Design, Synthesis, and Evaluation of Duocarmycin *O*-Amino Phenol Prodrugs Subject to Tunable Reductive Activation

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A series of *N*-acyl *O*-amino derivatives of *seco*-CBI-indole$_2$ are reported and examined as prototypical members of a unique class of reductively activated (cleaved) prodrugs of the duocarmycin and CC-1065 family of antitumor agents. These prodrugs were designed to be potentially preferentially activated in hypoxic tumor environments which carry an intrinsically higher concentration of "reducing" nucleophiles (e.g., thiols) capable of activating such derivatives by nucleophilic cleavage of a weak N—O bond. A remarkable range of stabilities and a resulting direct correlation with in vitro/in vivo biological potencies was observed for these prodrugs, even enlisting subtle variations in the electronic and steric environment around the weak N—O bond. An in vivo evaluation of several of the prodrugs demonstrates that some approach the potency and exceed the efficacy of the free drug itself (CBI-indole$_2$), suggesting the prodrugs may offer an additional advantage related to a controlled or targeted release.

Introduction

CC-1065 (**1**)[1a] and duocarmycin SA (**2**)[1b] represent the parent members of a class of antitumor agents that derive their biological activity from their ability to selectively alkylate duplex DNA (Figure 1).[1–3] The study of the natural products, their synthetic unnatural enantiomers,[4] and key analogues has defined many of the fundamental features that control the alkylation selectivity, efficiency, and catalysis,[5] ultimately providing a detailed understanding of the relationship between structure, reactivity, and biological activity.[3–5]

Among the most widely studied duocarmycin analogue is CBI[6] (1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one[a]), Figure 2. This simplified alkylation subunit is not only synthetically more accessible but it exhibits an enhanced chemical stability (4×) and biological potency (4×) relative to the alkylation subunit found in **1**, approaching the stability and potency of duocarmycin SA (**2**) and yatakemycin derivatives. Derivatives bearing this modified alkylation subunit have been shown to exhibit efficacious antitumor activity in animal models at doses that reflect this potency.[7] Thus, it is on this scaffold that new design concepts are often evaluated.

A unique feature of this class of molecules is the observation that the phenol synthetic precursors possess indistinguishable biological properties (DNA alkylation efficiency and selectivity, in vitro cytotoxic activity, in vivo antitumor activity) in comparison to the cyclopropane derivatives themselves. These phenol precursors undergo in situ Ar-3′ spirocyclization with the displacement of an appropriate leaving group to afford the cyclopropane found in the natural products (Figure 2). This reliable behavior of the precursor phenols has provided the basis on which the development of prodrugs has been conducted.[8] Thus, protection of the phenol, which prevents spirocyclization to the cyclopropane, provides precursors that are inactive, yet readily yield the active products upon deprotection. Development of this protection and release strategy has been pursued, where phenol release in vivo is coupled with features that may allow for selective tumor activation or delivery.[9] Despite the attractiveness of such an approach, only a surprisingly small number of such studies have been disclosed.[10–12]

We recently reported an initial study examining the potential for *N*-acyl *O*-amino derivatives of such phenols to serve as duocarmycin prodrugs subject to reductive activation, with the expectation being that these compounds might be capable of preferential activation in hypoxic tumors.[12] At this initial stage of the studies, it was not known whether such derivatives possessed the required reactivity for effective chemical cleavage and release of the free drug or the necessary stability for their synthesis and subsequent storage. Consequently, it is remarkable that the initial studies not only established that such derivatives possessed the requisite combination of chemical stability and cleavage reactivity, but that one of the initially explored derivatives (**5**) demonstrated effective in vivo release of the free drug, leading to more efficacious antitumor activity than the free drug itself and with a potency that approached that of the free drug.[12] Alternative approaches to incorporate reductive activation into this class of molecules have been disclosed but target an intrinsic enzyme activity that differentiates normal versus tumor cells.[11] However, in many cases (e.g., mitomycin) on which the design principles are based,

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[a] Abbreviations: BnSH, benzyl thiol; CBI, 1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one; LiHMDS, lithium bis(trimethylsilyl)amide; THF, tetrahydrofuran; TFA, trifluoroacetic acid; EtOAc, ethyl acetate; DMAP, 4-dimethylaminopyridine; DMF, dimethylformamide; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene.
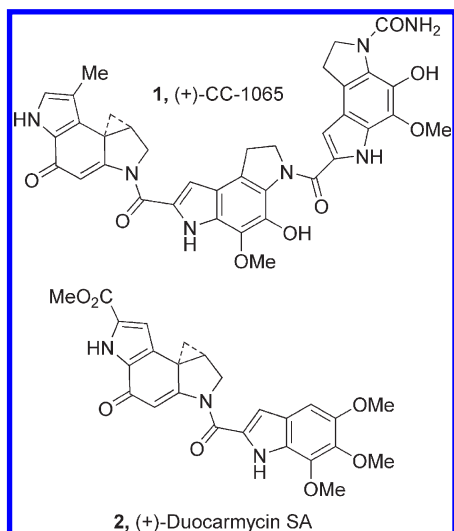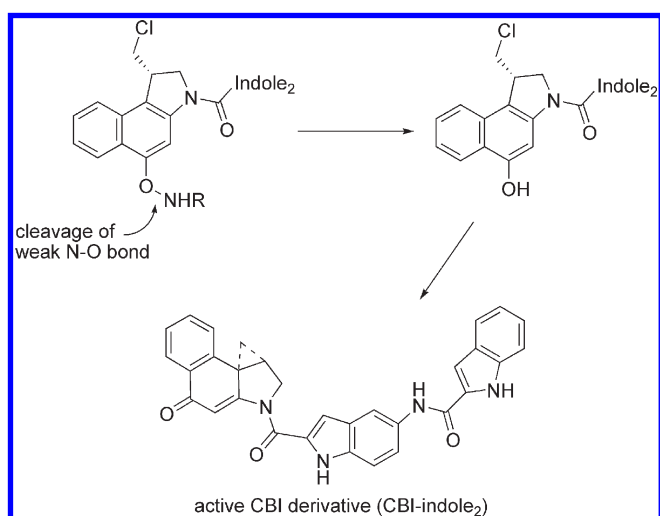
Figure 1. Natural products.

Figure 2. Prodrug design.

it may not be the enzymatic reduction itself but rather the reoxidation of the drug in normal cells (vs hypoxic tumor environments) that protects them from damage. Systems utilized for reductive activation have been reviewed,[13] and such agents include mitomycin C and other aziridoquinones, nitro-aromatics, *N*-oxides, various metal complexes, azides, and di- and trisulfides. Those most relevant to the design disclosed herein are a class of natural products (FR900482 and its related congeners)[14] as well as a dehydromonocrotaline progenitor[15] incorporating hydroxylamine hemiketals that are irreversibly activated by reductive cleavage of a N−O bond. The approach outlined herein is not designed for reversible or enzymatic reductive activation but rather for irreversible cleavage of a weak N−O bond by reducing nucleophiles (Figure 2).[12] The key question was whether one can take advantage of the fact that hypoxic tumor environments contain a higher concentration of reducing nucleophiles (i.e., glutathione) over normal cells capable of activating such agents.[16] Further contributing to the properties of the prodrugs and their sites of release, it has been reported that intracellular glutathione (thiol) concentrations are 100-fold higher than in the extracellular matrix, suggesting preferential intracellular versus plasma release of such drugs.[16,17]
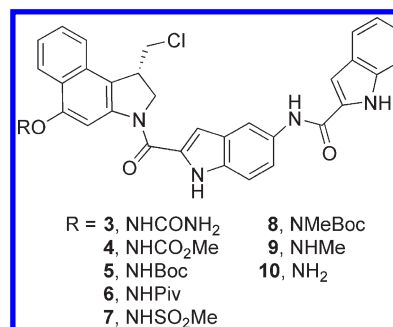

Figure 3. N−O prodrugs.

Herein, we report an examination of the impact of the electronic and steric features of the functionality on the nitrogen atom of the *O*-aminophenols that can be utilized to tune the reactivity of the N−O bond. Thus, a range of functional groups that affect both the electronic (amines vs ureas vs carbamates vs amides vs sulfonamides) and steric (*t*-butyl vs methyl carbamate) environment around nitrogen were examined. In addition, the introduction of steric bulk directly on the nitrogen atom was explored (*N*-methylation). It has been shown that the addition of alkyl groups adjacent to the bond undergoing in vivo cleavage can increase stability, presumably by interfering with the approach of incoming nucleophiles.[18]

To access the desired analogues (Figure 3), a direct S_N2 phenol displacement of an *O*-tosylated hydroxamide using the fully elaborated *seco*-CBI-indole$_2$ (**11**) was examined and utilized to provide the desired prodrugs in a single step.[12] In turn, the requisite hydroxamides were prepared in 2−4 steps from commercially available materials.

Chemistry

Synthesis. The starting *seco*-CBI-indole$_2$ (**11**) was synthesized by a 10-step sequence in 28% overall yield.[19] Deprotonation of **11** with LiHMDS in ether:1,4-dioxane (1:1) at 0 °C, followed by the addition of TsONHBoc[20] and maintaining the reaction mixture at room temperature (0.1 M) for 4 h resulted in consistent yields of 35−42% for compound **5** (Scheme 1). Remarkably, use of the lithium phenolate generated at 0 °C and the judicious choice of a nonpolar, aprotic solvent[12] prevents the competitive facile spirocyclization from occurring. Moreover, the *O*-amination reaction can be effectively conducted on the fully elaborated *seco*-CBI-indole$_2$ without competing sites of reaction, permitting a late stage introduction of the prodrug variations. In the case of **5**, separation of the desired product from unreacted *seco*-CBI-indole$_2$ (**11**) was not possible by silica gel chromatography. Therefore, deliberate spirocyclization of the remaining starting material **11** in the reaction product mixture with 1:1 saturated aqueous NaHCO$_3$:THF afforded the much more polar spirocyclized CBI-indole$_2$ (Figure 2), which was readily separated from **5** by conventional chromatography.

Applying these conditions to the more electron-rich urea substrate (TsONHCONH$_2$) provided only trace amounts of product **3**. After extensive optimization, small amounts of urea **3** were isolated by increasing the scale and concentration of the reaction to 0.2 M. Similarly, methyl carbamate **4** was formed in trace amounts using the original reaction conditions (TsONHCO$_2$Me). In this case, raising the reaction temperature to 50 °C and increasing the concentration to 0.2 M provided carbamate **4**. The *N*-trimethylacetyl prodrug **6** was formed in much higher conversions (TsONHCO*t*Bu), but the

Scheme 1
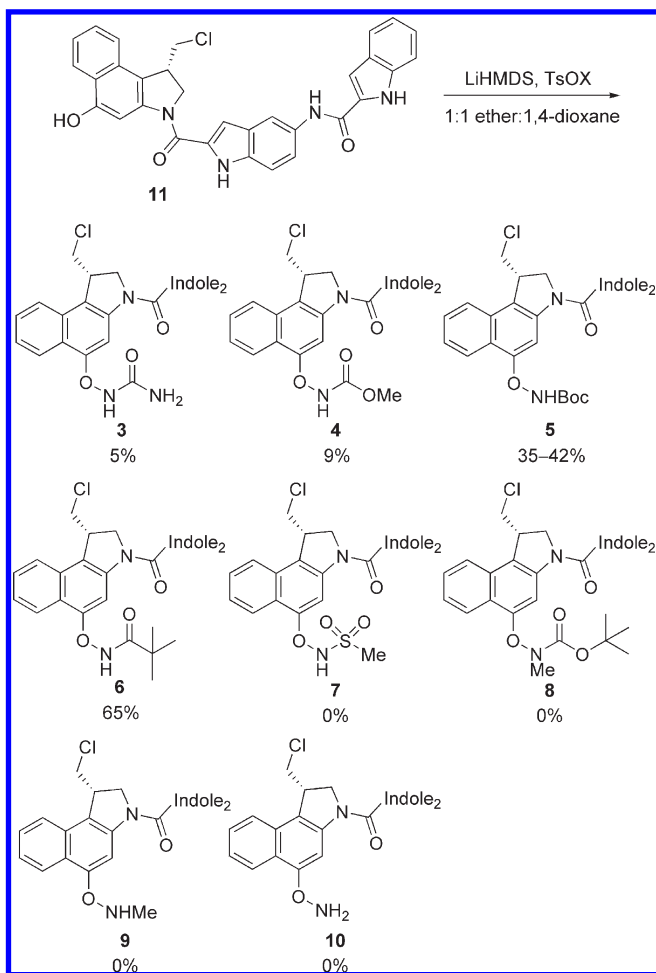
relative instability of its N−O bond prevented the use of chromatography for purification. However, precipitation of the product from THF using distilled hexanes afforded the desired prodrug **6** in 65% isolated yield. Although unrecognized at the time the synthesis was conducted, the yield of the *O*-amidation reactions were found to be inversely correlated with the relative stability of the products and directly correlated with the relative reactivity of the cleavable N−O bond.

As anticipated from the stability of **6**, initial efforts on the synthesis of the more electron-withdrawing methanesulfonyl prodrug **7** (Scheme 1) proved challenging. Not surprisingly, recovered phenol was observed in all of the attempts to prepare **7** (TsONHSO$_2$Me) and likely reflects not its lack of formation but rather its instability to isolation or even its detection (TLC). Since ongoing efforts conducted alongside efforts to generate **7** suggested it would possess a reactivity too high to be useful for our purposes, the synthesis of **7** was not pursued in depth. Similarly, the synthesis of *N*-Me-*t*-butyl carbamate **8** by direct N−O bond formation with TsONMeBoc failed to afford the desired product, presumably due to the steric hindrance on nitrogen. However, selective *N*-methylation of **5** with dimethyl sulfate and LiHMDS afforded the methylated product **8**. Efforts to remove the *t*-butyl carbamate from **8** to provide **9** were unsuccessful, leading only to complex reaction mixtures. Direct *O*-amination of *seco*-CBI-indole$_2$ (**11**) was also explored with several different aminating reagents but failed to provide **10**. Overall, the exploratory work led to the syntheses of the urea **3**,
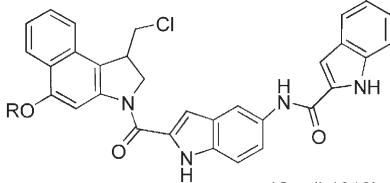
methyl carbamate **4**, *t*-butyl carbamate **5**, pivaloyl amide **6**, and *N*-Me-*t*-butyl carbamate **8** containing prodrugs that proved sufficient to span the range of useful reactivity.

Stability and Reactivity of the N−O Prodrugs and Release of Free Drug. The stability of the prodrugs and their ability to release the free drug **11** were examined under a wide variety of acidic, basic, and nucleophilic conditions. As expected, based on electronic properties, the relative stability followed the order of **3** and **8** > **4** > **5** > **6**, with the urea **3** and carbamate **8** proving stable to all conditions except strong acid, whereas **6** was cleaved under almost all conditions examined (Supporting Information Table S1). Although the trends in reactivity can be easily rationalized based on the electronic effect of the *N*-acyl group (**6** > **5** > **4** > **3**) or the steric effect of further *N*-methylation (**5** > **8**), the magnitude of the differences in reactivity represented, in our opinion, a remarkable range given the subtle changes in the structures. Derivatives **3**, **4**, **5**, and **8** proved robust to acidic conditions (1:1 TFA: CH$_2$Cl$_2$, 4 N HCl in EtOAc) and stable to mild base in nonpolar, aprotic solvents (Et$_3$N or DMAP, CH$_2$Cl$_2$) but showed diminished stability to base releasing **11** as the solvent polarity increased. All but **6** were stable to NaHCO$_3$ in THF and 1:1 THF:H$_2$O, but **5** underwent cleavage in NaHCO$_3$/DMF:H$_2$O (1:1), and both **4** and **5** were cleaved in DBU/MeCN to release **11**. Similarly, all the prodrugs proved stable in MeOH, but cleavage of **4**, **5**, and **6** to provide **11** was observed upon treatment with NaHCO$_3$ in MeOH (2 h, 23 °C). Most relevant to the potential source of cleavage under physiological conditions (i.e., thiols), **6** was rapidly cleaved by BnSH under all conditions examined, whereas **3**, **4**, **5**, and **8** were stable to treatment with BnSH in THF (72 h, 23 °C) or MeOH (72 h, 23 °C) and stable to treatment with BnSH in THF even in the presence of insoluble NaHCO$_3$ (2 h, 23 °C). However, all were cleaved to release **11** and provide CBI-indole$_2$ upon treatment with BnSH (100 equiv) in MeOH in the presence of NaHCO$_3$ (100 equiv), albeit at relative rates that reflect the N−O bond reactivity (**6** > **5** > **4** > **3**, **8**). The half-life of the prodrugs under these thiol cleavage conditions, releasing CBI-indole$_2$, were estimated to be: **6** (< 0.5 h), **5** (6 h), **4** (10 h), **8** (48 h), and **3** (35 h). The stability of these compounds was also assessed in pH 7.0 phosphate buffer, and no significant cleavage was observed for derivatives **3**, **4**, **5**, and **8** (72 h), whereas **6** was completely cleaved to release CBI-indole$_2$ with a $t_{1/2}$ of ca. 6 h. Finally, **5** was observed to exhibit a robust half-life in human plasma ($t_{1/2}$ = 3−6 h) with clean release of **11** and its subsequent spirocyclization to CBI-indole$_2$.[12]

Additionally, we have shown that prodrug **5** is incapable of alkylating DNA in cell-free systems, indicating that any activity observed in the following cell-based assay or in vivo model requires cleavage of the prodrug N−O bond with release of the free drug **11**.[12]

Biological Properties

In Vitro Cytotoxic Activity. Both enantiomers of prodrugs **3**−**6** and **8** were tested for cell growth inhibition in a cytotoxic assay with L1210 cells (Figure 4). Importantly, all the prodrugs exhibited potent cytotoxic activity (>100-fold more active than the phenol methyl ether of **11**),[12] and the natural (1*S*)-enantiomers were on average 30-fold more potent than the unnatural (1*R*)-enantiomers, analogous to the enantiomer pairs of **11**, indicating the release of the free drug under the assay conditions. The relative order of activity for the compounds correlated with their observed reactivity for release
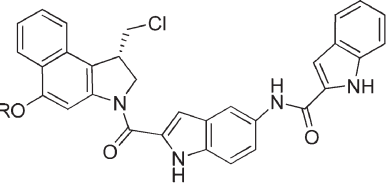
Figure 4. Summary data for N−O prodrugs.

R, Prodrug	IC$_{50}$ (L1210)	
	natural (nM)	unnatural (nM)
1 (CC-1065)	0.020	0.020
2 (duocarmycin SA)	0.010	0.100
H, **11**	0.030	0.90
NHPiv, **6**	0.080	3.5
NHBoc, **5**	0.10	4.0
NHCO$_2$Me, **4**	0.50	9.0
NMeBoc, **8**	0.90	30
NHCONH$_2$, **3**	1.1	30

of **11** under the conditions described above. The unstable pivoloyl amide prodrug **6** was the most potent derivative (IC$_{50}$ = 80 pM), exhibiting a slightly greater potency than *t*-butyl carbamate **5** (IC$_{50}$ = 100 pM) and approaching the potency of the free drug **11** itself (IC$_{50}$ = 30 pM). Urea **3** (IC$_{50}$ = 1.1 nM) was ten times less potent than **5**, which is a testament to its stability due to the less electron-withdrawing nature of the urea functionality. Further, **5** was found to be five times more potent than the methyl carbamate **4** (IC$_{50}$ = 500 pM), indicating that the steric size of the carbamate distal from the N−O bond may subtly decrease its stability (see above), increase its rate of in situ free drug release, and increase its cytotoxic activity. The addition of the *N*-methyl group in **8** increased the stability of the compound considerably (above), and a resulting 9-fold decrease in cytotoxic potency was observed relative to **5**. On the basis of these results, the productive N−O bond cleavage and free drug release proved much more sensitive to the subtle differences in the electron-withdrawing properties of the *N*-acyl group than initially expected. It is notable that the enantiomeric pairs of each prodrug exhibited the same distinguishable differences in cytotoxic potency as **11** (ca. 30-fold), consistent with, but not proving, simple in vitro chemical versus enzymatic reductive cleavage of the N−O bond. Most significant in these assessments was the fact that the biological activity (potency) of the prodrugs in this cellular functional assay correlates remarkably well with their corresponding reactivity for free drug release.

In Vivo Antitumor Activity. From the stability studies and the results of in vitro cytotoxic assays, a clear depiction of the relationship between relative N−O cleavage ability and functional activity in a cell-based assay was defined. However, it was not clear how these features might translate to in vivo use of the prodrugs. Clearly, they must be sufficiently reactive to effectively release the free drug, yet sufficiently stable to permit its controlled and potentially more selective release. Because the pivoloyl amide prodrug **6** was found to be unstable in pH 7.0 phosphate buffer ($t_{1/2}$ = 6 h), it was not examined alongside **3**−**5**. The key question being addressed was whether the more reactive or more stable N−O prodrugs in the remaining series (**3**−**5**) performed best in an in vivo tumor model. Consequently, the in vivo antitumor activity of

Compd	Dose μg/kg[a]	MSP days[b]	T/C[c]	Surviving Mice[d]
none	0	22	100	0/10
11	10	42	192	1/10
11	30	51	230	2/10
11	60	78	352	5/10
11	100	73	333	4/10
3	50	29	133	0/10
3	100	33	152	0/10
3	250	39	177	1/10
3	500	71	323	4/10
4	10	24	110	0/10
4	30	29	133	0/10
4	60	28	126	0/10
4	100	27	122	0/10
4	200	32	145	0/10
5	10	50	225	2/10
5	30	58	265	2/10
5	60	69	315	4/10
5	100	86	389	6/10

[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 120 days (terminated).

Figure 5. In vivo antitumor activity (L1210, ip).

3−**5** was assessed alongside the parent drug **11** in a widely employed antitumor model consisting of L1210 murine leukemia cells implanted ip into DBA/2J mice (Figure 5). A dose range (10−100 μg/kg) and a dosing schedule (administered three times ip on days 1, 5, and 9) found suitable for related parent drugs including CBI-indole$_2$ was used and extended to 2-fold and 5-fold higher doses for **4** and **3**, respectively.[7] Although the free drug **11** exhibited a narrow range of dose dependent efficacy, exhibiting its maximal effect at 60 μg/kg and less effective efficacy at 30 μg/kg (too little drug) or 100 μg/kg (too much drug), **5** exhibited increasing efficacy up to and including the 100 μg/kg dose and it matched or exceeded the efficacy of the free drug **11** at each dose. Herein, higher doses of **5** were not examined in this comparison, but the results of two independent examinations of **5** in which the studies were conducted for as long as 365 days[12] complement the observations made herein and more clearly define the enhanced efficacy of **5** over **11**. In all cases, the dose at which a maximal response was achieved proved similar for **5** and the free drug **11**, but with **5** showing improved efficacy. Prodrugs **3** and **4** were also active, albeit only at higher doses. In both instances, the initial examination was conducted likely without reaching their optimal dosing. However, importantly, the studies reveal that each of the prodrugs release the free drug **11** in the in vivo model (reductive activation), that the relative potency in vivo parallels the relative cleavage ability of the prodrug N−O bond and the relative in vitro cytotoxic activity that even the subtle structural changes examined herein likely span the useful range of relative reactivities and that either the rate of release or the site of release can lead to enhancements in the efficacy of the drug. The *t*-butyl carbamate prodrug **5** exhibited the most robust antitumor activity, providing 6/10 long-term survivors at the highest dose examined (100 μg/kg). Especially notable is that the in vivo study was conducted for

120 days with meaningful numbers of long-term survivors (untreated controls average life span = 22 days), suggesting cures with **5** even without a dosing optimization. Finally, it is worth highlighting that the compounds are extraordinarily potent, requiring less than 1 mg of sample to conduct the in vivo antitumor testing and indicating that clinical amounts of such agents could easily be supplied by chemical synthesis.

Conclusions

A unique series of *N*-acyl *O*-aminophenol prodrugs of CBI-indole$_2$ were explored. These prodrugs, subject to reductive activation through nucleophilic cleavage of a weak N—O bond, were found to exhibit predictable and tunable N—O cleavage properties subject to both electronic and steric control of their relative reactivity and to exhibit a remarkable range of reactivity given the subtle nature of the structural changes examined. All were found to effectively release the free drug both in functional cellular assays and in vivo antitumor models, and the most effective of the derivatives examined (**5**) exhibited potent and efficacious antitumor activity approaching the potency of the free drug **11** and surpassing its in vivo efficacy. Further characterization of **5** is in progress and will be reported in due course. Remarkable in these studies is that the small structural changes in moving from the urea to carbamate to amide-based prodrug proved sufficient to result in large differences in both the stability and in vitro/in vivo potency of these agents. Coupled with the addition of steric bulk on or adjacent to the N—O bond, a useful range of stabilities and biological potencies was observed for these duocarmycin prodrugs.

Experimental Section

General. Reagents and solvents were purchased reagent-grade and used without further purification. THF was freshly distilled from sodium benzophenone ketyl. All reactions were performed in oven-dried glassware under an argon atmosphere. Evaporation and concentration in vacuo was performed at 20 °C and atmospheric pressure. Further drying of the new compounds was carried out at ca. 5 mbar. TLC: precoated SiO$_2$ 60 F$_{254}$ glass plates from EMD, visualization by UV light (254 or 366 nm). Analytical reverse-phase HPLC was performed using a Waters HPLC on a Waters Nova-Pac HR C18 6 μm, 60 Å column (25 mm × 100 mm) using a gradient of MeCN/water with 0.07% TFA at flow rate of 5 mL/min and with UV detection at λ = 254 and 280 nm. NMR (^{1}H or ^{13}C): Varian Inova-400, Bruker DRX-500, and Bruker DRX-600 NMR spectrophotometers at 298 K. 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), quintuplet (qt), multiplet (m), or broad signal (br). IR spectra: Thermo Scientific Nicolet 380 FT-IR spectrophotometer 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 compounds was determined as >95% as determined by HPLC analysis.

***N*-(2-(1-(Chloromethyl)-5-(ureidooxy)-2,3-dihydro-1*H*-benzo-[*e*]indole-3-carbonyl)-1*H*-indol-5-yl)-1*H*-indole-2-carboxamide (Urea Prodrug 3).** A solution of *seco*-CBI-indole$_2$ (**11**, 73.0 mg, 0.137 mmol) in 1:1 ether:dioxane (0.8 mL) at 0 °C was treated with LiHMDS (0.410 mL, 0.410 mmol, 1.0 M in THF). The solution was stirred for 30 min, after which **18** (94.2 mg, 0.410 mmol) was added and the solution was warmed to room temperature. The reaction was stirred for 4 h, diluted with ethyl acetate, washed with H$_2$O and saturated aqueous NaCl, and dried (MgSO$_4$). The solution was concentrated under reduced pressure, and the result-ing residue was purified by PTLC (1:1 THF:toluene, eluted 2×)

to afford **3** as a pale-yellow solid (4.1 mg, 5.1%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 11.92 (s, 1H), 11.73 (s, 1H), 10.20 (s, 1H), 8.26 (s, 1H), 7.93 (d, J = 7.8 Hz, 1H), 7.90 (d, J = 7.8 Hz, 1H), 7.68 (d, J = 7.8 Hz, 1H), 7.66 (t, J = 7.2 Hz, 1H), 7.63 (d, J = 9.0 Hz, 1H), 7.50 (t, J = 8.4 Hz, 1H), 7.50 (d, J = 11 Hz, 1H), 7.48 (d, J = 11 Hz, 1H), 7.47 (s, 1H), 7.43 (s, 1H), 7.25 (s, 1H), 7.22 (t, J = 7.8 Hz, 1H), 7.07 (t, J = 7.2 Hz, 1H), 6.93 (s, 1H), 5.49 (s, 2H), 4.77 (dd, J = 10, 4.8 Hz, 1H), 4.48 (d, J = 10 Hz, 1H), 4.23 (m, 1H), 3.80 (dd, J = 13, 3.6 Hz, 1H), 3.52 (dd, J = 14, 3.6 Hz, 1H). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 162.1, 159.5, 158.3, 157.5, 138.7, 136.6, 133.8, 132.0, 131.9, 131.7, 131.0, 129.9, 128.0, 127.6, 127.03, 126.96, 126.1, 125.5, 123.5, 121.6, 120.2, 119.8, 113.0, 112.4, 112.3, 111.5, 107.5, 106.9, 103.4, 66.5, 60.9, 43.4. IR (film) ν_{max} 3307, 2958, 2889, 1647, 1521 cm^{-1}. ESI-TOF HRMS *m*/*z* 593.1681 (M + H$^+$, C$_{32}$H$_{25}$ClN$_6$O$_4$ requires 593.1698).

1*S*-**3**: [α]$_D^{23}$ +11 (*c* 0.20, THF), natural enantiomer.
1*R*-**3**: [α]$_D^{23}$ −10 (*c* 0.11, THF), unnatural enantiomer.

Methyl(3-(5-(1*H*-indole-2-carboxamido)-1*H*-indole-2-carbonyl)-1-(chloromethyl)-2,3-dihydro-1*H*-benzo[*e*]indol-5-yl)oxycarbamate (Methylcarbamate Prodrug 4). A solution of *seco*-CBI-indole$_2$ (**11**, 76.1 mg, 0.142 mmol) in 1:1 ether:dioxane (2.5 mL) at 0 °C was treated with LiHMDS (0.427 mL, 0.427 mmol, 1.0 M in THF). The solution was stirred for 30 min, after which **17** (105 mg, 0.427 mmol) was added and the solution was heated at 50 °C for 1 h. The reaction mixture was diluted with ethyl acetate, washed with H$_2$O and saturated aqueous NaCl, and dried (MgSO$_4$). The solution was concentrated under reduced pressure, and the result-ing residue was purified by PTLC (1:1 THF:toluene, eluted 2×) to afford **4** as a pale-yellow solid (8.0 mg, 9.2%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 11.92 (s, 1H), 11.73 (s, 1H), 10.20 (s, 1H), 8.42 (s, 1H), 8.27 (s, 1H), 7.92 (d, J = 7.2 Hz, 1H), 7.694 (d, J = 8.4 Hz, 1H), 7.689 (t, J = 9.0 Hz, 1H), 7.62 (d, J = 9.0 Hz, 1H), 7.52 (t, J = 7.2 Hz, 1H), 7.50 (d, J = 7.2 Hz, 1H), 7.48 (d, J = 10 Hz, 1H), 7.43 (s, 1H), 7.38 (s, 1H), 7.31 (s, 1H), 7.22 (t, J = 7.2 Hz, 1H), 7.07 (t, J = 6.6 Hz, 1H), 4.81 (dd, J = 10, 4.8 Hz, 1H), 4.47 (d, J = 10 Hz, 1H), 3.71 (m, 1H), 3.43 (dd, J = 11, 3.0 Hz, 1H), 3.37 (s, 3H), 3.33 (d, J = 10 Hz, 1H). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 161.9, 159.5, 155.8, 154.2, 137.4, 136.6, 133.8, 132.4, 132.2, 131.9, 131.7, 129.9, 129.6, 128.5, 128.3, 127.0, 126.9, 126.7, 125.1, 123.5, 121.6, 120.2, 119.8, 113.1, 112.34, 112.28, 107.2, 106.9, 103.4, 66.5, 60.3, 51.2, 42.9. IR (film) ν_{max} 3271, 2923, 2853, 1713, 1646, 1597, 1517 cm^{-1}. ESI-TOF HRMS *m*/*z* 608.1699 (M + H$^+$, C$_{33}$H$_{26}$ClN$_5$O$_5$ requires 608.1695).

1*S*-**4**: [α]$_D^{23}$ −33 (*c* 0.44, THF), natural enantiomer.
1*R*-**4**: [α]$_D^{23}$ +32 (*c* 0.31, THF), unnatural enantiomer.

***tert*-Butyl(3-(5-(1*H*-indole-2-carboxamido)-1*H*-indole-2-carbo-nyl)-1-(chloromethyl)-2,3-dihydro-1*H*-benzo[*e*]indol-5-yl)oxy-carbamate (*tert*-Butylcarbamate Prodrug 5).** A solution of *seco*-CBI-indole$_2$ (**11**, 106 mg, 0.191 mmol) in 1:1 ether:dioxane (6 mL) at 0 °C was treated with LiHMDS (0.594 mL, 0.594 mmol, 1.0 M in THF). The solution was stirred for 30 min, after which **13** (171 mg, 0.594 mmol) was added and the solution was warmed to room temperature and stirred for 4 h. The reaction mixture was diluted with ethyl acetate, washed with H$_2$O and saturated aqueous NaCl, and dried (MgSO$_4$). The solution was concentrated under reduced pressure and purified by PTLC (1:1 THF:toluene, eluted 2×) to afford **5** as a pale-yellow solid (52.1 mg, 42%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.80 (s, 1H), 11.73 (s, 1H), 10.84 (br s, 1H), 10.20 (s, 1H), 8.28 (s, 1H), 8.26 (s, 1H), 8.06 (d, J = 8.5 Hz, 1H), 7.91 (d, J = 8.5 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.63 (t, J = 7.5 Hz, 1H), 7.60 (dd, J = 9.0, 2.0 Hz, 1H), 7.54 (t, J = 7.5 Hz, 1H), 7.50 (d, J = 8.5 Hz, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.44 (s, 1H), 7.29 (s, 1H), 7.22 (t, J = 7.5 Hz, 1H), 7.07 (t, J = 7.5 Hz, 1H), 4.92 (t, J = 9.5 Hz, 1H), 4.67 (dd, J = 11, 2.0 Hz, 1H), 4.44 (m, 1H), 4.11 (dd, J = 11, 3.0 Hz, 1H), 4.04 (dd, J = 11, 6.5 Hz, 1H), 1.27 (s, 9H). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 160.1, 159.5, 155.5, 151.4, 141.5, 139.1, 136.7, 133.4, 131.8, 131.7, 130.8, 129.5, 128.0, 127.7, 127.1, 124.9, 124.3, 123.5, 122.4, 121.9, 121.6, 119.8, 119.4, 112.9, 112.3, 112.2, 110.4, 106.0, 103.4, 80.8, 54.9, 47.7, 41.2, 26.2. IR (film) ν_{max} 3271, 2973, 1714, 1632, 1517 cm^{-1}.

ESI-TOF HRMS *m/z* 650.2167 (M + H⁺, C₃₆H₃₂ClN₅O₅ requires 650.2165).

1*S*-**5**: [α]²³_D +6.6 (*c* 0.58, THF), natural enantiomer.

1*R*-**5**: [α]²³_D −6.0 (*c* 0.40, THF), unnatural enantiomer.

***N*-(2-(1-(Chloromethyl)-5-(pivalamidooxy)-2,3-dihydro-1*H*-benzo[*e*]indole-3-carbonyl)-1*H*-indol-5-yl)-1*H*-indole-2-carboxamide (Trimethylacetamide Prodrug 6).** A solution of *seco*-CBI-indole₂ (**11**, 33.9 mg, 0.0634 mmol) in 1:1 ether:dioxane (0.1 mL) at 0 °C was treated with LiHMDS (0.190 mL, 0.190 mmol, 1.0 M in THF). The solution was stirred for 30 min, after which **15** (51.6 mg, 0.190 mmol) was added and the solution was warmed to room temperature and stirred for 4 h. The reaction mixture was diluted with ethyl acetate, washed with H₂O and saturated aqueous NaCl, and dried (MgSO₄). The solution was concentrated and purified by recrystallization (THF:hexanes) ×4 to afford **6** as a pale-yellow solid (45.5 mg, 65%). ¹H NMR (DMSO-*d₆*, 400 MHz) δ 11.87 (s, 1H), 11.72 (s, 1H), 10.17 (s, 1H), 8.25 (s, 1H), 8.09 (br s, 1H), 8.08 (d, *J* = 8.8 Hz, 1H), 7.94 (d, *J* = 8.4 Hz, 1H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.583 (d, *J* = 8.8 Hz, 1H), 7.575 (t, *J* = 6.8 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.45 (t, *J* = 8.4 Hz, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 7.43 (s, 1H), 7.26 (s, 1H), 7.22 (t, *J* = 8.0 Hz, 1H), 7.07 (t, *J* = 7.6 Hz, 1H), 4.87 (t, *J* = 10 Hz, 1H), 4.63 (dd, *J* = 11, 2.0 Hz, 1H), 4.34 (m, 1H), 4.08 (dd, *J* = 11, 3.2 Hz, 1H), 3.97 (dd, *J* = 12, 7.2 Hz, 1H), 1.20 (s, 9H). ¹³C NMR (THF-*d₈*, 150 MHz) δ 161.6, 160.7, 159.1, 153.6, 143.8, 138.4, 135.0, 133.7, 133.6, 132.9, 131.7, 129.4, 128.3, 126.5, 124.9, 124.6, 124.5, 123.7, 122.6, 120.9, 120.1, 118.3, 113.9, 113.1, 112.8, 106.9, 106.7, 103.2, 56.2, 50.2, 47.6, 44.3, 30.0. IR (film) ν_max 3442, 3347, 3222, 2968, 1650, 1613, 1552 cm⁻¹. ESI-TOF HRMS *m/z* 656.2030 (M + Na⁺, C₃₆H₃₂Cl-N₅O₄ requires 656.2035).

1*S*-**6**: [α]²³_D +9.0 (*c* 0.20, THF), natural enantiomer.

1*R*-**6**: [α]²³_D −8.8 (*c* 0.25, THF), unnatural enantiomer.

***tert*-Butyl(3-(5-(1*H*-indole-2-carboxamido)-1*H*-indole-2-carbonyl)-1-(chloromethyl)-2,3-dihydro-1*H*-benzo[*e*]indol-5-yl)oxy-(methyl)carbamate (*N*-Methyl-*tert*-Butylcarbamate Prodrug 8).** A solution of **5** (15.2 mg, 0.0234 mmol) in THF (0.1 mL) at −78 °C was treated with LiHMDS (23 µL, 0.023 mmol, 1.0 M in THF) and was stirred for 15 min. Me₂SO₄ (2.2 µL, 0.023 mmol) was added, and the solution was warmed to room temperature and stirred for 2 h. The solvent was removed under a stream of N₂, and the residue was purified by PTLC (1:1 THF:toluene) to afford **8** as a pale-yellow solid (5.5 mg, 35%). ¹H NMR (DMSO-*d₆*, 600 MHz) δ 11.79 (s, 1H), 11.74 (s, 1H), 10.19 (s, 1H), 8.28 (s, 1H), 8.25 (s, 1H), 8.07 (d, *J* = 7.8 Hz, 1H), 7.89 (d, *J* = 8.4 Hz, 1H), 7.68 (d, *J* = 7.8 Hz, 1H), 7.64 (t, *J* = 7.8 Hz, 1H), 7.60 (d, *J* = 8.4 Hz, 1H), 7.54 (t, *J* = 7.8 Hz, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.48 (d, *J* = 7.8 Hz, 1H), 7.44 (s, 1H), 7.29 (s, 1H), 7.22 (t, *J* = 7.8 Hz, 1H), 7.07 (t, *J* = 7.2 Hz, 1H), 4.92 (t, *J* = 10 Hz, 1H), 4.68 (d, *J* = 10 Hz, 1H), 4.45 (m, 1H), 4.11 (dd, *J* = 11, 3.0 Hz, 1H), 4.04 (dd, *J* = 11, 6.6 Hz, 1H), 3.39 (s, 3H), 1.35 (s, 9H). ¹³C NMR (DMSO-*d₆*, 150 MHz) δ 160.1, 159.8, 157.1, 146.9, 141.5, 136.6, 133.4, 131.8, 131.7, 130.8, 129.5, 127.7, 127.0, 125.2, 124.0, 123.48, 123.46, 122.7, 122.0, 121.6, 119.8, 112.8, 112.3, 112.2, 110.3, 106.9, 106.0, 103.3, 82.2, 66.9, 54.8, 47.7, 41.1, 26.9. IR (film) ν_max 3286, 2976, 1715, 1632, 1502 cm⁻¹. ESI-TOF HRMS *m/z* 686.2153 (M + Na⁺, C₃₇H₃₄Cl-N₅O₅ requires 686.2141).

1*S*-**8**: [α]²³_D +24 (*c* 0.45, THF), natural enantiomer.

1*R*-**8**: [α]²³_D −22 (*c* 0.43, THF), unnatural enantiomer.

***tert*-Butyl *N*-Hydroxycarbamate (12).** A solution of hydroxylamine hydrochloride (5.0 g, 72.0 mmol) and (BocO)₂CO (15.7 g, 72.0 mmol) in 1:1 THF:H₂O (160 mL) at 0 °C was treated with NaHCO₃ (12.1 g, 144 mmol). The solution was stirred at 0 °C for 2 h, after which it was diluted with ethyl acetate, washed with saturated aqueous NaHCO₃, H₂O, and saturated aqueous NaCl, and dried (MgSO₄). The solvent was removed under reduced pressure to provide **12** as a white solid (9.25 g, 96%). ¹H NMR (acetone-*d₆*, 400 MHz) δ 8.39 (s, 1H), 7.64 (s, 1H), 1.40 (s, 9H).

***tert*-Butyl *N*-Tosyloxycarbamate (13).** A solution of **12** (3.83 g, 28.8 mmol) and TsCl (5.23 g, 27.4 mmol) in THF (40 mL) at 0 °C

was treated with triethylamine (4.00 mL, 28.8 mmol). The resulting suspension was stirred at 0 °C for 2 h. The solution was filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in ethyl acetate, washed with 1 N HCl, H₂O, and saturated aqueous NaCl, and dried (MgSO₄). The solution was concentrated, and the resulting yellow solid was triturated with hexanes to provide **13** as a pure white solid (4.1 g, 52%). ¹H NMR (acetone-*d₆*, 400 MHz) δ 10.03 (s, 1H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.50 (d, *J* = 8.4 Hz, 2H), 2.48 (s, 3H), 1.29 (s, 9H).

***tert*-Butyl Pivaloyl(*N*-tosyloxy)carbamate (14).** A solution of **13** (1.00 g, 3.48 mmol) and trimethylacetyl chloride (2.14 mL, 17.4 mmol) in THF (10 mL) at 0 °C was treated with triethylamine. The solution was warmed to room temperature and stirred overnight (16 h). The reaction mixture was diluted with ethyl acetate, washed with H₂O and saturated aqueous NaCl, and dried (MgSO₄). The solution was concentrated and the residue purified by flash chromatography to provide **14** as a white solid (1.29 g, 99%). ¹H NMR (acetone-*d₆*, 400 MHz) δ 7.90 (d, *J* = 8.4 Hz, 2H), 7.59 (d, *J* = 8.0 Hz, 2H), 2.51 (s, 3H), 1.30 (s, 9H), 1.28 (s, 9H). ¹³C NMR (acetone-*d₆*, 150 MHz) δ 183.7, 175.4, 148.4, 132.9, 131.9, 131.3, 87.4, 45.1, 28.7, 27.6, 22.6. IR (film) ν_max 2978, 2936, 1808, 1737 cm⁻¹. ESI-TOF HRMS *m/z* 394.1294 (M + Na⁺, C₁₇H₂₅NO₆S requires 394.1295).

***N*-(Tosyloxy)pivalamide (15).** A vial containing **14** (91 mg, 0.245 mmol) was treated with 4 N HCl in EtOAc (10 mL). The reaction mixture was stirred at room temperature for 2 h. The solvent was evaporated under a stream of N₂ to provide **15** as the HCl salt (68 mg, 91%). ¹H NMR (acetone-*d₆*, 400 MHz) δ 10.88 (s, 1H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 8.0 Hz, 2H), 2.46 (s, 3H), 1.08 (s, 9H). ¹³C NMR (acetone-*d₆*, 150 MHz) δ 177.8, 147.7, 133.1, 131.4, 130.5, 54.0, 28.1, 22.6. IR (film) ν_max 3197, 2977, 1695 cm⁻¹. ESI-TOF HRMS *m/z* 272.0941 (M + H⁺, C₁₂H₁₇NO₄S requires 272.0951).

Methyl Hydroxycarbamate (16). A solution of hydroxylamine hydrochloride (2.00 g, 28.8 mmol) in H₂O (30 mL) at 0 °C was treated with KOH (4.85 g, 86.4 mmol), and the reaction mixture was stirred for 10 min. Dimethyl carbonate (2.42 mL, 28.8 mmol) was added dropwise, and the reaction mixture was stirred at 5 °C for 8 h. The reaction mixture was acidified with the addition of 1 N HCl and extracted with diethyl ether (3 × 100 mL). The ether layers were combined and concentrated under reduced pressure to provide **16** as a clear oil (308 mg, 12%). ¹H NMR (acetone-*d₆*, 400 MHz) δ 8.83 (s, 1H), 3.63 (s, 3H).

Methyl Tosyloxycarbamate (17). A solution of **16** (308 mg, 3.38 mmol) and TsCl (537 mg, 2.82 mmol) in diethyl ether (5 mL) at 0 °C was treated with triethylamine (0.471 mL, 3.38 mmol). The resulting suspension was stirred at 0 °C for 1 h, after which the solution was diluted with ethyl acetate, washed with H₂O and saturated aqueous NaCl, and dried (MgSO₄). The solution was concentrated under reduced pressure to provide **17** as an off-white solid (350 mg, 51%). ¹H NMR (acetone-*d₆*, 400 MHz) δ 10.25 (br s, 1H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.50 (d, *J* = 8.4 Hz, 2H), 3.60 (s, 3H), 2.48 (s, 3H). ¹³C NMR (acetone-*d₆*, 125 MHz) δ 158.3, 147.9, 133.0, 131.6, 131.2, 54.5, 22.6. IR (film) ν_max 3222, 2964, 1730 cm⁻¹. ESI-TOF HRMS *m/z* 268.0250 (M + Na⁺, C₉H₁₁NO₅S requires 268.0250).

1-(Tosyloxy)urea (18). A solution of hydroxyurea (2.00 g, 26.3 mmol) and TsCl (4.80 g, 25.0 mmol) in DMF (20 mL) at 0 °C was treated with triethylamine (3.67 mL, 26.3 mmol). The solution was stirred for 3 h at 0 °C, diluted with ethyl acetate, washed with H₂O and saturated aqueous NaCl, and dried (MgSO₄). The solution was concentrated under reduced pressure and purified by flash chromatography to provide **18** as a white solid (716 mg, 12%). ¹H NMR (acetone-*d₆*, 400 MHz) δ 9.33 (s, 1H), 7.89 (d, *J* = 8.4 Hz, 2H), 7.50 (d, *J* = 8.0 Hz, 2H), 6.23 (br s, 2H), 2.48 (s, 3H). ¹³C NMR (acetone-*d₆*, 125 MHz) δ 160.7, 147.9, 132.9, 131.7, 131.2, 22.6. IR (film) ν_max 3444, 3233, 3052, 2855, 1698 cm⁻¹. ESI-TOF HRMS *m/z* 253.0250 (M + Na⁺, C₈H₁₀N₂O₄S requires 253.0253).

In Vivo Antitumor Activity. DBA/2J mice were injected intraperitoneal (ip) with syngeneic L1210 cells (1 × 10⁶) on day 0.

Ten mice were randomly assigned to treatment groups for compounds **3**−**5**, **11**, or vehicle at doses of 50, 100, 250, and 500 μg/kg/inj for **3**, 10, 30, 60, 100, and 200 μg/kg/inj for **4**, and 10, 30, 60, and 100 μg/kg/inj for **5** and **11**. Compounds **3**−**5** and **11** were formulated with 30% DMSO plus 0.7% glucose solution and were ip injected consecutively on days 1, 5, and 9. Animals were monitored daily and weighed three times per week following injection of tumor cells. Tumor burden in the peritoneal cavity and survivorship were determined for animals in each treatment group.

Supporting Information Available: Stability data for prodrugs **3**−**6** and **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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