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A POTENT, SIMPLE DERIVATIVE OF AN ANALOG OF THE CC-1065 ALKYLATION SUBUNIT

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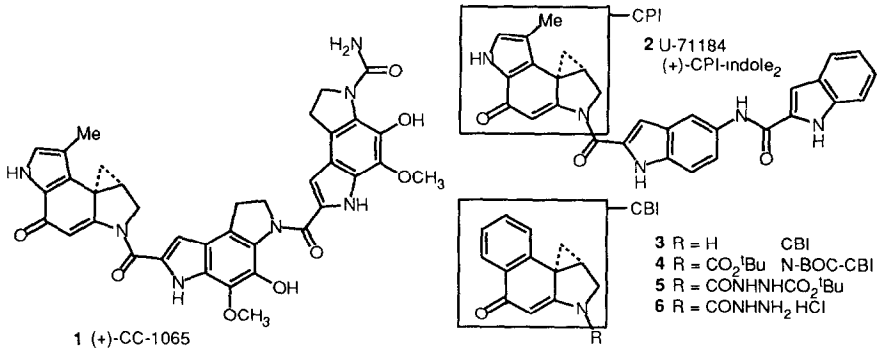
Abstract: A simple semicarbazide derivative of CBI, an analog of the authentic CPI alkylation subunit of CC-1065, exhibits enhanced in vitro cytotoxic potency and DNA alkylation properties (ca. 100x) derived from noncovalent electrostatic DNA binding.

(+)-CC-1065 (**1**), a potent antitumor antibiotic isolated from cultures of *Streptomyces zelensis*, possesses potent in vitro cytotoxic activity, broad spectrum antimicrobial activity, and in vivo antitumor activity.[2] In a series of investigations, the site and mechanism of the antitumor activity have been correlated with the (+)-CC-1065 DNA alkylation within A-T rich minor groove regions which has been shown to proceed by 3′-adenine *N*-3 alkylation of the electrophilic cyclopropane present in the CPI left-hand subunit.[2-3] In the conduct of extensive efforts to separate the delayed toxicity characteristic of the natural product[4] from its productive antitumor activity, the preparation of a series of agents that bear the authentic CPI alkylation subunit have been detailed that maintain the potent in vitro cytotoxic activity and possess more efficacious in vivo antitumor activity than (+)-CC-1065 but which lack the characteristic delayed toxicity of the natural product.[2-6] The prototype of such clinical candidates introduced at UpJohn is U71184 (**2**).[5,6] Characteristic of the natural product and such agents has been the hydrophobic central and right-hand subunits that serve a functional role of providing pronounced DNA binding affinity[2] and specificity[7-10] to the agent effectively delivering the selective electrophile to accessible adenine alkylation sites. In contrast, simple agents lacking the additional hydrophobic central and right-hand subunits exhibit modest cytotoxic potency (*ca.* 10000x (+)-CC-1065), weak DNA alkylation capabilities (*ca.* 10000x (+)-CC-1065), and efficacious antitumor activity albeit at higher dosage levels (*ca.* 100x).[2,3,5,6]



In our own complementary studies designed to address the structural origin of the (+)-CC-1065 sequence-selective DNA alkylation,[7-15] we have prepared agents that possess modified alkylation subunits including those that possess the more stable 1,2,9,9a-tetrahydrocyclopropa[1,2-*c*]benz[1,2-*e*]indol-4-one (CBI) left-hand subunit[19] Herein, we detail the preparation and preliminary evaluation of **5** and **13**, simple derivatives of the synthetic CBI alkylation subunit which possesses enhanced DNA alkylation capabilities and in vitro cytotoxic potency by virtue of stabilizing electrostatic versus hydrophobic DNA binding. That is, in place of the DNA binding affinity derived from the hydrophobic binding and stabilizing van der Waals contacts provided by the central and right-hand subunits of **1-2**, the simple electrostatic binding affinity provided by the protonated amine of **5** and **13** with anionic DNA proved sufficient to substantially enhance the DNA alkylation intensity and in vitro cytotoxic activity

The semicarbazide of (+)-CBI and its seco chloride precursor were prepared as detailed in Scheme I. Treatment of bis-(2,4-dinitrophenyl)carbonate (**8**)[16] with *tert*-butyl carbazate (**7**, 1 equiv, 24°C, 2 h, EtOAc) provided **9**[17] (61%) and a convenient acylating agent for introduction of the *tert*-butyloxycarbonyl protected hydrazide. *N*-deprotection of **10** (3 N HCl/EtOAc, 24°C, 20 min, quant) followed by immediate treatment of the unstable amine hydrochloride salt **11** with **9** (1.3 equiv, 1 equiv Et_3N, 24°C, 5.5 h, THF, 91%) provided **12** in excellent yield[18] Acid-catalyzed *tert*-butyloxycarbonyl deprotection of **12** provided **13**[18] and exposure of **12** or **13** to 5% aq. $NaHCO_3$-THF (24°C) provided **5** or **6**, respectively.

The results of the preliminary in vitro cytotoxic evaluation of the *N*-semicarbazide of (+)-CBI conducted on its more stable seco precursor **13**[19] are detailed in Table 1 along with the comparative results from the evaluation of **3-4** and **12**. Notably, agent **13** possessing the free amine exhibited more potent in vitro cytotoxic activity than its precursor possessing the *tert*-butylcarbazate (**12**, *ca.* 100x) or *N*-BOC-CBI (**4**) itself, and proved to be only 100x less potent than (+)-CC-1065.

Consistent with the trends observed in the relative cytotoxic potency of the agents, the intensity of DNA alkylation similarly increased with the introduction of the free semicarbazide. Singly 5′-^{32}P-end-labeled double-stranded DNA constituting SV40 DNA nucleotides no. 5238-138 (144 base-pairs) cloned into the *Sma* I site of M13mp10[12] was treated with the agents at a range of concentrations. Removal of the unreacted agent through ethanol precipitation of the DNA, redissolution of the alkylated DNA in aqueous buffer, thermally-induced cleavage of the DNA at the sites of covalent alkylation (100°C, 30 min) revealed the agent sites of alkylation and their relative intensities, Figure 1.[12] Consistent with the relative trends observed with the in vitro cytotoxic activity of the agents, the agent **13** bearing the protonated amine exhibited a more intense alkylation of DNA than the corresponding carbazate **12** (*ca* 10-100x) or *N*-BOC-CBI (**4**, ≥ 1000x) and proved to be only 100-1000x less effective than (+)-CC-1065.

Scheme I.

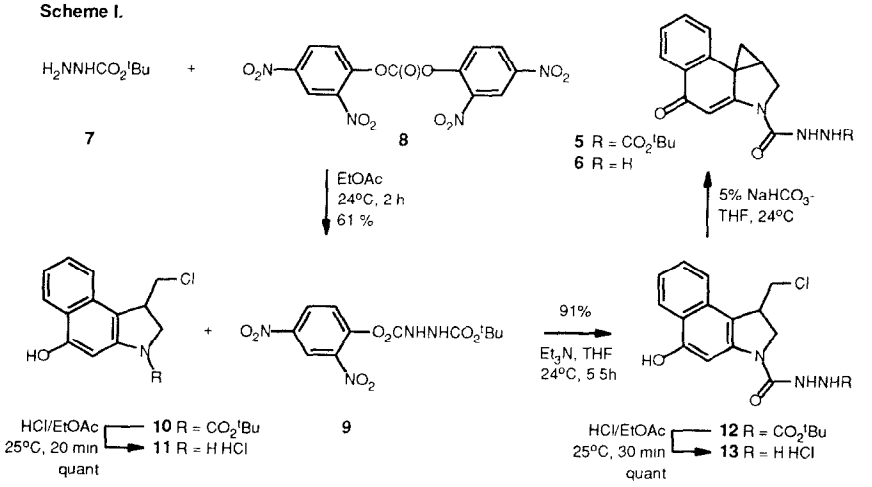


Table 1.	(±)-13	(±)-12	(+)-CC-1065 (1)	(±)-*N*-BOC-CBI (4)	(±)-CBI (3)
IC_{50} (L1210, μg/mL)[a]	0.001	0.1	0.00001	0.05	2
Relative IC_{50}	(1)	(0.01)	(100)	(0.02)	(0.0005)
Relative Intensity of DNA Alkylation[b]					
4°C, 24 h	0.1	0.01-0.001	100	-[c]	-[c]
37°C, 24 h	1	0.1-0.01	100	0.001	-[c]

[a]IC_{50}, Inhibitory concentration for 50% cell growth (L1210 mouse lymphocytic leukemia) relative to untreated controls. [b]Relative intensity of alkylation (thermally-induced strand cleavage) at the high affinity alkylation site [5′-d(AATAA)-3′] within w794 DNA (4°C or 37°C, 24 h)[12] determined using a scanning densitometer. [c]No alkylation observed at 10^{-1} M agent concentration.

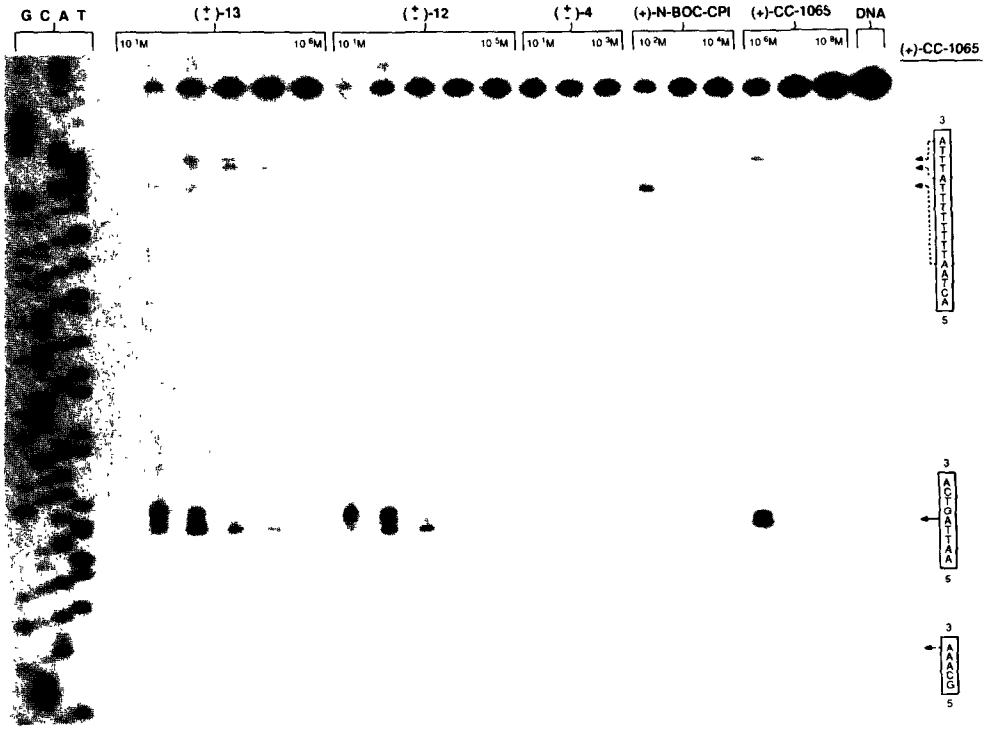


Figure 1. Thermally-induced strand cleavage of double-stranded DNA (SV40 fragment, 144 bp, nucleotide no. 138-5238, clone w794)[12] after 24 h incubation at 37°C followed by removal of unbound agent and 30 min incubation at 100°C, 8% denaturing polyacrylamide gel, and autoradiography. Lane 1-4, Sanger G, C, A, and T sequencing reactions; lanes 5-10, (±)-**13** (1 x 10^{-1} - 1 x 10^{-6} M); lanes 11-15 (±)-**12** (1 x 10^{-1} - 1 x 10^{-5} M); lanes 16-18, (±)-**4** (*N*-BOC-CBI, 1 x 10^{-1} - 1 x 10^{-3} M); lanes 19-21, (+)-*N*-BOC-CPI (1 x 10^{-2} - 1 x 10^{-4} M); lanes 22-24, (+)-CC-1065 (**1**, 1 x 10^{-6} - 1 x 10^{-8} M); lane 25, control DNA.

Thus, the introduction of a positively charged functionality (protonated amine) onto the simple CBI alkylation subunit served to enhance the DNA alkylation intensity of the agent presumably by providing noncovalent electrostatic DNA binding affinity to the agent. Consistent with the enhancement in the DNA alkylation intensity (*ca.* 100x), the in vitro cytotoxic activity of the agents increased correspondingly (*ca* 100x)

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17. For **9**: mp 105-107°C (EtOAc); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm) 8.93 (d, 1H, *J* = 2.7 Hz, C3-H), 8.51 (dd, 1H, *J* = 2.7, 9 0 Hz, C5-H), 7.62 (d, 1H, *J* = 9.0 Hz, C6-H), 7 05 (bs, 1H, NH), 6.43 (bs, 1H, NH), 1 48 (s, 9H, $C(CH_3)_3$); IR (KBr) ν_{max} 1754, 1738, 1612 cm^{-1}.
18. For **12**: white solid; mp 221°C (dec.); ^{1}H NMR ($CDCl_3$-DMF-d_7, 200 MHz, ppm) 9 82 (s, 1H, OH), 8.25 (d, 1H, *J* = 8 Hz, C6-H), 7.82 (s, 1H, C4-H), 7.64 (d, 1H, *J* = 2 Hz, NH), 7.58 (d, 1H, *J* = 8 Hz, C9-H), 7 46 (ddd, 1H, *J* = 1 4, 7, 8 Hz, C8-H), 7.30 (ddd, 1H, *J* = 1.4, 7, 8 Hz, C7-H), 6.86 (br s, 1H, NH), 4.24 (dd, 1H, *J* = 3, 10 Hz, C2-H), 4.17 (t, 1H, *J* = 10 Hz, C2-H), 3.98 (m, 1H, C1-H), 3.92 (dd, 1H, *J* = 3, 11 Hz, C*H*HCl), 3.37 (t, 1H, *J* = 11 Hz, CH*H*Cl), 1.50 (s, 9H, $C(CH_3)_3$); IR (KBr) ν_{max} 3408, 2926, 1718, 1654, 1584, 1522, 1476, 1394, 1246, 1160, 862, 758 cm^{-1}; UV (THF) λ 318 (ε = 9500), 308 (ε = 8200), 260 (ε = 31000), 254 nm (ε 32000); FABHRMS (DTT/DTE) *m/e* 392.1364 ($C_{19}H_{22}ClN_3O_4$ + H$^+$ requires 392.1377). For **13**, white solid, mp 225°C (dec.), ^{1}H NMR ($CDCl_3$-DMSO-d_6, 300 MHz, ppm) 10.0 (br s, -NH_3^+), 9 96 (s, 1H, CONH), 9.77 (s, 1H, OH), 8.18 (d, 1H, *J* = 8.3 Hz, C6-H), 7.78 (s, 1H, C4-H), 7.63 (d, 1H, *J* = 8.3 Hz, C9-H), 7 48 (t, 1H, *J* = 7 4 Hz, C8-H), 7.30 (t, 1H, *J* = 7 5 Hz, C7-H), 4.26 (m, 2H, C2-H), 4 07 (m, 1H, C1-H), 3 93 (dd, 1H, *J* = 2, 11 Hz, C*H*HCl), 3.51 (t, 1H, *J* = 10 Hz, CH*H*Cl); IR (KBr) ν_{max} 3400 (br), 3200 (br), 2926, 1670, 1632, 1584, 1520 cm^{-1}; UV (DMF) λ_{max} 322 (ε = 9300), 310 (sh, ε = 7900), 270 nm (ε = 23000), FABHRMS (DTT/DTE) *m/e* 292.0867 ($C_{14}H_{14}ClN_3O_2$ + H$^+$ requires 292.0853).
19. The seco agents **12** and **13** display biological properties (in vitro cytotoxic activity) indistinguishable from **5** and **6** but withstand prolonged storage better than **5** and **6**.