Bioorganic & Medicinal Chemistry Letters, Vol.1, No.2, pp. 115-120, 1991
Printed in Great Britain

0960-894X/91 $3.00 + .00

SYNTHESIS AND PRELIMINARY EVALUATION OF (+)-CBI-INDOLE$_2$: AN ENHANCED FUNCTIONAL ANALOG OF (+)-CC-1065

Dale L. Boger,* Takayoshi Ishizaki,[1a] Subas M. Sakya, Stephen A. Munk[1b]
Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, USA

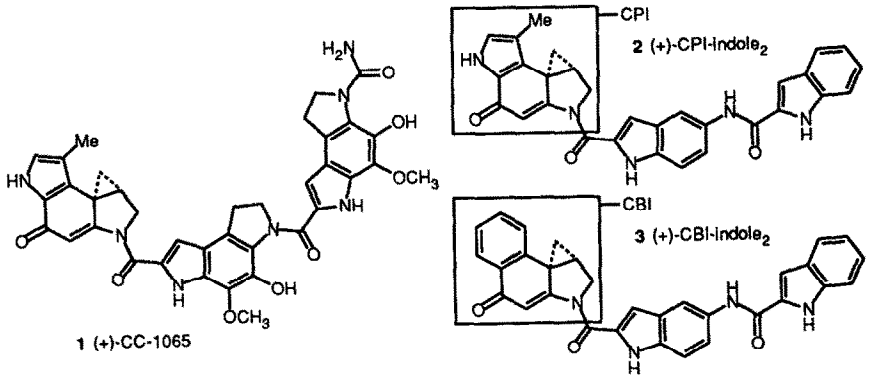
Paul A. Kitos and Qing Jin
Department of Biochemistry, University of Kansas, Lawrence, Kansas 66045, USA

Jeffrey M. Besterman
Department of Cell Biology, Glaxo Research Institute, Research Triangle Park, NC 27709, USA

(*Received* 21 *January* 1991)

Abstract. The synthesis and comparative preliminary evaluation of (+)-CBI-indole$_2$ (3) are detailed in efforts that further demonstrate a direct relationship between the chemical stability of the agents and their biological potency/DNA alkylation intensity.

(+)-CC-1065 (1), a potent antitumor antibiotic isolated from cultures of *Streptomyces zelensis*, possesses exceptionally potent in vitro cytotoxic activity, broad spectrum antimicrobial activity, and in vitro 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 five base-pair A-T rich minor groove regions[2-4] that has been shown to proceed by 3′-adenine *N*-3 alkylation of the electrophilic cyclopropane present in the CPI left-hand subunit.[2-3] Despite the highly potent in vitro and in vivo antitumor activity of (+)-CC-1065 derived through its selective alkylation of DNA, clinical development of the natural product has been precluded by its delayed toxicity.[5] Thus, in the conduct of extensive efforts to separate the delayed toxicity of the agent from its productive antitumor properties, the preparation of a series of agents that bear the authentic (+)-CC-1065 CPI alkylation subunit have been detailed that maintain the exceptionally potent in vitro cytotoxic activity and possess more efficatious in vivo antitumor activity than (+)-CC-1065 but whichlack the delayed toxicity of the natural product.[2-3,6-7] The prototype of such clinical candidates introduced at Upjohn is



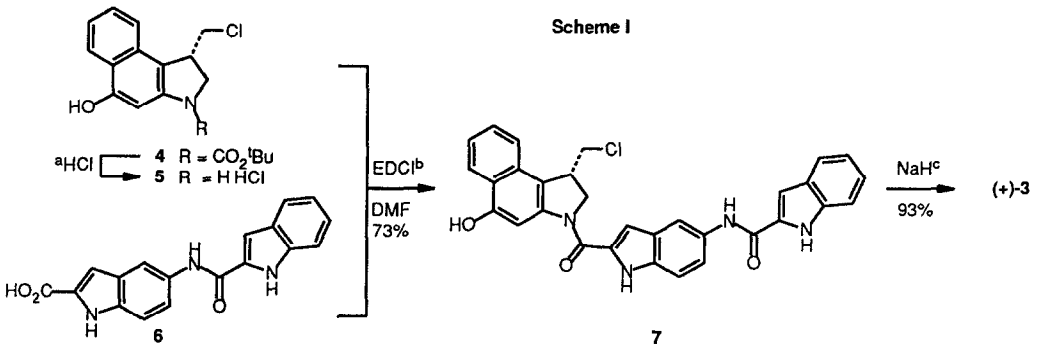
U71184 (**2**, (+)-CPI-indole$_2$).[6-7] In our own complementary studies designed to address the structural origin of the (+)-CC-1065 sequence-selective DNA alkylation,[8-15] we have prepared agents that possess modified alkylation subunits including those that possess the more stable 1,2,9,9a-tetrahydrocycloprop[1,2-*c*]benz[1,2-*e*]indol-4-one (CBI) left-hand subunit.[14]

In the conduct of these studies and in contrast to earlier proposals,[2] we have detailed an inverse relationship between the solvolytic reactivity and the cytotoxic potency/DNA alkylation intensity of an agent in which the synthetically more accessible, chemically more stable CBI-based agents exhibited greater cytotoxic potency and a more intense DNA alkylation intensity than the corresponding CPI-based agent.[14-15] Herein, we report the extension of these observations to the preparation and preliminary evaluation of a potentially clinically significant agent, (+)-CBI-indole$_2$ (**3**), that additionally support the observation of the *inverse* relationship between the agent chemical reactivity and the agent biological potency/DNA alkylation intensity.

The preparation of (+)-**3** is detailed in Scheme I and follows closely the protocols introduced in early studies.[8,14] Thus, acid-catalyzed deprotection of (+)-*N*-BOC-CBI (**4**)[14] and subsequent immediate coupling of the unstable indoline hydrochloride salt **5** with **6** in the *absence* of external base cleanly provided **7** (73%). As detailed in earlier studies,[14] attempts to promote the coupling of **5** with carboxylic acids such as **6** in the presence of external base (e.g., K_2CO_3, $NaHCO_3$) has led to the observation of competitive cyclization of **5** to CBI resulting in diminished conversions to **7**. Alkylative closure of **7** to (+)-**3** was most effectively accomplished by treatment with sodium hydride (2 equiv) at 0°C and provided (+)-**3** in excellent yield (93%).[16]

Results of the preliminary in vitro cytotoxic evaluation (L1210) of (+)-**3** and its comparison with (+)-**2** are detailed in Table 1. Following the *precise* trends observed in earlier studies,[14] (+)-**3** proved to be 4x more potent than (+)-**2** and correlates exceptionally well with the observation that the agent proved to be approximately 4x more stable to acid-catalyzed solvolysis, Table 1.

Scheme I



(a) 3N anhydrous HCl/EtOAc, 24°C, 20 min, 100%. (b) 3 equiv EDCI, 1.0 equiv **6**, DMF, 24°C, 8.5 h, 73%. (c) 2 equiv NaH, THF-DMF (1:1), 0°C, 1 h, 93%.

Table 1.	(+)-CBI-indole$_2$ [(+)-3]	(+)-CPI-indole$_2$ [(+)-2]
Relative Intensity (4°C, 24 h) of DNA Alkylation[a] (%)	1.0 (93%)	0.1 (5-10%)
k (rel) at 37°C[b]	1.0	0.07
Relative Stability[c]	1.0	0.27
Relative Cytotoxic Activity (L1210)[d]	1.0	0.25

[a]Relative intensity of alkylation (thermally-induced strand cleavage) at the high affinity alkylation site [5′-d(AATTA)-3′] within w794 DNA determined using a scanning densitometer. % Reaction (%) is expressed as the % of the total alkylation at this site when the alkylation is taken to > 90% completion, the relative intensity of alkylation at this point is compared to (+)-3 (4°C, 24 h) = 1.0. [b]Relative first order rate constants for alkylation at the high affinity site taken from plots of intensity of DNA cleavage vs time (37°C; 10^{-5} M agent: 12, 24, 48, 96, 192 h). [c]Taken from references 11, 14-15. Solvolysis studies conducted spectrophotometrically (UV) at pH = 3 (50% buffer-MeOH, buffer = 4:1:20 (v:v:v) 0.1 M citric acid, 0.2 M Na_2HPO_4, and H_2O). *k*(*N*-BOC-CBI) = 1.45 ± 0.01 x 10^{-6} sec^{-1}, $t_{1/2}$ = 133 h; *k*(*N*-BOC-CPI) = 5.26 ± 0.08 x 10^{-6} sec^{-1}, $t_{1/2}$ = 36.7 h. [d]Relative IC_{50} for in vitro cytotoxic activity against L1210 mouse lymphocytic leukemia. (+)-3, 5 x 10^{-6} μg/mL (10 pM) and (+)-2, 20 x 10^{-6} μg/mL (40 pM).

This observation of an inverse[14-15] relationship between the agent solvolytic reactivity and cytotoxic potency further translates into the relative intensity of DNA alkylation exhibited by the agents. That is, *the rate and intensity of DNA alkylation follows the same inverse relationship: (+)-3 > (+)-2*. Singly 5′ ^{32}P end-labeled double-stranded DNA constituting SV40 DNA nucleotide no. 5238-138 (144 base-pairs) cloned into the *Sma* I site of M13mp10[4] was treated with the agents at 4°C (24 h) 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), and gel electrophoresis of the resultant DNA alongside Sanger dideoxynucleotide sequencing reactions revealed the agent sites of covalent alkylation and their relative intensities of covalent alkylation, Figure 1. Consistent with the relative in vitro cytotoxic activity of the agents, the less reactive and more stable agent, (+)-3, exhibits a more intense alkylation of DNA at 4°C than (+)-2.

As detailed elsewhere, this more productive covalent modification of DNA by the more stable agent presumably arises in part from the enhanced agent availability (stability, selectivity).[14-15] However, when the alkylation reactions were conducted under conditions of where excess DNA is present and under conditions where the agents are effectively sequestered by double-stranded DNA, the results suggest that nonproductive solvolysis and alkylation selectivity differences account for only part of the distinctions in the observed DNA alkylation intensity at 4°C. Under such conditions the DNA alkylation reaction follows first order kinetics with respect to agent concentration (10^{-5} - 10^{-6} M agent) and the comparisons made additionally reflect the relative rates of DNA alkylation by the two agents which follow the unexpected order of (+)-CBI-indole$_2$ > (+)-CPI-indole$_2$: k (3)/k (2) = 14 (37°C), Table 1 and Figure 2.

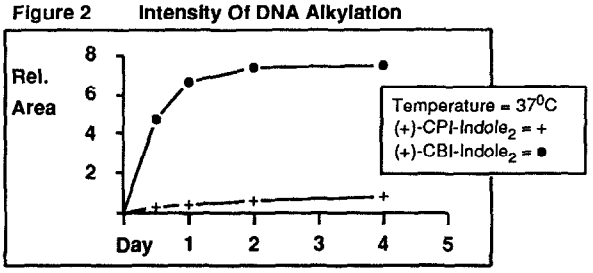


Figure 2. Plot of the relative area (optical density) of end-labeled DNA derived from the alkylation and thermal cleavage at the high affinity alkylation site (5′-AATTA) within clone w794 versus time at 37°C (10^{-5} M agent). The optical density determinations were obtained using a laser scanning densitometer. The results at day 4 represent 50% consumption of the end-labeled DNA for (+)-CBI-indole$_2$.

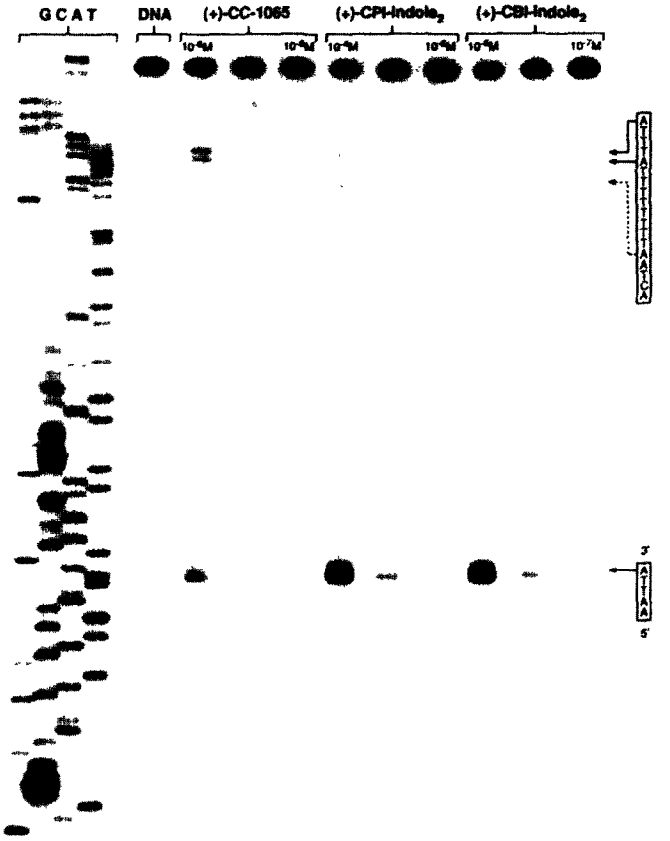


Figure 1. Thermally-induced strand cleavage of double-stranded DNA (SV40 fragment, 144 b.p., nucleotide no. 138-5238, clone w794)[4] after 24 h incubation at 4°C followed by removal of unbound agent and 30 min incubation at 100°C, 8% denaturing polyacrylamide gel and autoradiography. Lanes 1-4, Sanger G, C, A, and T reactions; lane 5, control DNA; lanes 6-8, (+)-CC-1065 (1, 1 x 10^{-6} - 1 x 10^{-8} M); lanes 9-11, (+)-CPI-indole$_2$ (2, 1 x 10^{-4} - 1 x 10^{-6} M); lanes 12-14, (+)-CBI-indole$_2$ (3, 1 x 10^{-5} - 1 x 10^{-7} M).

Table 2. In Vivo Antitumor Activity (L1210)[a]

dose (μg/kg-day)	T/C: (+)-**3**, (+)-CBI-indole$_2$	T/C: (+)-**2**, (+)-CPI-indole$_2$
5	120	-
10	156	114
14	-	133
19	162	113
29	> 175[b]	118
39	> 200[b]	129

[a]Ip tumor inoculation (L1210), sc agent delivery on day 1, 4, 6, and 8. T/C = Average life span of treated animals/Average life span of untreated control animals x 100. Control mean lifetime = 8.5 days. [b]Survivors at day 18 (1/6 at 29 μg/kg, 4/6 at 39 μg/kg) were sacrificed and examined for evidence of residual tumor. No evidence of residual tumor found. The reported optimum activity of (+)-**2**: Ip-implanted P388 leukemia, ip drug treatment on days 1, 5, and 9, optimum dose = 3 x 25 μg/kg-day (T/C = 259, 4/6 30-day survivors), reference 7.

A preliminary examination of the in vivo antitumor activity of (+)-**3** revealed significant activity (T/C > 200) at dosage levels and with a drug treatment protocol (sc agent delivery) for which (+)-**2** was found to be marginally active (T/C = 113-133), Table 2. Thus, the preliminary studies illustrate that the productive antitumor activity of agents related to (+)-CC-1065 may reside in agents possessing alternative DNA alkylation subunits and that more potent agents may be derived through introduction of solvolytically more stable alkylation subunits. The excellent inverse relationship between the cytotoxic potency of the agents and their solvolytic reactivity suggests that an agents relative stability may constitute the relevant feature in the further design of functional analogs. Potentially contributing to the distinctions in the properties of the agents examined herein may be the unexpected observation of the more rapid (rate) and more productive (intensity) DNA alkylation by the more stable agent.

Acknowledgments. This work was supported by the National Institutes of Health (DLB, CA41986) and the Wesley Foundation (8812007, PAK). We thank Professor M. Bina and her group for the gift of M13mp10 clones (w794).

References

1. (a) On sabbatical leave from Kyorin Pharmaceutical Co., Ltd., Tochigi, Japan, 1988-1990. (b) American Cancer Society postdoctoral fellow 1988-1991 (PF-3311).
2. Warpehoski, M.; Hurley, L. H. *Chem. Res. Toxicol.* **1988**, *1*, 315.
3. Hurley, L. H.; Warpehoski, M. A.; Lee, C.-S.; McGovren, J. P.; Scahill, T. A.; Kelly, R. C.; Mitchell, M. A.; Wicnienski, N. A.; Gebhard, I.; Johnson, P. D.; Bradford, V. S. *J. Am. Chem. Soc.* **1990**, *112*, 4633.
4. Boger, D. L.; Munk, S. A.; Zarrinmayeh, H.; Ishizaki, T.; Haught, J.; Bina, M. *Tetrahedron* **1991**, *47*, in press.

5. McGovren, J. P.; Clarke, G. L.; Pratt, E. A.; DeKoning, T. F. *J. Antibiot.* **1984**, *37*, 63.
6. Wierenga, W.; Bhuyan, B. K.; Kelly, R. C.; Krueger, W. C.; Li, L. H.; McGovren, J. P.; Swenson, D. H.; Warpehoski, M. A. *Adv. Enzyme Regul.* **1986**, *25*, 141.
7. Warpehoski, M. A.; Gebhard, I.; Kelly, R. C.; Krueger, W. C.; Li, L. H.; McGovren, J. P.; Prairie, M. D.; Wicnienski, N.; Wierenga, W. *J. Med. Chem.* **1988**, *31*, 590.
8. Boger, D. L.; Coleman, R. S. in *Studies in Natural Products Chemistry*, A-u-Rahman, Ed.; Elsevier: Amsterdam, 1989, Vol. 3, 301.
9. Boger, D. L.; Coleman, R. S. *J. Am. Chem. Soc.* **1988**, *110*, 4796.
10. Boger, D. L.; Coleman, R. S.; Invergo, B. J.; Sakya, S. M.; Ishizaki, T.; Munk, S. A.; Zarrinmayeh, H.; Kitos, P. A.; Thompson, S. C. *J. Am. Chem. Soc.* **1990**, *112*, 4623.
11. Boger, D. L.; Wysocki, R. J., Jr. *J. Org. Chem.* **1989**, *54*, 1238. Boger, D. L.; Wysocki, R. J., Jr.; Ishizaki, T. *J. Am. Chem. Soc.* **1990**, *112*, 5230.
12. Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. *J. Am. Chem. Soc.* **1990**, *112*, 8961.
13. Boger, D. L.; Coleman, R. S.; Invergo, B. J.; Zarrinmayeh, H.; Kitos, P. A.; Thompson, S. C.; Leong, T.; McLaughlin, L. W. *Chem.-Biol. Interactions* **1990**, *73*, 29.
14. Boger, D. L.; Ishizaki, T.; Wysocki, R. J., Jr.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. *J. Am. Chem. Soc.* **1989**, *111*, 6461. Boger, D. L.; Ishizaki, T. *Tetrahedron Lett.* **1990**, *31*, 793. Boger, D. L.; Ishizaki, T.; Kitos, P. A.; Suntornwat, O. *J. Org. Chem.* **1990**, *55*, 5823.
15. Boger, D. L.; Munk, S. A.; Ishizaki, T. *J. Am. Chem. Soc.*, in press.
16. For (+)-**3**: mp > 240°C; $[\alpha]_D^{23}$ = +114° (c = 0.03, DMF); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.86 (br s, 1H, NH), 11.73 (br s, 1H, NH), 10.19 (s, 1H, NH), 8.24 (d, 1H, *J* = 2.6 Hz, C4′-H), 8.02 (d, 1H, *J* = 8.0 Hz, C5-H), 7.67 (d, 1H, *J* = 7.8 Hz, C4′′-H), 7.63 (m, 2H, C6-H and C7-H), 7.47 (m, 4H), 7.29 (s, 1H, C3′-H or C3′′-H), 7.25 (m, 2H, C8-H and C6′′-H), 7.07 (t, 1H, *J* = 7.3 Hz, C5′′-H), 6.98 (s, 1H, C3-H), 4.65 (dd, 1H, *J* = 4.9, 10.2 Hz, C1-H), 4.53 (apparent d, 1H, *J* = 10.2 Hz, C1-H), 3.20 (m, 1H, obscured by H_2O, C9a-H), 1.77 (dd, 1H, *J* = 4.2, 7.4 Hz, C9-H), 1.73 (t, 1H, *J* = 4.2 Hz, C9-H); IR (KBr) ν_{max} 3432, 1648, 1522, 1384, 1266, 1126, 744 cm^{-1}; UV (DMF) λ_{max} 316 (ε = 45000), 274 nm (ε = 25000); FABMS (*m*-nitrobenzyl alcohol), *m/e* 499 (M^+ + H); HRFABMS (*m*-nitrobenzyl alcohol), *m/e* 499.1792 ($C_{31}H_{22}N_4O_3$ + H requires 499.1770).