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Synthesis and Preliminary Cytotoxicity Study of Glucuronide Derivatives of CC-1065 Analogues

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Abstract—Glucuronide derivatives of CBI-bearing CC-1065 analogues have been synthesized, and their cytotoxicities tested against U937 leukemia cells. The new compounds show potent antitumor activity in vitro. Compounds **1** and **2**, and their corresponding glucuronides **3** and **4** have IC_{50} values of 0.6, 0.1, 1.4 and 0.6 nM, respectively. Glucuronide **3** is approximately 2-fold less toxic than its hydroxyl counterpart **1**, and glucuronide **4** is approximately 6-fold less toxic than its hydroxyl counterpart **2**. Glucuronides **3** and **4** may have limited use in the ADEPT approach. However, they may be used as antitumor agents in a conventional way.

Introduction

Anticancer agents generally act on metabolically active or rapidly proliferating cells, and cannot distinguish between cancer and normal cells. Thus, toxicity to normal cells limits the amount of drugs that can be given to patients. Therefore, much research has focused on the development of more specific therapeutic strategies to reduce toxicity to normal cells. One approach is antibody-directed enzyme prodrug therapy (ADEPT).[1,2] In this approach, an enzyme is conjugated to a tumor-specific antibody, which selectively localizes the enzyme to the tumor cell surface. Subsequent administration of a prodrug substrate of the enzyme leads to the enzyme-catalyzed release of the free drug at the tumor site. This strategy addresses the stoichiometry, controlled drug release and poor antibody penetration problems associated with the use of monoclonal antibody-drug conjugates.[3] Since the drug is released enzymatically, a single enzyme can generate a large amount of free drug at the tumor site. Consequently, a small amount of antibody can be used to reduce immunogenicity.

It is important that the free drug in the ADEPT approach is highly toxic. This will reduce the amount of the monoclonal antibody required, thereby reducing side effects. CC-1065 (Fig. 1) is among the most potent antitumor agents discovered.[4] It binds to double-stranded B-DNA within the minor groove with the sequence preference for 5′-d(A/GNTTA)-3′ and 5′-d(AAAAA)-3′, and alkylates the N3 position of the 3′-adenine with its left-hand CPI segment.[5] CC-1065 also inhibits gene transcription by interfering with binding of the TATA box binding protein to its target DNA.[6] Despite its high potency and broad spectrum of antitumor activity, CC-1065 cannot be used in humans because it causes delayed death in experimental animals.[7] To pursue compounds possessing the potent antitumor activity but devoid of the toxic side effects of the parent compound, many CC-1065 analogues have been synthesized.[8,9] To take advantage of the potent antitumor activity of the CC-1065 class of compounds and the ADEPT approach, we chose to make glucuronide derivatives as prodrug candidates (Fig. 2).

Glucuronide derivatives were selected because the level of β-glucuronidase is higher in human breast cancers than normal tissue,[10] and glucuronide derivatives appear to be less toxic than prodrugs incorporating other chemical groups.[11] Furthermore, glucuronide derivatives should be stable in blood after iv administration because the β-glucuronidase concentration in human serum is very low.[12] Although several organs including liver, GI tract, spleen, and lung do contain endogenous β-glucuronidases,[13] mammalian tissues also express uridine 5′-diphosphoglucuronyl transferase, a class of xenobiotic detoxification enzymes that can reverse the reaction catalyzed by β-glucuronidase.[14]

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We previously demonstrated that a butyramido substituent on the right-hand side of the CC-1065 analogues produced compounds with enhanced cytotoxicity.[9b,h,n,15] Thus, a butyramido group was selected to link together the sugar and the toxic CC-1065 analogues. Glucuronides **3** and **4** are expected to be less toxic than their hydroxyl counterparts **1** and **2**. However, the glucuronic acid moieties of **3** and **4** are expected to be cleaved by β-glucuronidase after removal of the ester groups on the sugar by carboxylate esterase (Fig. 3). Glucuronides bearing methyl and acetyl groups on the sugar may not be good substrates for β-glucuronidase. However, the ester groups of compounds **3** and **4** are expected to be cleaved by cellular carboxylate esterase, which is abundant in human serum, and the resulting products are expected to be substrates for β-glucuronidase. Herein, we report synthesis and preliminary cytotoxic effects of new glucuronide derivatives of CBI-bearing CC-1065 analogues.

Results and Discussion

Chemical synthesis

Target compound **4** can be dissected into three parts, namely the left-hand CBI, the middle-indoles and the right-hand glucuronate (Fig. 4). Because of the lengthy process and low overall yield in the synthesis of CBI, coupling the left-hand CBI with the indole(s)-glucuronate as the final synthetic step represents the best route for us. Retro-synthetic analysis shows that the key intermediate is **8**. Scheme 1 illustrates synthesis of **8** and its glucuronate conjugate **12**. Ethyl 5-nitroindole-2-carboxylate, **5**, was reduced to amine **6** by hydrogenation over Pd/C. The latter was treated with γ-butyrolactone to produce the key intermediate **7** with 44% overall yield from **5**. Basic hydrolysis of **7** using sodium hydroxide solution afforded the key intermediate **8** with 65% yield. The carboxylic acid group of **8** was selectively protected by treatment with benzyl bromide in the presence of sodium hydrogen carbonate in DMF to afford benzyl ester **9** with 71% yield. Through Koenigs–Knorr reaction, **9** was transformed to **11** by treatment with **10** catalyzed by silver triflate and silver carbonate. Hydrogenation of **11** over Pd/C smoothly removed the benzyl protective group, affording acid **12** with 66% overall yield from **9**.

Scheme 2 illustrates synthesis of acid **18**. Treatment of amine **6** with $(BOC)_2O$ produced **13**, which was then hydrolyzed using NaOH followed by HCl neutralization,

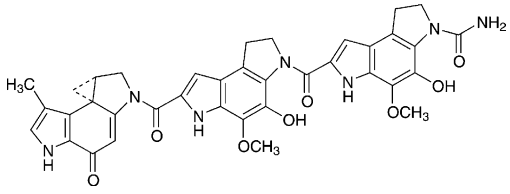


Figure 1. Structure of (+)-CC-1065.

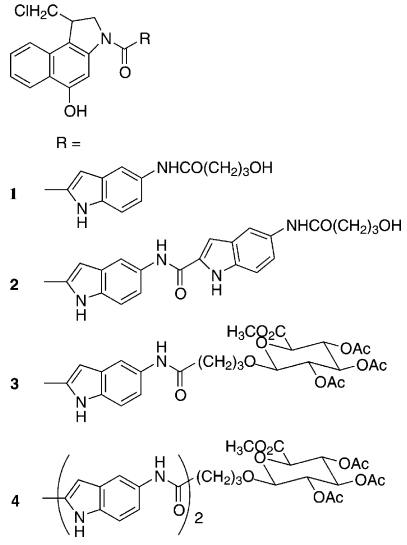


Figure 2. Structures of new agents.

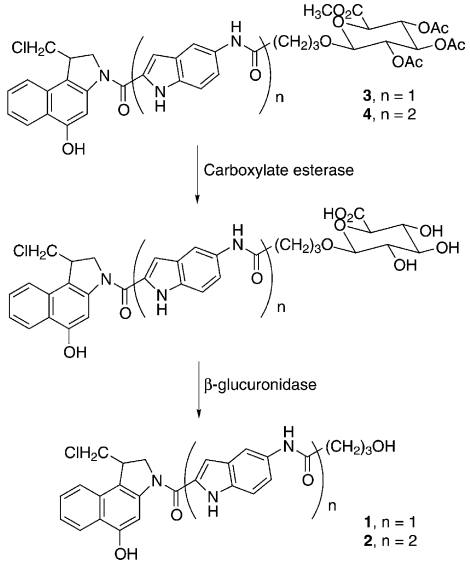


Figure 3. Proposed in situ conversion of prodrugs to free drugs.

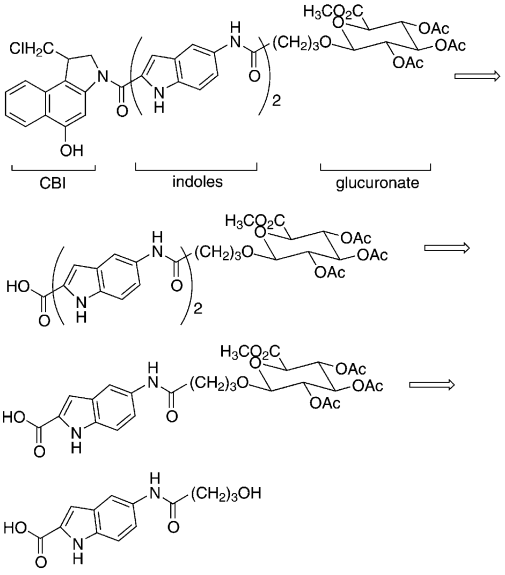
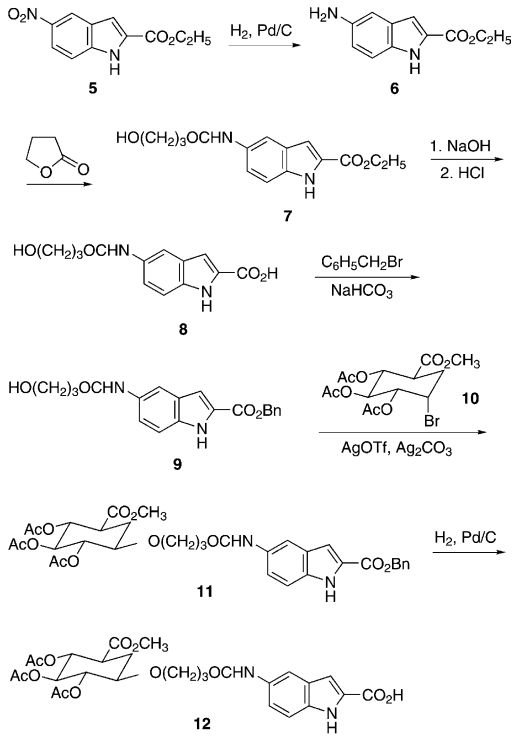
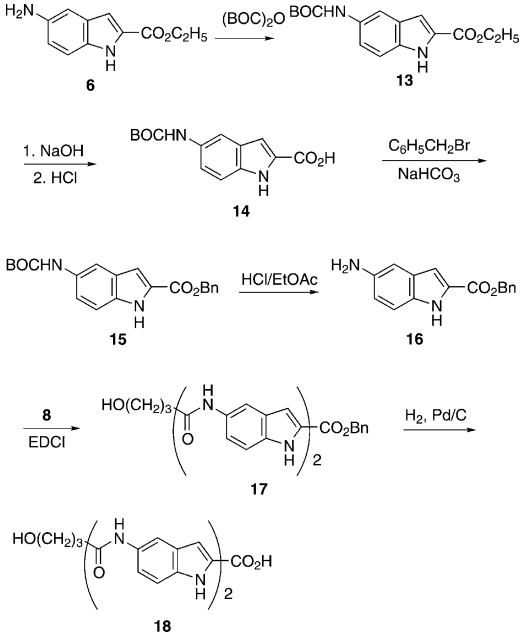


Figure 4. Retro-synthetic analysis.

affording acid **14** with 86% yield. Similar to the synthesis of **8**, the carboxylic acid group of **14** was protected as a benzyl ester by treatment with benzyl bromide in the presence of sodium hydrogen carbonate in DMF affording **15** with 84% yield. The protective group of **15** was removed by treatment with hydrochloride in ethyl acetate, affording **16**. The latter was coupled to **8** in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI), affording **17** (60% yield from **15**). The benzyl protective group of **17** was removed by hydrogenation over Pd/C, affording acid **18** with 84% yield.



Scheme 1. Synthesis of **12**.



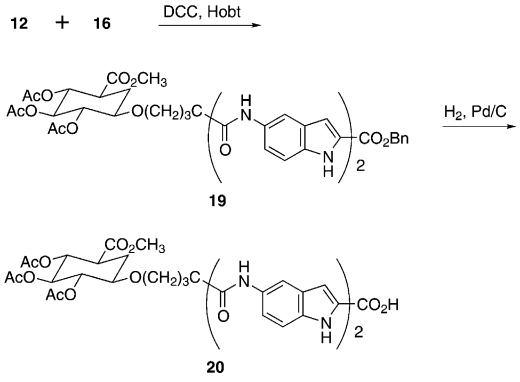
Scheme 2. Synthesis of **18**.

Scheme 3 illustrates synthesis of acid **20**. Acid **12** was coupled to amine **16** using 1,3-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazol (HOBT) in DMF produced **19** with 80% yield. The benzyl group of **19** was removed by hydrogenation over Pd/C to afford **20** with 86% yield.

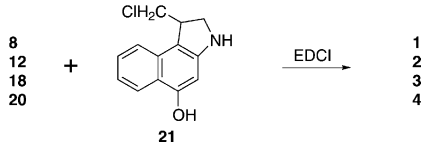
Scheme 4 illustrates the final synthesis of free drugs, **1** and **2**, and prodrugs, **3** and **4**. A solution of 1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (CBI), which was synthesized according to a reported procedure, was treated with hydrogen chloride in ethyl acetate to produce intermediate **21**.[16] The latter was coupled, respectively, to acids **8**, **12**, **18**, and **20** in the presence of EDCI in DMF, affording the target compounds **1–4**.

Cytotoxicity studies

Compounds **1–4** were tested against U937 monocytic leukemia cells, and the results are shown in Table 1. The IC_{50} values of compounds **1** and **2** are 0.6 and 0.1 nM, respectively, against U937 cells in vitro. The IC_{50} values of glucuronides **3** and **4** are 1.4 and 0.6 nM, respectively. Glucuronide **3** is approximately 2-fold less toxic than its hydroxyl counterpart **1**, and glucuronide **4** is approximately 6-fold less toxic than its hydroxyl counterpart **2**.



Scheme 3. Synthesis of **20**.



Scheme 4. Synthesis of **1–4**.

Table 1. Cytotoxicity of compounds **1–4** against U937 leukemia cells in vitro[a]

Compd	IC_{50} (nM)[b]
1	0.6
2	0.1
3	1.4
4	0.6

[a]Cells were incubated with drugs for 48 h and the experiments were performed according to our previously published method..[9n]

[b]IC_{50} values are defined as the minimal drug concentration necessary to inhibit incorporation of [^{3}H]thymidine by 50%, and are the averages of three experiments.

The drugs are designed that the acetyl groups of the glucuronide will be removed in-situ to generate a glucuronic acid derivative, which will be a substrate of β-glucuronidase. When the glucuronide was first incubated with carboxylate esterase and β-glucuronidase, and then tested for cytotoxicity, surprisingly, we found no difference between the cytotoxicity of the glucuronide with or without prior incubation with carboxylate esterase and β-glucuronidase (data not shown). There are two possibilities for this result. First, the acetyl groups may not be removed by the esterase, and second, even if the acetyl groups are removed, the glucuronic acid derivative produced may not be a good substrate for the β-glucuronidase. As a result, glucuronides 3 and 4 may not be good prodrug candidates for the ADEPT approach. However, they may be used as antitumor agents in a conventional way.

Experimental

Chemistry

Melting points were measured using a Mel-Temp II and are uncorrcted. ^{1}H NMR spectra were recorded at ambient temperature on an NT-360 spectrometer. High-resolution mass spectra (FABHRMS) were recorded on a modified MS50 mass spectrometer equipped with a VG 11-250J data system. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA, USA, and the results were within ±0.4% of the theoretical values unless otherwise noted. Analytical thin-layer chromatography was performed on silica-coated plastic plates (silica gel 60 F-254, Merck) and visualized under UV light. Preparative separations were performed by flash chromatography on silica gel (Merck, 70–230 mesh). Tetrahydrofuran (THF) was dried by distillation from sodium-benzophenone ketyl. Dimethylformamide (DMF) and triethylamine were dried over molecular sieves (4A) before use. The above solvents were stored over molecular sieves (4A). All other solvents were used as received and were reagent grade, where available.

5-[(4-Hydroxy)butyramido]indole-2-carboxylic acid (8). A solution of **5** (2.5 g, 10.7 mmol) in ethyl acetate (200 mL) was treated with 10% palladium on activated carbon (0.6 g), and was then hydrogenated for 1 h at room temperature. The reaction mixture was filtered through Celite, and the filter cake was washed with ethyl acetate. The combined organic solution was concentrated in vacuo. Without further purification, the resulting oil was dissolved in butyrolactone (20 mL), and the solution was stirred for 18 h at 130 °C. The product was purified by flash chromatography eluting with 30% hexane in ethyl acetate to give **7** (1.37 g, 44% yield from **5**) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 11.72 (s, 1H, NH), 9.73 (s, 1H, CONH), 8.00–7.07 (m, 4H, Ar-H), 4.47–4.44 (t, 1H, J = 4.8 Hz, OH), 4.36–4.30 (q, 2H, J = 7.0, 14.2 Hz, CH_2CH_3), 3.47–3.42 (q, 2H, J = 6.1, 11.4 Hz, $HOCH_2$), 2.36–2.32 (t, 2H, J = 7.4 Hz, $COCH_2$), 1.77–1.73 (m, 2H, $CH_2CH_2CH_2$), 1.36–1.32 (t, 3H, J = 7.2, CH_2CH_3). A solution of **7** (1.0 g, 3.4 mmol) in methanol (150 mL) was treated with 3 N sodium hydroxide solution (20 mL), and was stirred for 18 h at room temperature. The solution was then concentrated in vacuo. Water was added (100 mL). The solution was neutralized with 20% hydrochloric acid, and the precipitate was filtered to afford **8** (723 mg, 65% yield) as a brown solid, mp 194–195 °C. ^{1}H NMR (DMSO-d_6) δ 12.80 (brs, 1H, COOH), 11.60 (s, 1H, NH), 9.72 (s, 1H, CONH), 7.99–7.02 (m, 4H, Ar-H), 3.47–3.43 (t, 2H, J = 6.8, $HOCH_2$), 2.37–2.32 (t, 2H, J = 7.4, $COCH_2$), 1.77–1.73 (m, 2H, $CH_2CH_2CH_2$). Anal. ($C_{13}H_{14}N_2O_4$), C, H, N.

Benzyl 5-[(4-hydroxy)butyramido]indole-2-carboxylate (9). A solution of **8** (810 mg, 3.1 mmol) in DMF (15 mL) was treated with sodium bicarbonate (1.6 g, 19.0 mmol) and benzyl bromide (0.54 mL, 4.5 mmol). The reaction mixture was stirred for 18 h at room temperature. The reaction was quenched with water (80 mL), and the product was extracted with ethyl acetate (50 mL × 5). The solution was dried using sodium sulfate, and was concentrated in vacuo. The product was purified by flash chromatography eluting with 50% hexane in ethyl acetate to give **9** (778 mg, 71% yield) as a solid, mp 191–192 °C. ^{1}H NMR (DMSO-d_6) δ 11.78 (s, 1H, NH), 9.73 (s, 1H, CONH), 8.00–7.14 (m, 9H, Ar–H), 5.37 (s, 2H, $CH_2C_6H_5$), 4.46–4.44 (t, 1H, J = 5.2 Hz, OH), 3.47–3.42 (q, 2H, J = 6.6, 11.7 Hz, $HOCH_2$), 2.36–2.32 (t, 2H, J = 7.4, $COCH_2$), 1.78–1.71 (m, 2H, $CH_2CH_2CH_2$). Anal. ($C_{20}H_{20}N_2O_4 + 0.3H_2O$), C, H, N.

5-[4-(Methyl 2,3,4-tri-*O*-acetyl-1-deoxy-β-D-glucuronate)butyramido]indole-2-carboxylic acid (12). Molecular sieves (1 g) were added to a solution of **9** (778 mg, 2.21 mmol) in THF (20 mL) and CH_2Cl_2 (20 mL), and the temperature was cooled to −60 °C under nitrogen. Methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate (**10**, 1.32 g, 3.3 mmol), silver trifluoromethanesulfonate (852 mg, 3.3 mmol) and silver carbonate (922 mg, 3.3 mmol) was added sequentially, and the reaction mixture was stirred for 10 min. The temperature was allowed to warm up to room temperature, and the stirring was continued for an additional 2 h in the dark. The mixture was filtered through Celite, and the filter cake was washed with ethyl acetate. The solution was dried with sodium sulfate, and then was concentrated in vacuo. The product was purified by flash chromatography eluting with 50% hexane in ethyl acetate to give **11** as white foam. ^{1}H NMR (acetone-d_6) δ 8.88 (s, 1H, CONH), 7.95 (s, 1H, Ar–H), 7.47–7.21 (m, 9H, Ar–H), 5.86–5.85 (d, 1H, J = 4.5 Hz, sugar C1-H), 5.38 (s, 2H, $CH_2C_6H_5$), 5.22–5.21 (t, 1H, J = 2.4 Hz, sugar C2-H), 5.16–5.14 (m, 1H, sugar C4-H), 4.34–4.30 (m, 2H, sugar C3-H, C5-H), 3.76 (s, 3H, $COOCH_3$), 3.63–3.59 (m, OCH_2), 2.47–2.44 (t, 2H, J = 6.7 Hz, CH_2CO), 2.17 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 2.03–2.00 (m, 2H, $CH_2CH_2CH_2$), 1.75 (s, 3H, CH_3CO). Without further purification, the foam was dissolved in ethyl acetate (15 mL). The mixture was treated with 10% palladium on activated charcoal (600 mg), and was hydrogenated for 1 h at room temperature. The mixture was filtered through Celite, and the filter cake was washed with ethyl acetate. The combined organic

solution was concentrated in vacuo to give **12** (846 mg, 66% yield from **9**) as a white foam, which was crystallized from petroleum ether, mp 94–96 °C. ^{1}H NMR (DMSO-d_6) δ 11.43 (s, 1H, NH), 9.69 (s, 1H, CONH), 7.94–6.91 (m, 4H, Ar–H), 5.88–5.87 (d, 1H, J = 4.2 Hz, sugar C1-H), 5.09–5.07 (t, 1H, J = 3.2 Hz, sugar C2-H), 5.05–5.02 (m, 1H, sugar C4-H), 4.42–4.40 (d, 1H, J = 6.2 Hz, sugar C5-H), 4.36–4.35 (t, 1H, J = 3.3 Hz, sugar C3-H), 3.69 (s, 3H, $COOCH_3$), 3.51–3.48 (t, 2H, J = 6.4 Hz, OCH_2), 2.37–2.33 (t, 2H, J = 7.6 Hz, CH_2CO), 2.05 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 1.82–1.79 (m, 2H, $CH_2CH_2CH_2$), 1.65 (s, 3H, CH_3CO). FABHRMS calcd for $C_{26}H_{30}N_2O_3$ + Na 601.1646; found 601.1633.

Ethyl 5-(*tert*-butyloxycarbonylamino)indole-2-carboxylate (13). A solution of **6**, which was made from **5** (2.5 g, 10.7 mmol), in ethyl acetate (150 mL) was treated with di-*tert*-butyl dicarbonate (5.8 g, 26.6 mmol), and the reaction mixture was stirred for 18 h at room temperature. The reaction was quenched with water (40 mL), and the ethyl acetate solution was separated. The solution was dried with sodium sulfate, and was then concentrated in vacuo to give **13** (2.61 g, 80%) as a yellow solid, mp 190–192 °C. ^{1}H NMR (DMSO-d_6) δ 11.69 (s, 1H, NH), 9.13 (s, 1H, CONH), 7.79–7.04 (m, 4H, Ar-H), 4.35–4.30 (q, 2H, J = 6.8, 13.9 Hz, CH_2CH_3), 1.48 (s, 9H, $C(CH_3)_3$), 1.35–1.31 (t, 3H, J = 6.7, CH_2CH_3). Anal. ($C_{16}H_{20}N_2O_4$), C, H, N.

5-(*tert*-Butyloxycarbonylamino)indole-2-carboxylic acid (14). A solution of **13** (2.61 g, 8.6 mmol) in methanol (200 mL) was treated with 3 N sodium hydroxide solution (50 mL), and the reaction mixture was stirred for 18 h at room temperature. The solvent was removed. Water (100 mL) was added. The solution was neutralized with 20% hydrochloric acid, and the precipitate was filtered to give **14** (2.03 g, 86%) as a yellow solid, mp 192–193 °C. ^{1}H NMR (DMSO-d_6) δ 12.83 (brs, 1H, COOH), 11.69 (s, 1H, NH), 9.13 (s, 1H, CONH), 7.79–7.04 (m, 4H, Ar-H), 1.48 (s, 9H, $C(CH_3)_3$). Anal. ($C_{14}H_{16}N_2O_4 + 0.5H_2O$), C, H, N.

Benzyl 5-(*tert*-butyloxycarbonylamino)indole-2-carboxylate (15). A solution of **14** (2.03 g, 7.4 mmol) in DMF (50 mL) was treated with sodium bicarbonate (1.8 g, 21.4 mmol) and benzyl bromide (4 mL, 33.6 mmol). The reaction mixture was stirred for 18 h at room temperature. Water (50 mL) was added, and the product was extracted with ethyl acetate (80 mL × 3). The solution was dried with sodium sulfate, and was concentrated in vacuo to give **15** (2.27 g, 84% yield) as a white solid, mp 180–182 °C. ^{1}H NMR (DMSO-d_6) δ 11.93 (s, 1H, NH), 7.51–7.02 (m, 9H, Ar-H), 5.38 (s, 2H, $CH_2C_6H_5$), 1,48 (s, 9H, $C(CH_3)_3$). Anal. ($C_{21}H_{22}N_2O_4 + 0.25H_2O$), C, H, N.

5-[5-[4-(Hydroxy)butyramido]-1*H*-indol-2-ylcarbonyl]-amino]-1*H*-indol-2-carboxylic acid (18). A solution of **15** (280 mg, 0.77 mmol) in ethyl acetate (20 mL) saturated with hydrogen chloride was refluxed for 30 min. The suspension was concentrated in vacuo to give a solid, which was then treated with triethylamine (6 mL) in ethyl acetate (20 mL). The reaction mixture was stirred at room temperature for 10 min, and was filtered. The solution was concentrated in vacuo to afford **16**. Without further purification, the latter was dissolved in DMF (1 mL), and treated with **8** (200 mg, 0.76 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI, 440 mg, 2.30 mmol). The reaction mixture was stirred for 18 h at room temperature. The mixture was then diluted with water (20 mL), and was extracted with ethyl acetate (50 mL × 3). The organic layer was dried with sodium sulfate, and concentrated in vacuo. The product was purified by flash chromatography eluting with ethyl acetate to give **17** (232 mg, 60% yield) as a pale-yellow solid, mp 242–243 °C. ^{1}H NMR (DMSO-d_6) δ 11.87 (s, 1H, NH), 11.55 (s, 1H, NH), 10.08 (s, 1H, CONH), 9.71 (s, 1H, CONH), 8.13–7.22 (m, 13H, Ar-H), 5.39 (s, 2H, $CH_2C_6H_5$), 4.47–4.45 (t, 1H, J = 5.1 Hz, OH), 3.48–3.44 (q, 2H, J = 6.4, 11.9 Hz, $HOCH_2$), 2.38–2.33 (t, 2H, J = 7.5 Hz, $COCH_2$), 1.78–1.74 (m, 2H, $CH_2CH_2CH_2$). Without further purification, a solution of **17** (230 mg, 0.45 mmol) in DMF (1 mL) and methanol (20 mL) was treated with 10% palladium in activated carbon (20 mg), and hydrogenated for 1 h at room temperature. The mixture was filtered through Celite, and the filter cake washed with methanol. The combined organic solution was concentrated in vacuo to give **18** (160 mg, 84%) as a white solid, mp 249–251 °C. ^{1}H NMR (DMSO-d_6) δ 12.75 (s, 1H, COOH), 11.68 (s, 1H, NH), 11.54 (s, 1H, NH), 10.05 (s, 1H, CONH), 9.70 (s, 1H, CONH), 8.12–7.09 (m, 8H, Ar-H), 4.47–4.44 (t, 1H, J = 5.4 Hz, OH), 3.49–3.44 (q, 2H, J = 6.4, 11.0 Hz, $HOCH_2$), 2.38–2.34 (t, 2H, J = 7.4 Hz, $COCH_2$), 1.80–1.74 (m, 2H, $CH_2CH_2CH_2$). FABHRMS calcd for $C_{22}H_{20}N_4O_5$ 421.1511; found 421.1481.

5-[5-[4-(Methyl 2,3,4-tri-*O*-acetyl-1-deoxy-β-D-glucuronate)butyramido]-1*H*-indol-2-ylcarbonyl]amino]-1*H*-indol-2-carboxylic acid (20). A solution of **16** (95 mg, 0.35 mmol) in DMF (1 mL) was treated with **12** (200 mg, 0.35 mmol), 1, 3-dicyclohexylcarbodiimide (DCC, 214 mg, 1.04 mmol) and 1-hydroxybenzotriazole hydrate (HOBT, 140 mg, 1.04 mmol). The reaction mixture was stirred at room temperature for 2 h, which was then diluted with water (10 mL), and extracted with ethyl acetate (50 mL × 3). The solution was dried with sodium sulfate, and concentrated in vacuo. The product was purified by flash chromatography eluting with a solution of hexane and ethyl acetate (3/7, v/v) to afford **19** (230 mg, 80% yield) as a yellow solid, mp 143–145 °C. ^{1}H NMR (DMSO-d_6) δ 11.87 (s, 1H, NH), 11.56 (s, 1H, NH), 10.08 (s, 1H, CONH), 9.73 (s, 1H, CONH), 8.14–7.22 (m, 13H, Ar-H), 5.89–5.88 (d, 1H, J = 4.4 Hz, sugar C1-H), 5.39 (s, 2H, $CH_2C_6H_5$), 5.09–5.08 (t, 1H, J = 2.9 Hz, sugar C2-H), 5.05–5.02 (m, 1H, sugar C4-H), 4.43–4.41 (d, 1H, J = 6.7 Hz, sugar C5-H), 4.37–4.35 (t, 1H, J = 3.4 Hz, sugar C3-H), 3.69 (s, 3H, $COOCH_3$), 3.52–3.49 (t, 2H, J = 6.4 Hz, OCH_2), 2.39–2.34 (t, 2H, J = 7.7 Hz, $COCH_2$), 2.05 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 1.84–1.80 (m, 2H, $CH_2CH_2CH_2$), 1.66 (s, 3H, CH_3CO). Without further purification, a solution of **19** (230 mg, 0.28 mmol) in ethyl acetate (35 mL) was treated with 10% palladium on activated charcoal (20 mg), and was then hydrogenated for 1 h at

room temperature. The mixture was filtered through Celite, and the filter cake was washed with ethyl acetate. The combined organic solution was concentrated in vacuo to give **20** (176 mg, 86% yield) as a yellow oil, which crystallized from ether on standing, mp 76–79 °C. ^{1}H NMR (DMSO-d_6) δ 12.81 (brs, 1H, COOH), 11.67 (s, 1H, NH), 11.54 (s, 1H, NH), 10.05 (s, 1H, CONH), 9.72 (s, 1H, CONH), 8.12–7.08 (m, 8H, Ar-H), 5.89–5.88 (d, 1H, J = 4.7 Hz, sugar C1-H), 5.09–5.08 (t, 1H, J = 3.0 Hz, sugar C2-H), 5.05–5.03 (m, 1H, sugar C4-H), 4.42–4.41 (d, 1H, J = 6.6 Hz, sugar C5-H), 4.37–4.35 (t, 1H, J = 3.2 Hz, sugar C3-H), 3.69 (s, 3H, $COOCH_3$), 3.52–3.49 (t, 2H, J = 5.8 Hz, OCH_2), 2.39–2.34 (t, 2H, J = 7.8 Hz, $COCH_2$), 2.05 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 1.84–1.80 (m, 2H, $CH_2CH_2CH_2$), 1,66 (s, 3H, CH_3CO). FABHRMS calcd for $C_{35}H_{36}N_4O_{14}$ + Na 759.2126; found 759.2161.

3-[5-[(4-Hydroxy)butyramido]-1*H*-indol-2′-yl]carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (1). A solution of (±)-CBI (15 mg, 0.076 mmol) in 3 mL of ethyl acetate saturated with hydrogen chloride was stirred at room temperature for 30 min in the dark. The suspension was concentrated in vacuo to give intermediate **21**. The latter was dissolved in DMF (0.5 mL), and was treated with **8** (20 mg, 0.078 mmol) and EDCI (16.5 mg, 0.086 mmol). The reaction mixture was stirred at room temperature overnight, and the product was purified by thin layer chromatography eluting 50% hexane in ethyl acetate to give **1** (12 mg, 36% yield) as a yellow solid. ^{1}H NMR (acetone-d_6) δ 10.73 (s, 1H, NH), 9.27 (s, 1H, Ar-OH), 9.08 (s, 1H, CONH), 8.27–7.20 (m, 9H, Ar–H), 4.84–4.80 (m, 2H, NCH_2), 4.33–4.26 (m, 1H, $ClCH_2CH$), 4.09–4.05 (dd, 1H, J = 3.6, 11.2 Hz, ClC*H*H), 3.85–3.79 (dd, 1H, J = 8.5, 11.2 Hz, ClCH*H*), 3.73–3.69 (t, 1H, J = 5.6, CH_2OH), 3.66–3.61 (q, 2H, J = 5.9, 11.5 Hz, CH_2OH), 2.51–2.47 (t, 2H, J = 7.1, Hz, $COCH_2$), 1.92–1.89 (m, 2H, $CH_2CH_2CH_2$). FABHRMS calcd for $C_{26}H_{25}ClN_3O_4$ 478.1533; found 478.1527.

Compounds **2**–**4** were synthesized using a procedure similar to that described for the synthesis of **1**.

3-[5-[5-[4-(Hydroxy)butyramido]-1*H*-indol-2-ylcarbonyl]-amino]-1*H*-indol-2-yl]carbonyl]-1-(chloromethyl)-5-hydroxy-1, 2-dihydro-3*H*-benz[*e*]indole (2). Yellow solid (19% yield). ^{1}H NMR (DMF-d_6) δ 11.74 (s, 1H, NH), 11.62 (s, 1H, NH), 10.47 (s, 1H, Ar–OH), 10.44 (s, 1H, CONH), 9.89 (s, 1H, CONH), 8.43–7.06 (m, 13H, Ar-H), 4.95–4.86 (m, 1H, NC*H*H), 4.76–4.72 (m, 1H, NCH*H*), 4.60–4.54 (brs, 1H, CH_2OH), 4.34–4.28 (m, 1H, $ClCH_2CH$), 4.15–4.11 (dd, 1H, J = 3.2, 10.7 Hz, ClC*H*H), 3.98–3.93 (dd, 1H, J = 8.2, 11.1 Hz, ClCH*H*), 3.66–3.64 (m, 2H, CH_2OH), 2.52–2.48 (t, 2H, J = 7.4, Hz, $COCH_2$), 1.92–1.85 (m, 2H, $CH_2CH_2CH_2$). FABHRMS calcd for $C_{35}H_{31}ClN_5O_5$ 636.2014; found 636.2017.

3-[5-[4-(Methyl 2,3,4-tri-*O*-acetyl-1-deoxy-β-D-glucuronate) butyramido]-1*H*-indol-2′-yl]carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (3). Yellow solid (12% yield). ^{1}H NMR (DMF-d_6) δ 11.56 (s, 1H, NH), 10.5 (brs, 1H, OH), 9.84 (s, 1H, CONH), 8.25–7.23 (m, 9H, Ar-H), 5.99–5.98 (d, 1H, J = 4.1 Hz, sugar C1-H), 5.22–5.20 (t, 1H, J = 2.9 Hz, sugar C2-H), 5.15–5.13 (m, 1H, sugar C4-H), 4.90–4.84 (t, 1H, J = 8.6, NC*H*H), 4.75–4.71 (dd, 1H, J = 1.1, 12.2 Hz, NCH*H*), 4.51–4.48 (m, 2H, sugar C3-H, C5-H), 4.33–4.28 (m, 1H, $ClCH_2CH$), 4.14–4.10 (dd, 1H, J = 2.6, 10.6 Hz, ClC*H*H), 3.96–3.91 (dd, 1H, J = 7.8, 10.9 Hz, ClCH*H*), 3.77 (s, 3H, $COOCH_3$), 3.63–3.59 (t, 2H, J = 6.9 Hz, OCH_2), 2.50–2.46 (t, 2H, J = 7.1 Hz, $COCH_2$), 2.10 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 1.96–1.90 (m, 2H, $CH_2CH_2CH_2$), 1,72 (s, 3H, CH_3CO). FABHRMS calcd for $C_{39}H_{40}ClN_3O_{13}$ + Na 816.2147; found 816.2152.

3-[5-[5-[4-(Methyl 2,3,4-tri-*O*-acetyl-1-deoxy-β-D-glucuronate) butyramino]-1*H*-indol-2-ylcarbonyl]amino]-1*H*-indol-2-yl]carbonyl]-1-(chloromethyl)-5-hydroxy-1, 2-dihydro-3*H*-benz[*e*]indole (4). Yellow solid (15% yield). ^{1}H NMR (DMF-d_6) δ 11.61 (s, 2H, 2 NH), 10.20 (s, 1H, CONH), 9.79 (s, 1H, CONH), 8.38–7.27 (m, 13H, Ar-H), 5.99–5.98 (d, 1H, J = 4.6 Hz, sugar C1-H), 5.22–5.20 (t, 1H, J = 3.1 Hz, sugar C2-H), 5.16–5.13 (m, 1H, sugar C4-H), 4.91–4.86 (t, 1H, J = 8.7 Hz, NC*H*H), 4.77–4.73 (dd, 1H, J = 1.8, 11.4 Hz, CH*H*Cl), 4.51–4.48 (m, 2H, sugar C3-H, C5-H), 4.34–4.32 (m, 1H, $ClCH_2CH$), 4.15–4.11 (dd, 1H, J = 3.4, 11.2 Hz, ClC*H*H), 3.98–3.92 (dd, 1H, J = 8.0, 11.1 Hz, ClCH*H*), 3.77 (s, 3H, $COOCH_3$), 3.63–3.59 (t, 2H, J = 6.6 Hz, OCH_2), 2.50–2.46 (t, 2H, J = 7.3 Hz, $COCH_2$), 2.10 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 1.94–1.90 (m, 2H, $CH_2CH_2CH_2$), 1,72 (s, 3H, CH_3CO). FABHRMS calcd for $C_{48}H_{46}ClN_5O_{14}$ + Na 974.2628; found 974.2625.

Cytotoxicity studies

Cell lines. The human monocytic leukemia, U937, was obtained from the ATCC, and cultured in RPMI-1640 plus 10% FCS in the absence of antibiotics. The cells were routinely tested for mycoplasma contamination, and in all cases found to be negative.

The drugs were dissolved in DMF to provide a stock solution of 1 mg/mL, and were stored at −20 °C. For each experiment, drug solutions were freshly prepared from the stock solution by addition of sterile water to afford concentrations suitable for the experiment.

The cytotoxic effects of the drugs were measured by inhibition of DNA synthesis. U937 cells in RPMI-1640 plus 10% FCS medium were seeded at 5×10^4 cells/well in a 96-well plate. Drugs (10 μL) at increasing concentrations were added to each well and the total volume was adjusted to 0.1 mL/well using the same medium. The plate was incubated for 24 h at 37 °C followed by addition of 10 μL of ^{3}H-thymidine (20 μCi/mL). The plate was incubated for another 24 h, and the cells were harvested and radioactivity was counted using the Packard Matrix 96 beta counter. The results are expressed as the percent growth inhibition (IC_{50}), calculated as follows: percent growth inhibition (IC_{50}) = [(total cpm −experimental cpm)/total cpm] × 100.

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