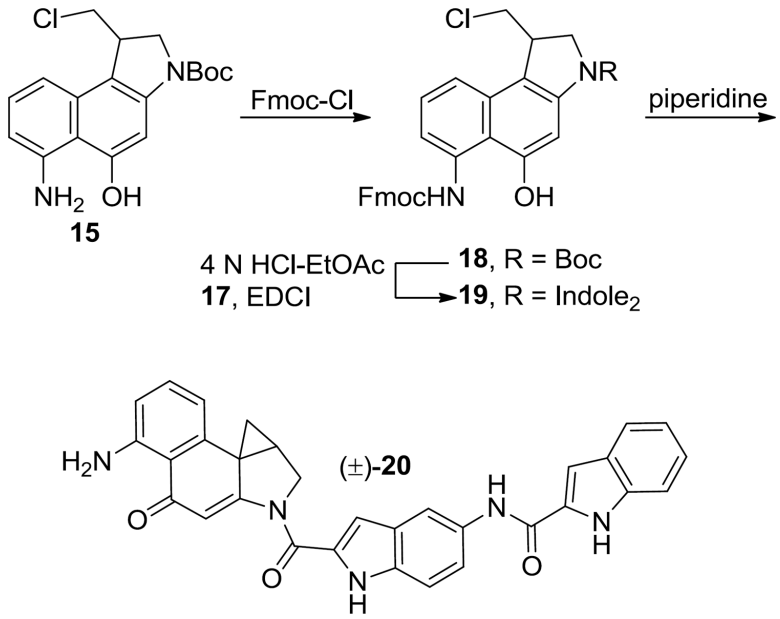
Figure 6.
In vivo antitumor activity (L1210, ip).



Scheme 1.



Scheme 2.



Scheme 3.