In Vitro Optimization of Non-Small Cell Lung Cancer Activity with Troxacitabine, L-1,3-Dioxolane-cytidine, Prodrugs

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L-1,3-Dioxolane-cytidine, a potent anticancer agent against leukemia, has limited efficacy against solid tumors, perhaps due to its hydrophilicity. Herein, a library of prodrugs were synthesized to optimize in vitro antitumor activity against non-small cell lung cancer. N^4-Substituted fatty acid amide prodrugs of 10−16 carbon chain length demonstrated significantly improved antitumor activity over L-1,3-dioxolane-cytidine. These in vitro results suggest that the in vivo therapeutic efficacy of L-1,3-dioxolane-cytidine against solid tumors may be improved with prodrug strategies.

Introduction

L-1,3-Dioxolane-cytidine (L-OddC; (−)-2′-deoxy-3′-oxacytidine) is the first L-nucleoside analogue to exhibit anticancer activity.[1] Currently, L-1,3-dioxolane-cytidine is being evaluated in phase II/III clinical trials for the treatment of acute myelogenous leukemia and phase I/II dose ranging trials for refractory pancreatic cancer. L-1,3-Dioxolane-cytidine has the same intracellular activation pathway as common antitumor nucleosides (gemcitabine and cytarabine), which involves the formation of the triphosphate and incorporation into DNA causing chain termination.[2] Unlike the aforementioned nucleosides, L-1,3-dioxolane-cytidine has a unique pattern of cellular uptake and metabolism, which may render it insusceptible to the common mechanisms of resistance to nucleoside analogues. It has been shown that L-1,3-dioxolane-cytidine can be transported into cells by passive diffusion rather than by nucleoside-specific membrane transporters, such as the equilibrative nucleoside transporters (ENT) and concentrative nucleoside transporters (CNT), and thus may not be subject to ENT and CNT mediated resistance.[3] However, this may be cell type dependent. In addition, L-1,3-dioxolane-cytidine is resistant to deoxycytidine deaminase (dCD), thus retaining its activity against tumors having high dCD levels.[4] In contrast to the pharmacokinetic behavior of nucleoside analogues with a D-configuration, which are characterized by rapid disappearance from plasma due to dCD-mediated deamination, L-1,3-dioxolane-cytidine exhibited a robust plasma half-life (82 h) and a systemic clearance comparable to the glomerular filtration rate.[5] Despite L-1,3-dioxolane-cytidine's relatively long intracellular retention and low systemic clearance, pharmacokinetic studies indicated that it is slowly accumulated in cancer cells in comparison to other carrier-transported nucleosides. L-1,3-Dioxolane-cytidine, like most other anticancer nucleosides, is a hydrophilic agent and must be administered intravenously in a frequent dosage schedule, which may result in greater toxicity than a single dose schedule.[6]

In view of these advantages and drawbacks, in the present work, a library of 20 L-1,3-dioxolane-cytidine prodrugs were synthesized to evaluate the structure−activity relationship between prodrug lipophilicity and antitumor activity. It is possible that improving the lipophilicity of the compounds may enhance their cellular uptake and thus enhance the efficacy of the parent drug. Prodrug strategies have been valuable to overcome undesirable pharmaceutical properties for a variety of drugs, thus optimizing their clinical application.[7] Typically, prodrugs are synthesized to impede the clearance of the active compounds and improve cellular uptake. Capecitabine and sapacitabine represent amide prodrugs of pyrimidines that were created to improve the bioavailability of their parent compounds, 5-fluorouracil and 2′-cyano-2′-deoxy-cytosine, respectively.[8,9] These compounds utilized prodrug strategies to avoid the relatively quick clearance of their active compounds, thus enhancing the bioavailability. However, L-1,3-dioxolane-cytidine is not a substrate for enzyme degradation and primarily requires modification to improve cellular uptake. In our current study, the amino group of the cytosine moiety was modified to increase the prodrugs' lipophilicity to investigate the change in in vitro antitumor activity.

Table 1. Cytotoxic Activity on Two Non-Small Cell Lung Cancer Cell Lines (A549 and SW1573) and Calculated LogP for L-1,3-Dioxolane-cytidine Prodrugs **6a**−**t**

cmpd	IC_{50}[a] (μM), A549	IC_{50}[a] (μM), SW1573	LogP[b]	cmpd	IC_{50}[a] (μM), A549	IC_{50}[a] (μM), SW1573	LogP[b]
L-1,3-dioxolane-cytidine	0.68	2.3	−0.66	**6j**	0.030	0.150	7.02
gemcitabine	13.1	8.3	−0.90	**6k**	0.500	0.900	7.86
cytarabine	620	10 500	−2.24	**6l**	18.67	22.00	0.00
6a	6.87	10.70	−0.73	**6m**	2.97	8.80	0.83
6b	6.60	8.07	0.34	**6n**	7.37	14.00	1.25
6c	5.23	9.13	0.76	**6o**	8.53	13.67	1.17
6d	1.04	2.40	1.60	**6p**	8.67	15.33	1.33
6e	0.046	0.276	2.43	**6q**	0.41	2.77	1.73
6f	0.024	0.040	2.85	**6r**	4.53	8.87	2.00
6g	0.015	0.03	3.68	**6s**	3.50	10.03	1.04
6h	0.004	0.020	5.35	**6t**	9.83	14.33	2.29
6i	0.025	0.125	6.19				

[a] SRB test data expressed as the mean of three experiments. [b] Calculated using ChemDraw Ultra 8.0.

Results and Discussion

The advent of combinatorial techniques has provided a significant impact on the process of drug discovery. In fact, in

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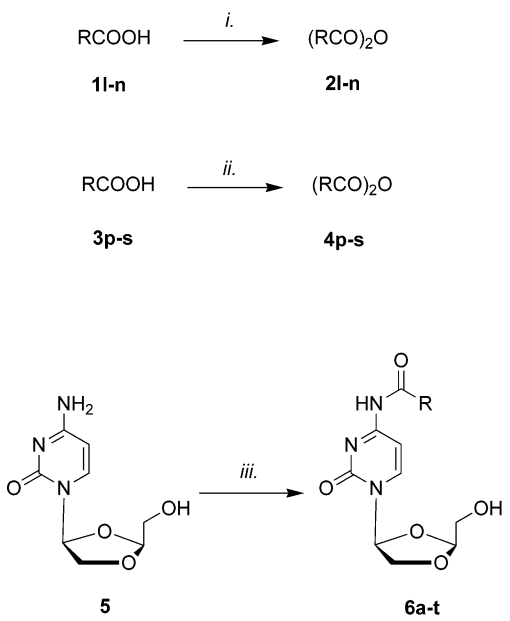
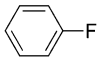
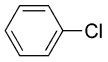
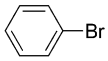
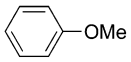
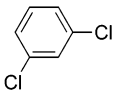
Scheme 1. Parallel Synthesis of L-1,3-Dioxolane-cytidine Prodrugs[a]



Compd	R	Compd	R
6a	CH_3	6l	
6b	$(CH_2)_2CH_3$	6m	
6c	$(CH_2)_3CH_3$		
6d	$(CH_2)_5CH_3$	6n	
6e	$(CH_2)_7CH_3$	6o	
6f	$(CH_2)_8CH_3$		
6g	$(CH_2)_{10}CH_3$	6p	
6h	$(CH_2)_{14}CH_3$	6q	
6i	$(CH_2)_{16}CH_3$		
6j	$(CH_2)_{18}CH_3$	6r	
6k	$(CH_2)_{20}CH_3$	6s	
		6t	

[a] Reagents and conditions: (*i*) DCC, CH_2Cl_2, rt, 24 h; (*ii*) Et_3N, CH_3CN, Cl_3CCN, Ph_3P, rt, 1 h; (*iii*) $(RCO)_2O$, MeOH, 55 °C, 6 h.

the past fifteen years, combinatorial chemistry linked to high-throughput screening techniques has resulted in the discovery of a variety of biologically active compounds.[10] Solution-phase combinatorial approaches have recently become of interest as an alternative to the solid-phase method for drug discovery and lead optimization.[11,12] We describe herein the synthesis of a library of L-1,3-dioxolane-cytidine prodrugs **6a**−**t** using a straightforward parallel solution-phase approach (Scheme 1). L-1,3-Dioxolane-cytidine **5**, synthesized according to the procedure from our laboratory,[13] was dissolved in anhydrous methanol and treated, in an Argonaut Quest 210 organic synthesizer with 20 different acid anhydrides. Some of these anhydrides (**2l**−**n** and **4p**−**s**), which are not commercially available, have been prepared by two different procedures as described in Scheme 1.[14,15] After 6 h at 55 °C, the reaction mixtures were simply filtered and then purified on a small silica flash column with gradient elution.

Compounds **6a**−**t** were evaluated using the sulforhodamide-B (SRB) assay in two non-small cell lung cancer cell lines (A549 and SW1573), and the antitumor activity was compared with the parent compound (L-1,3-dioxolane-cytidine), gemcitabine, and cytarabine (Table 1). These cell lines were chosen because they were characterized earlier for sensitivity to gemcitabine and for their ability to phosphorylate gemcitabine and L-1,3-dioxolane-cytidine to their corresponding monophosphates. In addition, non-small cell lung cancer is routinely being treated with gemcitabine containing regimens.

The L-1,3-dioxolane-cytidine prodrugs synthesized herein contained linear, cycloalkyl, and aromatic amides to increase the lipophilicity of the nucleoside analogues. Theoretically, increasing the lipophilicity of the prodrugs may aid the passive diffusion of these compounds, leading to an increase in intracellular accumulation and thus an increase in efficacy. The growth inhibition (Figure 1) indicates that analogues **6e**−**k**, with long linear aliphatic chains ($\geq$8 CH_2), are clearly more potent in vitro than L-1,3-dioxolane-cytidine, with IC_{50} values in the nanomolar range. These in vitro results suggest that lipophilic L-1,3-dioxolane-cytidine prodrugs are indeed more active against solid tumors in vitro than L-1,3-dioxolane-cytidine and possibly warrant further evaluation in vivo. In fact, a good inverse nonlinear correlation between IC_{50} and LogP values was found for all the linear-chain aliphatic prodrugs, as shown in Figure 2. Both cell lines indicated that compound **6h** (16 carbon chain) possesses the optimal activity for the linear amide moieties, however, it was similar to the 10- and 12-carbon chain prodrugs in the SW1573 cell line.

The cycloalkyl and aromatic derivatives (**6l**−**t**) did not exhibit an improved potency over L-1,3-dioxolane-cytidine, despite their improved lipophilicity. The *p*-chloro analogue **6q** did exhibit a slightly better potency in the A549 cell line, but not in the SW1573 cell line. Because this could be viewed as cell type dependent, it may be of interest to study the aromatic amide prodrugs further. It is likely that the cycloalkyl and aromatic moieties at the N_4 position of compounds **6l**−**t** may render these compounds poor substrates for intracellular amidases. We therefore speculate that the amidase-catalyzed hydrolysis of the prodrugs may play an important role, allowing the release of L-1,3-dioxolane-cytidine into the intracellular compartment for triphosphate formation. Thus, the improved activity profile of prodrugs **6e**−**k** could have been the result of an increased uptake due to the lipophilicity, as well as amidase-catalyzed hydrolysis. However, the detailed pharmacological and biochemical interactions for these prodrugs is beyond the scope of the current study.

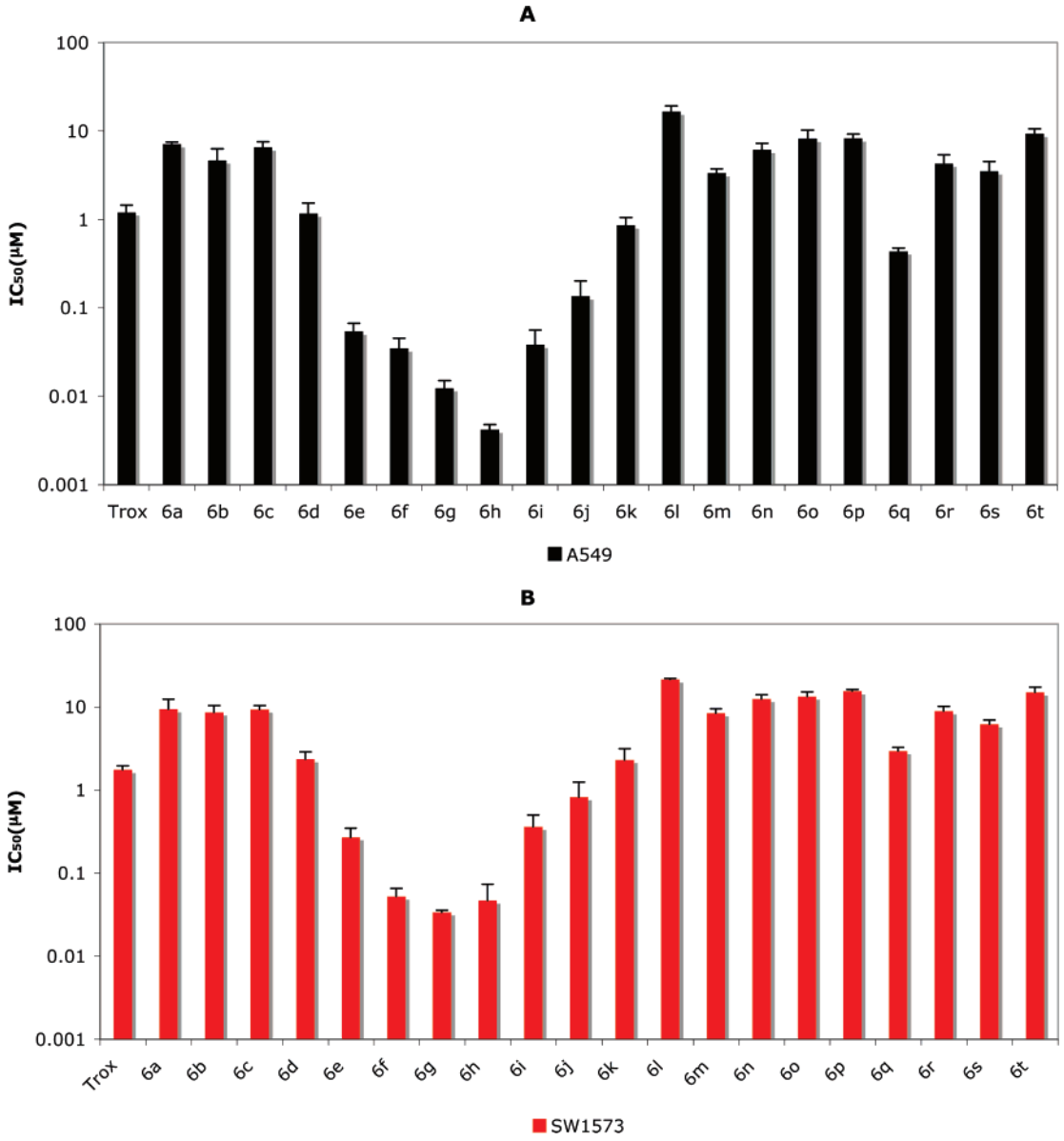


Figure 1. Activity profile for the L-1,3-dioxolane-cytidine prodrugs on two non-small cell lung cancer cell lines: IC_{50} values are expressed as the mean of three experiments + SEM (μM): (A) A549 data and (B) SW1573 data.

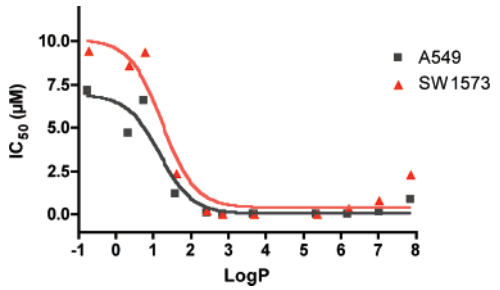


Figure 2. Correlation between LogP and IC_{50} (μM) for linear chain aliphatic prodrugs on both non-small cell lung cancer cell lines A549 and SW1573. LogP was estimated using ChemDraw 8.0 ultra. IC_{50} values are expressed as the mean of three experiments.

In summary, a straightforward methodology for the parallel synthesis of novel L-1,3-dioxolane-cytidine prodrugs **6a**−**t** has been developed. Some of these compounds exhibit improved antitumor activity against A549 and SW1573 non-small cell lung cancer cell lines. We have demonstrated that by systematic modification of a hydrophilic nucleoside, we were able to significantly improve the antitumor activity of the hydrophilic nucleoside L-1,3-dioxolane-cytidine. Herein, the linear amide prodrugs of L-1,3-dioxolane-cytidine were optimized for solid tumor non-small cell lung cancers in vitro. These finding suggest that compounds such as compound **6h** have better in vitro efficacy than L-1,3-dioxolane-cytidine and should be studied in vivo against solid tumors. In view of these interesting preliminary findings, additional in vitro and in vivo pharmacological and biochemical evaluations of L-1,3-dioxolane-cytidine prodrugs are warranted. Particular attention should be paid to the more lipophilic prodrugs, as they may present some difficulty for water solubility and thus bioavailability for in vivo studies. Furthermore, enzymatic stability, such as hydrolysis by amidase, and toxicity should be examined before the in vivo efficacy and PK studies are performed.

Experimental Section

General Considerations. Parallel synthesis was performed on an Argonaut Quest 210 organic synthesizer. Melting points were determined on a Mel-temp II laboratory device and are uncorrected. NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer; chemical shifts are reported in parts per million (δ) and signals are quoted as s (singlet), d (doublet), t (triplet), m

(multiplet), and dd (double of doublets). UV spectra were obtained on a Beckman DU-650 spectrophotometer. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. TLC was performed on Uniplates (silica gel) purchased from Analtech. Co. and Elemental analysis were performed by Atlantic Microlab Inc., Norcross, GA. All commercially available anhydrides were used without further purification. Cyclopropanecarboxylic, cyclopenthanecarboxylic, and cyclohexanecarboxylic anhydrides were synthesized according to a reported procedure and used without further purification.[14] 4-Fluoro, 4-chloro, 4-bromobenzoic anhydride, as well as 2,4-dichlorobenzoic anhydride, were synthesized according to a reported procedure.[15]

Chemical Synthesis. General Procedure for the Parallel Solution-Phase Synthesis of 4-(*N*-Acyl-substituted)-L-OddC Prodrugs (6a−t). L-1,3-Dioxolane-cytidine **5** (2 g) was dissolved in anhydrous MeOH (20 mL) and 1 mL of the solution (100 mg, 1 equiv of **5**) was added to each microfrit-equipped reaction vessel, followed by 9 mL of methanol. The appropriate anhydrides (3 equiv) were then added, and the reaction mixture was stirred vigorously (upward stroke = 50%, time = 1 s) at 55 °C for 6 h in an Argonaut Quest 210 organic synthesizer. After 6 h, the reaction vessels were drained, and the collected crude material was evaporated to dryness under reduced pressure and purified on a short flash column (gradient elution, 60% hexane/40% ethyl acetate−100% ethyl acetate).

(−)-(2*S*,4*S*)-1-[2-(Hydroxymethyl)-1,3-dioxolan-4-yl]-4-*N*-acetyl-cytosine (6a): Yield = 92%; mp 176.0−178.0 °C; $[\alpha]_D^{24}$ −68.005 (*c* 0.04, MeOH); UV (H_2O) λ_{max} 246 nm (ϵ 9033 pH 2), 246 nm (ϵ 16 703 pH 7.4), 270 nm (ϵ 9760 pH 11); ^{1}H NMR ($CDCl_3$) δ 8.60 (d, 1H, J = 7.32 Hz), 6.24 (m, 1H), 5.12 (m, 1H), 4.33−4.25 (m, 2H), 3.90 (m, 2H), 2.21 (s, 3H); ^{13}C NMR (CD_3OD) δ 180.1, 171.6, 163.1, 156.8, 145.1, 106.1, 96.2, 83.4, 72.0, 60.2, 23.2; IR (neat) 1716, 1654, 1562, 1494 cm^{-1}

(−)-(2*S*,4*S*)-1-[2-(Hydroxymethyl)-1,3-dioxolan-4-yl]-4-*N*-butyryl-cytosine (6b): Yield = 82%; mp 118.0−120.0 °C; $[\alpha]_D^{24}$ −48.43 (*c* 0.042, MeOH); UV (H_2O) λ_{max} 246 nm (ϵ 16 430 pH 2), 246 nm (ϵ 18 353 pH 7.4), 244 nm (ϵ 10 155 pH 11); ^{1}H NMR ($CDCl_3$) δ 8.49 (d, 1H, J = 7.33 Hz), 7.44 (d, 1H, J = 7.33 Hz), 6.20 (m, 1H), 5.12 (m, 1H), 4.34−4.23 (m, 2H), 3.97 (m, 2H), 2.45 (t, 2H, J = 7.32 Hz), 1.70 (m, 2H), 0.97 (t, 3H, J = 7.32 Hz); ^{13}C NMR ($CDCl_3$) δ 171.6, 162.5, 155.6, 144.9, 105.7, 96.2, 83.6, 72.7, 61.1, 39.6, 18.3, 13.6; IR (neat) 1693, 1651, 1554, 1500 cm^{-1}.

(−)-(2*S*,4*S*)-1-[2-(Hydroxymethyl)-1,3-dioxolan-4-yl]-4-*N*-valeryl-cytosine (6c): Yield = 84%; mp 143.0−144.0 °C; $[\alpha]_D^{24}$ −53.34 (*c* 0.5, MeOH); UV (H_2O) λ_{max} 246 nm (ϵ 13 393 pH 2), 246 nm (ϵ 14 436 pH 7.4), 246 nm (ϵ 12 188 pH 11); ^{1}H NMR ($CDCl_3$) δ 8.49 (d, 1H, J = 7.32 Hz), 7.44 (d, 1H, J = 7.32 Hz), 6.20 (m, 1H), 5.12 (m, 1H), 4.32 (m, 1H), 4.23 (m, 1H), 3.97 (m, 2H), 2.47 (t, 2H, J = 7.32 Hz), 1.65 (m, 2H), 1.36 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 173.8, 162.8, 155.6, 145.2, 105.8, 96.4, 83.5, 72.6, 61.0, 37.4, 26.9, 22.2, 13.7; IR (neat) 1690, 1648, 1552, 1494 cm^{-1}.

(−)-(2*S*,4*S*)-1-[2-(Hydroxymethyl)-1,3-dioxolan-4-yl]-4-*N*-heptanoyl-cytosine (6d): Yield = 84%; mp 151.0−153.0 °C; $[\alpha]_D^{25}$ −56.82 (*c* 0.044, MeOH); UV (H_2O) λ_{max} 246 nm (ϵ 14 223 pH 2), 246 nm (ϵ 15 665 pH 7.4), 246 nm (ϵ 14 519 pH 11); ^{1}H NMR ($CDCl_3$) δ 8.49 (d, 1H, J = 7.32 Hz), 7.44 (d, 1H, J = 7.32 Hz), 6.19 (m, 1H), 5.12 (m, 1H), 4.33 (m, 1H), 4.23 (m, 1H), 3.97 (m, 2H), 2.46 (t, 2H, J = 7.32 Hz), 1.66 (m, 8H), 1.28 (m, 4H), 0.89 (t, 3H, J = 7.32 Hz); ^{13}C NMR ($CDCl_3$) δ 173.7, 162.7, 155.6, 145.2, 105.8, 96.4, 83.5, 72.7, 61.0, 37.7, 31.5, 28.8, 24.8, 22.5, 14.0; IR (neat) 1693, 1649, 1553, 1494 cm^{-1}.

(−)-(2*S*,4*S*)-1-[2-(Hydroxymethyl)-1,3-dioxolan-4-yl]-4-*N*-nonanoyl-cytosine (6e): Yield = 72%; mp 135.0−137.0 °C; $[\alpha]_D^{27}$ −51.58 (*c* 0.03, MeOH); UV (H_2O) λ_{max} 246 nm (ϵ 14 101 pH 2), 246 nm (ϵ 15 201 pH 7.4), 246 nm (ϵ 11 582 pH 11); ^{1}H NMR ($CDCl_3$) δ 8.49 (d, 1H, J = 7.81 Hz), 7.45 (d, 1H, J = 7.81 Hz), 6.19 (m, 1H), 5.12 (m, 1H), 4.33 (m, 1H), 4.23 (m, 1H), 3.97 (m, 2H), 2.46 (t, 2H, J = 7.32 Hz), 1.66 (m, 2H), 1.28 (m, 10H), 0.88 (t, 3H, J = 7.32 Hz); ^{13}C NMR ($CDCl_3$) δ 173.7, 162.7, 155.6, 145.2, 105.8, 96.3, 83.5, 72.7, 61.0, 37.7, 31.8, 29.1, 24.9, 22.6, 14.1; IR (neat) 1689, 1649, 1553, 1496 cm^{-1}.

(−)-(2*S*,4*S*)-1-[2-(Hydroxymethyl)-1,3-dioxolan-4-yl]-4-*N*-decanoyl-cytosine (6f): Yield = 66%; mp 141.0−143.0 °C; $[\alpha]_D^{25}$ −35.59 (*c* 0.028, MeOH); UV (H_2O) λ_{max} 246 nm (ϵ 11850 pH 2), 246 nm (ϵ 13056 pH 7.4), 246 nm (ϵ 8256 pH 11); ^{1}H NMR ($CDCl_3$) δ 8.49 (d, 1H, J = 7.32 Hz), 7.44 (d, 1H, J = 7.32 Hz), 6.19 (m, 1H), 5.12 (m, 1H), 4.33 (m, 1H), 4.24 (m, 1H), 3.97 (m, 2H), 2.45 (t, 2H, J = 7.32 Hz), 1.66 (m, 2H), 1.29 (m, 12H), 0.88 (t, 3H, J = 7.32 Hz); ^{13}C NMR ($CDCl_3$) δ 173.6, 162.7, 155.6, 145.2, 105.7, 96.3, 83.5, 72.7, 61.0, 37.8, 31.9, 29.4, 29.3, 29.2, 29.1, 24.9, 22.7, 14.1; IR (neat) 1690, 1650, 1553, 1499 cm^{-1}.

(−)-(2*S*,4*S*)-1-[2-(Hydroxymethyl)-1,3-dioxolan-4-yl]-4-*N*-lauryl-cytosine (6g): Yield = 54%; mp 137.0−138.5 °C; $[\alpha]_D^{27}$ −61.75 (*c* 0.039, MeOH); UV (MeOH) λ_{max} 241 nm (ϵ 7442); ^{1}H NMR ($CDCl_3$) δ 8.48 (d, 1H, J = 7.32 Hz), 7.45 (d, 1H, J = 7.32 Hz), 6.19 (m, 1H), 5.12 (m, 1H), 4.33 (m, 1H), 4.24 (m, 1H), 3.97 (m, 2H), 2.46 (t, 2H, J = 7.32 Hz), 1.66 (m, 2H), 1.25 (m, 16H), 0.88 (t, 3H, J = 7.32 Hz); ^{13}C NMR ($CDCl_3$) δ 173.6, 162.7, 155.7, 145.2, 105.8, 96.3, 83.5, 72.7, 61.0, 37.7, 31.9, 29.6, 29.5, 29.4, 29.1, 24.9, 22.7, 14.1; IR (neat) 1690, 1650, 1553, 1499 cm^{-1}.

(−)-(2*S*,4*S*)-1-[2-(Hydroxymethyl)-1,3-dioxolan-4-yl]-4-*N*-palmitoyl-cytosine (6h): Yield = 23%; mp 134.5−135.5 °C; $[\alpha]_D^{29}$ −79.34 (*c* 0.02, MeOH); UV (MeOH) λ_{max} 244 nm (ϵ 11 145); ^{1}H NMR ($CDCl_3$) δ 8.46 (d, 1H, J = 7.32 Hz), 7.43 (d, 1H, J = 7.32 Hz), 6.19 (m, 1H), 5.13 (m, 1H), 4.34 (m, 1H), 4.26 (m, 1H), 3.98 (m, 2H), 2.42 (t, 2H, J = 7.32 Hz), 1.66 (m, 2H), 1.25 (m, 24H), 0.88 (t, 3H, J = 7.32 Hz); ^{13}C NMR ($CDCl_3$) δ 162.4, 150.0, 145.0, 105.6, 96.0, 83.6, 72.8, 61.1, 37.9, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 24.9, 22.7, 14.1; IR (neat) 1690, 1651, 1553, 1499 cm^{-1}.

(−)-(2*S*,4*S*)-1-[2-(Hydroxymethyl)-1,3-dioxolan-4-yl]-4-*N*-stearoyl-cytosine (6i): Yield = 20%; mp 131−133 °C; $[\alpha]_D^{29}$ −9.60 (*c* 0.5%, $CHCl_3$); UV (H_2O pH=7) λ_{max} 240 nm (ϵ 5593); ^{1}H NMR ($CDCl_3$) δ 8.46 (d, 1H, J = 7.4 Hz), 8.19 (bs, 1H), 7.43 (d, 1H, J = 7.4 Hz), 6.19 (m, 1H), 5.14 (m, 1H), 4.35 (m, 1H), 4.27 (m, 1H), 3.99 (m, 2H), 2.40 (t, 2H, J = 7.40 Hz), 1.68 (m, 2H), 1.25 (m, 28H), 0.88 (t, 3H, J = 7.00 Hz); ^{13}C NMR ($CDCl_3$) δ 173.4, 162.4, 155.5, 145.1, 105.6, 96.1, 83.6, 72.7, 61.0, 37.8, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 24.9, 22.7, 14.2; IR (neat) 1689, 1650, 1553, 1497 cm^{-1}.

(−)-(2*S*,4*S*)-1-[2-(Hydroxymethyl)-1,3-dioxolan-4-yl]-4-*N*-arachidoyl-cytosine (6j): Yield = 15%; mp 127−130 °C; $[\alpha\ ]_D^{29}$ −6.41 (*c* 0.5%, MeOH); UV (H_2O pH=7) λ_{max} 240 nm (ϵ 8412); ^{1}H NMR ($CDCl_3$) δ 8.47 (d, 1H, J = 7.4 Hz), 8.36 (br s, 1H), 7.43 (d, 1H, J = 7.4 Hz), 6.19 (m, 1H), 5.14 (m, 1H), 4.35 (m, 1H), 4.26 (m, 1H), 3.99 (m, 2H), 2.41 (t, 2H, J = 7.60 Hz), 1.66 (m, 2H), 1.25 (m, 32H), 0.88 (t, 3H, J = 6.60 Hz); ^{13}C NMR ($CDCl_3$) δ 173.4, 162.5, 155.6, 145.1, 105.7, 96.2, 83.6, 77.3, 73.7, 61.0, 37.8, 29.75, 29.73, 29.7, 29.66, 29.54, 29.5, 29.4, 29.37, 29.1, 24.9, 22.7, 14.2; IR (neat) 1699, 1656, 1553, 1499 cm^{-1}.

(−)-(2*S*,4*S*)-1-[2-(Hydroxymethyl)-1,3-dioxolan-4-yl]-4-*N*-behenoyl-cytosine (6k): Yield = 10%; mp 128−130 °C; $[\alpha]_D^{29}$ −79.34 (*c* 0.5%, $CHCl_3$); UV (H_2O pH = 7) λ_{max} 241 nm (ϵ 7618); ^{1}H NMR ($CDCl_3$) δ 8.51 (br s, 1H), 8.47 (d, 1H, J = 7.5 Hz), 7.43 (d, 1H, J = 7.5 Hz), 6.19 (m, 1H), 5.13 (m, 1H), 4.35 (m, 1H), 4.26 (m, 1H), 3.98 (m, 2H), 2.42 (t, 2H, J = 7.75 Hz), 1.67 (m, 2H), 1.25 (m, 36H), 0.88 (t, 3H, J = 7.25 Hz); ^{13}C NMR ($CDCl_3$) δ 173.2, 162.4, 145.1, 105.6, 96.1, 83.6, 83.58, 77.3, 72.8, 61.0, 37.9, 32.0, 29.8, 29.75, 29.7, 29.65, 29.5, 29.4, 29.35, 29.1, 24.9, 22.7, 14.2; IR (neat) 1691, 1654, 1554, 1500 cm^{-1}.

(−)-(2*S*,4*S*)-1-[2-(Hydroxymethyl)-1,3-dioxolan-4-yl]-4-*N*-cyclopropyl-cytosine (6l): Yield = 66%; mp 169.0−170.5 °C; $[\alpha]_D^{26}$ −62.83 (*c* 0.033, MeOH); UV (H_2O) λ_{max} 247 nm (ϵ 16 457 pH 2), 247 nm (ϵ 18 447 pH 7.4), 247 nm (ϵ 16 191 pH 11); ^{1}H NMR ($CDCl_3$) δ 8.48 (d, 1H, J = 7.32 Hz), 7.41 (d, 1H, J = 7.32 Hz), 6.21 (m, 1H), 5.10 (m, 1H), 4.31 (m, 1H), 4.23 (m, 1H), 3.95 (m, 2H), 1.86 (m, 1H), 1.07 (m, 2H), 0.92 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 174.6, 162.7, 155.8, 145.2, 105.9, 96.7, 83.4, 72.5, 61.0, 15.9, 9.7; IR (neat) 1709, 1651, 1560, 1491 cm^{-1}.

(−)-(2*S*,4*S*)-1-[2-(Hydroxymethyl)-1,3-dioxolan-4-yl]-4-*N*-cyclopentyl-cytosine (6m): Yield = 58%; mp 59.0 °C (decomposition); $[\alpha]_D^{27}$ −31.48 (*c* 0.031, MeOH); UV (H_2O) λ_{max} 247 nm (ϵ 15 014 pH 2), 247 nm (ϵ 16 397 pH 7.4), 247 nm (ϵ 12 296 pH

11); ^{1}H NMR ($CDCl_3$) δ 8.49 (d, 1H, J = 7.33 Hz), 7.43 (d, 1H, J = 7.33 Hz), 6.20 (m, 1H), 5.12 (m, 1H), 4.31 (m, 1H), 4.24 (m, 1H), 3.97 (m, 1H), 2.86 (m, 1H), 1.92−1.59 (m, 8H); ^{13}C NMR ($CDCl_3$) δ 176.7, 162.7, 155.8, 145.2, 105.8, 96.4, 83.5, 72.6, 61.0, 46.7, 30.1, 26.0; IR (neat) 1717, 1650, 1558, 1489 cm^{-1}.

(−)-(2*S*,4*S*)-1-[2-(Hydroxymethyl)-1,3-dioxolan-4-yl]-4-*N*-cyclohexyl-cytosine (6n): Yield = 64%; mp 77.0 °C (decomposition); $[\alpha]_D^{27}$ −76.27 (*c* 0.039, MeOH); UV (H_2O) λ_{max} 247 nm (ϵ 14 885 pH 2), 247 nm (ϵ 15 887 pH 7.4), 247 nm (ϵ 14 220 pH 11); ^{1}H NMR ($CDCl_3$) δ 8.49 (d, 1H, J = 7.32 Hz), 7.43 (d, 1H, J = 7.32 Hz), 6.20 (m, 1H), 5.12 (m, 1H), 4.32 (m, 1H), 4.24 (m, 1H), 3.97 (m, 1H), 2.39 (m, 1H), 1.90 (m, 2H), 1.80 (m, 2H), 1.69 (m, 1H), 1.45 (m, 2H), 1.24 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 176.4, 162.8, 155.8, 145.1, 105.8, 96.4, 83.5, 72.6, 61.0, 46.2, 29.2, 25.6, 25.4; IR (neat) 1738, 1654, 1558, 1489 cm^{-1}.

(−)-(2*S*,4*S*)-1-[2-(Hydroxymethyl)-1,3-dioxolan-4-yl]-4-*N*-benzoyl-cytosine (6o): Yield = 85%; mp 194.5−195.5 °C; $[\alpha]_D^{28}$ −56.71 (*c* 0.060, MeOH); UV (H_2O) λ_{max} 258 nm (ϵ 19 660 pH 2), 258 nm (ϵ 19 802 pH 7.4), 311 nm (ϵ 14 641 pH 11); ^{1}H NMR (CD_3OD) δ 8.67 (d, 1H, J = 7.32 Hz), 7.98 (d, 2H, J = 7.80 Hz), 7.63 (m, 2H), 7.54 (m, 2H), 6.24 (m, 1H), 5.11 (m, 1H), 4.31 (m, 1H), 4.27 (m, 1H), 3.90 (m, 2H); ^{13}C NMR (CD_3OD) δ 134.0, 129.7 129.0, 107.3, 98.0, 84.6, 73.3, 61.4; IR (neat) 1699, 1654, 1558, 1488 cm^{-1}.

(−)-(2*S*,4*S*)-1-[2-(Hydroxymethyl)-1,3-dioxolan-4-yl]-4-*N*-(*p*-fluorobenzoyl)-cytosine (6p): Yield = 61%; mp 163.0−165.0 °C; $[\alpha]_D^{27}$ −96.55 (*c* 0.036, MeOH); UV (H_2O) λ_{max} 259 nm (ϵ 23 324 pH 2), 258 nm (ϵ 24 194 pH 7.4), 314 nm (ϵ 21 234 pH 11); ^{1}H NMR (CD_3OD) δ 8.57 (d, 1H, J = 7.32 Hz), 7.98 (m, 2H), 7.63 (m, 2H), 7.55 (d, 1H, J = 7.32 Hz), 6.14 (m, 1H), 5.04 (m, 1H), 4.24 (m, 1H), 4.18 (m, 1H), 3.87 (m, 2H); ^{13}C NMR (CD_3OD) δ 145.4, 130.8, 116.1, 116.0, 106.3, 97.3, 83.6, 72.7, 60.7; IR (neat) 1695, 1650, 1560, 1487 cm^{-1}.

(−)-(2*S*,4*S*)-1-[2-(Hydroxymethyl)-1,3-dioxolan-4-yl]-4-*N*-(*p*-chlorobenzoyl)-cytosine (6q): Yield = 29%; mp 190.0−191.5 °C; $[\alpha]_D^{28}$ −84.15 (*c* 0.021, MeOH); UV (H_2O) λ_{max} 263 nm (ϵ 24 465 pH 2), 263 nm (ϵ 24 744 pH 7.4), 314 nm (ϵ 18 485 pH 11); ^{1}H NMR (CD_3OD) δ 8.53 (d, 1H, J = 7.32 Hz), 7.88 (m, 2H), 7.54 (d, 1H, J = 7.32 Hz), 7.42 (m, 2H), 6.16 (m, 1H), 5.06 (m, 1H), 4.26 (m, 1H), 4.21 (m, 1H), 3.88 (m, 2H); ^{13}C NMR (CD_3OD) δ 132.5, 130.3, 129.7, 107.0, 97.9, 84.3, 73.1, 61.1; IR (neat) 1698, 1651, 1557, 1483 cm^{-1}.

(−)-(2*S*,4*S*)-1-[2-(Hydroxymethyl)-1,3-dioxolan-4-yl]-4-*N*-(*p*-bromobenzoyl)-cytosine (6r): Yield = 56%; mp 189.0−191 °C; $[\alpha]_D^{26}$ −74.00 (*c* 0.039, MeOH); UV (H_2O) λ_{max} 265 nm (ϵ 28 850 pH 2), 265 nm (ϵ 30 085 pH 7.4), 316 nm (ϵ 23 659 pH 11); ^{1}H NMR (CD_3OD) δ 8.66 (d, 1H, J = 7.32 Hz), 7.88 (m, 2H), 7.71 (m, 2H), 7.59 (d, 1H, J = 7.32 Hz), 6.24 (m, 1H), 5.10 (m, 1H), 4.31 (m, 1H), 4.25 (m, 1H), 3.88 (m, 2H); ^{13}C NMR (CD_3OD) δ 146.4, 132.9, 130.7, 107.2, 98.0, 84.5, 73.2, 61.2; IR (neat) 1697, 1651, 1557, 1483 cm^{-1}.

(−)-(2*S*,4*S*)-1-[2-(Hydroxymethyl)-1,3-dioxolan-4-yl]-4-*N*-(*p*-methoxybenzoyl)-cytosine (6s): Yield = 68%; mp 181.0−181.5 °C; $[\alpha]_D^{27}$ −54.79 (*c* 0.028, MeOH); UV (H_2O) λ_{max} 303 nm (ϵ 28 704 pH 2), 302 nm (ϵ 29 283 pH 7.4), 302 nm (ϵ 25 191 pH 11); ^{1}H NMR (CD_3OD) δ 8.63 (d, 1H, J = 7.32 Hz), 7.97 (m, 2H), 7.58 (d, 1H, J = 7.32 Hz), 7.05 (m, 2H), 6.24 (m, 1H), 5.10 (m, 1H), 4.31 (m, 1H), 4.25 (m, 1H), 3.89 (m, 5H); ^{13}C NMR (CD_3OD) δ 146.5, 131.3, 115.1, 107.5, 98.1, 84.8, 73.3, 61.6, 56.1; IR (neat) 1646, 1567, 1488 cm^{-1}.

(−)-(2*S*,4*S*)-1-[2-(Hydroxymethyl)-1,3-dioxolan-4-yl]-4-N-(2,4-dichlorobenzoyl)-cytosine (6t): Yield = 84%; mp 185.0−187.0 °C; $[\alpha]_D^{25}$ −83.18 (*c* 0.036, MeOH); UV (H_2O) λ_{max} 253 nm (ϵ 22 561 pH 2), 253 nm (ϵ 23 043 pH 7.4), 305 nm (ϵ 21 989 pH 11); ^{1}H NMR (CD_3OD) δ 8.71 (d, 1H, J = 7.32 Hz), 7.62 (m, 2H), 7.56 (d, 1H, J = 7.32 Hz), 7.49 (m, 1H), 6.25 (m, 1H), 5.13 (m, 1H), 4.34 (m, 1H), 4.28 (m, 1H), 3.92 (m, 2H); ^{13}C NMR (CD_3OD) δ 147.0, 131.5, 131.0, 128.6, 107.5, 97.8, 84.8, 73.3, 61.5; IR (neat) 1705, 1647, 1557, 1490 cm^{-1}.

Biological Evaluation. The troxacitabine prodrugs were evaluated using two non-small cell lung cancer cell lines (A549 and SW1573). Prodrugs were dissolved in DMSO and diluted in media prior to treatment, with a final DMSO concentration less than 1%. The chemosensitivity assay used in this study was the sulforhodamine B (SRB) assay, as described earlier.[16] Cells were transferred on day 0 and treated with drug in triplicate on day 1. After incubation of 72 h, the cells were fixed for 1 h at 4 °C with 50% trichloroacetic acid, washed, air-dried, and stained with 0.4% SRB. The optical density was measured at 492 nm with a microplate reader (Tecan, Salzburg, Austria). Results were expressed as a percentage of control growth, 50% growth inhibition for the IC_{50}, and plotted to give the growth inhibition curve (Figure 2).

Supporting Information Available: Elemental analysis data is available for all compounds **6a**−**6t**. This data is available free of charge via the Internet at http://pubs.acs.org.

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