Role of the CC-1065 and Duocarmycin N^2 Substituent: Validation of a Direct Relationship between Solvolysis Chemical Stability and in Vitro Biological Potency

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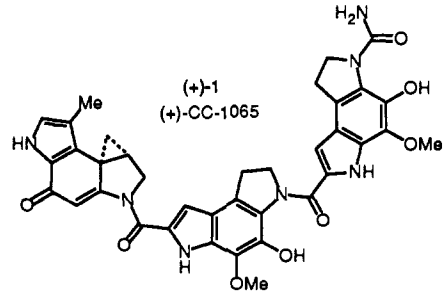
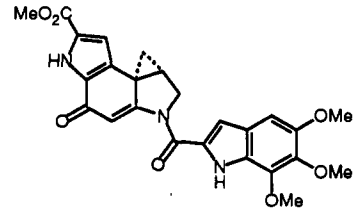
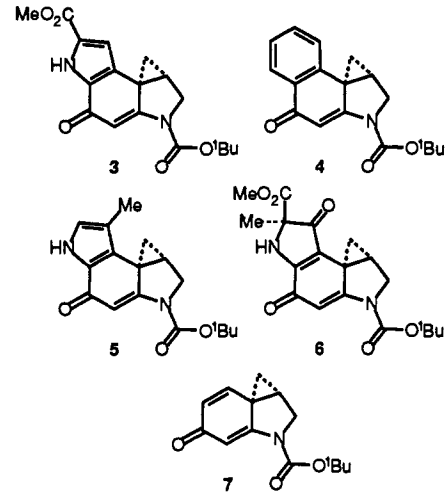
CC-1065[1] (**1**) and duocarmycin SA[2] (**2**) represent the parent members of a growing class[3] of exceptionally potent antitumor antibiotics that exert their effects through a sequence selective alkylation of DNA.[4-6] The stereoelectronically-controlled adenine N3 addition to the least substituted carbon of the activated cyclopropane has been shown to occur within selected AT-rich sites of the minor groove of DNA,[7-14] and extensive effort has been devoted to determine the origin of the DNA alkylation selectivity, to establish the link between DNA alkylation and the ensuing biological properties,[15] and to define the fundamental principles underlying the relationships between structure, chemical reactivity, and biological activity.





Initial studies conducted with simple derivatives of the (+)-CC-1065 alkylation subunit (CPI) led to the proposal that there exists a direct relationship between an agent's reactivity and in vitro cytotoxic potency (L1210, IC_{50})[16] and established the expectation that the biological potency may be enhanced as their electrophilic reactivity is increased. In a complementary series of studies conducted with agents containing deep-seated modifications in the alkylation subunit including **3-7**, the reverse relationship was observed[17,18] and the agents possessing the greatest chemical solvolysis stability exhibited the most potent in vitro cytotoxic activity. Moreover, a near linear relationship between solvolytic chemical stability and biological potency was observed and proved to be general with both simple and advanced analogs of the natural products.



	k (s^{-1}, pH 3)	$t_{1/2}$	IC_{50} (L1210)
3	1.1×10^{-6}	177 h	6 nM
4	1.5×10^{-6}	133 h	80 nM
5	5.3×10^{-6}	37 h	330 nM
6	1.7×10^{-5}	11 h	1000 nM
7	2.0×10^{-2}	0.01 h	18000 nM

As a consequence of these studies, we became interested in the inherent role of the CC-1065 and duocarmycin N^2 substituent. The simple derivatives **8-11** of (+)-CBI[19] were prepared for

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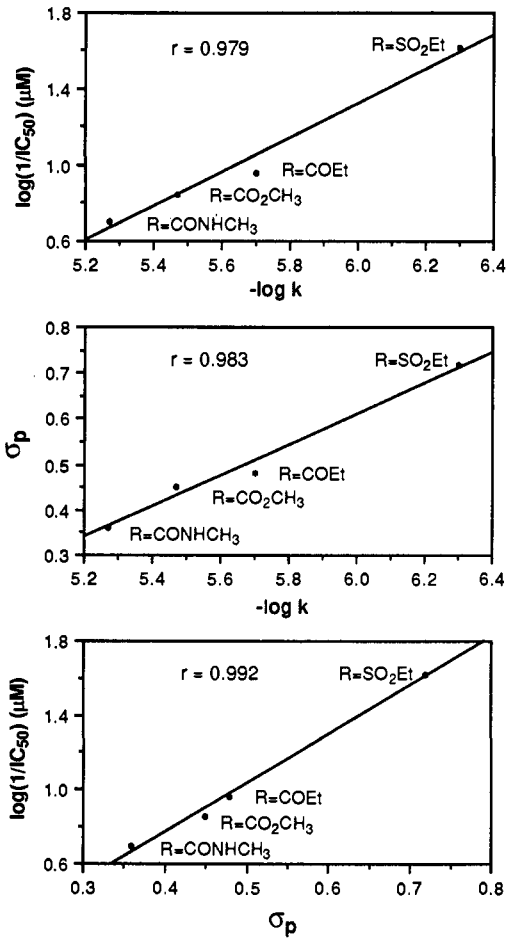
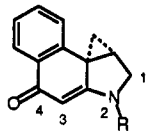


Figure 1.

examination and, by virtue of their structural similarities,[16] were expected to accurately reflect a potential relationship between functional reactivity and biological potency. The examination



R	k (s^{-1}, pH 3)	$t_{1/2}$	IC_{50} (L1210)	sigma
8 SO_2Et	0.5×10^{-6}	383 h	24 nM	0.72
9 COEt	2.0×10^{-6}	96 h	110 nM	0.48
10 CO_2CH_3	3.4×10^{-6}	57 h	140 nM	0.45
11 CONHMe	5.4×10^{-6}	36 h	200 nM	0.36

of **8-11** revealed a direct, linear relationship between the cytotoxic potency (L1210, log $1/IC_{50}$) and the solvolytic stability (-log k_{solv}, pH 3) of the agents (Figure 1). Thus, similar to the trend observed with **3-7**, the solvolytically more stable derivatives of CBI proved to be the most potent. Similarly, a linear relationship was found between the electron-withdrawing properties of the N^2 substituents (Hammett σ_p constant) and the solvolysis reactivity (-log k_{solv}, pH 3) of the agents with the strongest electron-withdrawing substituents providing the most stable agents (Figure 1). This latter relationship reflects the influence of the N^2 substituent on the ease of C4 carbonyl protonation required for catalysis of solvolysis and cyclopropyl ring cleavage with the stronger electron-withdrawing N^2 substituents exhibiting slower solvolysis rates. Less obvious but more fundamental, the observations were found to follow a predictable linear relationship between the cytotoxic potency (L1210, log $1/IC_{50}$) and the electron-withdrawing properties of the N^2 substituent (Hammett σ_p) with the strongest electron-withdrawing substituents providing the biologically most potent agents (Figure 1).

These fundamental correlations between the electron-withdrawing properties of the N^2 substituent, the functional reactivity of the agents, and the biological potency of the agents may prove useful in the predictable design of new analogs. For agents which possess sufficient reactivity to effectively alkylate duplex DNA,[20] the chemically more stable agents may be expected to constitute the biologically more potent agents. Presumably, this may be attributed to the more effective delivery of the more stable agents to their intracellular target, and the solvolysis rates may be taken to represent a general measure of the agent's relative functional reactivity. The consumption of the agent in route to its intracellular target need not be simply nonproductive solvolysis but may also be competitive alkylation of nonproductive extra- and intracellular sites as well, including the potential of nonproductive sites within duplex DNA. Since the chemically more stable agents provide thermodynamically less stable and more readily reversed addition products,[9] the observations may also represent a more effective thermodynamic partitioning of the agents to their productive intracellular target or site(s). These and related issues are presently under study.

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Supplementary Material Available: Characterization details of **8-11** are provided (2 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(20) We anticipate that this full relationship will be parabolic in nature displaying an optimal reactivity–stability/activity but that the studies detailed herein which span a 10-fold reactivity range lie in a range where the relationship is linear.