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Establishing the Parabolic Relationship between Reactivity and Activity for Derivatives and Analogues of the Duocarmycin and CC-1065 Alkylation Subunits

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CC-1065 (**1**),[1] the duocarmycins (**2**−**3**),[2,3] and yatakemycin (**4**)[4] are the parent members of a class of potent antitumor antibiotics that derive their properties through a sequence-selective DNA alkylation (Figure 1).[5−7] An extensive series of studies have characterized their structural features responsible for the DNA alkylation reaction and have established fundamental relationships between structure and activity.[5−10] Among the most important of these relationships is a direct correlation between chemical stability and biological potency (cytotoxic activity).[5,11] To date, this relationship covered a wide range of modified alkylation subunits with properties extending over a 10^6-fold range in both reactivities and activities.[5,12] Restricted to derivatives that possess sufficient reactivity to alkylate DNA, this relationship has been interpreted to reflect the ability of the chemically more stable derivatives to more effectively reach their biological target (DNA).[5,11,13]

Herein, we report an effective preparation and the examination of a novel series of N^2-aryl derivatives of 1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (CBI)[14,15] in which the electronic properties of the aryl *p*-substituent could be systematically varied to predictably alter the reactivity. As detailed below, the derivatives proved to be remarkably stable relative to the typical *N*-acyl derivatives, and they were found to exhibit a well-defined correlation between reactivity and biological potency. When combined with the results of preceding studies, this series served to complete a well-defined parabolic relationship between reactivity and activity.

The *N*-aryl derivatives were prepared using either a Buchwald−Hartwig[16] Pd(0)-catalyzed or a Barton[17] Cu(II)-catalyzed N-arylation of either enantiomer of CBI (**5**) (Scheme 1), enlisting precursor aryl chlorides or triarylbismuthines, respectively. The former provided effective couplings with electron-deficient aryl chlorides, affording **12**−**15** (0.05 equiv $Pd_2(dba)_3$, 0.1 equiv $(Cy)_2P$-(DMAbp),[18] 1.6 equiv Cs_2CO_3, THF, reflux, 3−6 h, 53−96%), but failed to provide products with electron-rich aryl halides under the conditions examined. In these instances, the Cu(II)-catalyzed coupling of **5** with the triarylbismuthines[19] (1.1 equiv $Cu(OAc)_2$, 1.0 equiv Et_3N, CH_2Cl_2, 25 °C, 24−36 h, 39−64%) provided **6**−**11** in good conversions.

The derivatives **6**−**15** proved to be remarkably stable, displaying readily measurable solvolysis reactivity only at pH 2. This reactivity followed a well-defined correlation with σ_p ($\rho = 0.17$) in which electron-withdrawing substituents enhance and electron-donating substituents decrease the solvolysis rate (Figure 2). Thus, the solvolysis stability correlates with the expected extent of cross-conjugated vinylogous amide stabilization of the cyclohexadienone structure. In turn, this extent of the vinylogous amide conjugation can be observed with the diagnostic N2−C3a bond length trends found in the X-rays of **7** (1.380(3) Å, R = OMe), **9** (1.395(4) Å, R = H), and **13** (1.399(6) Å, R = CN).[20]

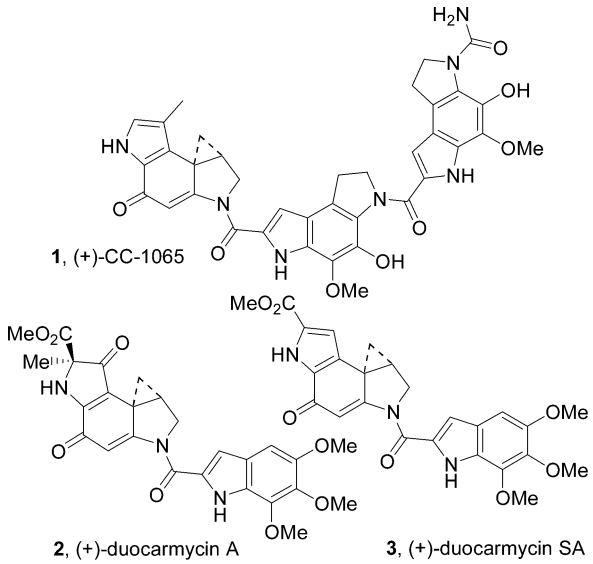
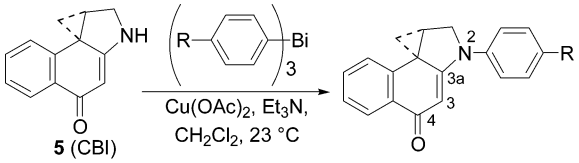
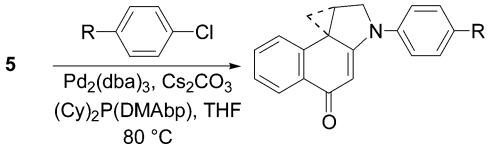


Figure 1.

Scheme 1



R = NMe_2 (**6**, 63%), OCH_3 (**7**, 53%), CH_3 (**8**, 39%)
R = H (**9**, 51%), F (**10**, 57%), Cl (**11**, 64%)



R = CO_2CH_3 (**12**, 65%), CN (**13**, 53%)
R = NO_2 (**14**, 96%), 2,4-NO_2 (**15**, 54%)

The biological properties (cytotoxic activity, L1210) of **6**−**14** also exhibited a well-defined trend correlating with their reactivity (Table S1 and Figure S1). Thus, electron-withdrawing substituents smoothly increase, whereas electron-donating substituents decrease, the cytotoxic activity of the derivatives. Notably, **14**, **13**, and **12** bearing electron-withdrawing substituents displayed cytotoxic potency (IC_{50} = 40, 140, and 330 nM, respectively) comparable to that of simple *N*-acyl-CBI derivatives (e.g., *N*-Boc-CBI, IC_{50} = 80 nM), that of the naturally occurring alkylation subunits (e.g., *N*-Boc-MeCPI, IC_{50} = 300 nM), and analogous to that commonly associated with efficacious antitumor drugs (e.g., mitomycin, IC_{50}

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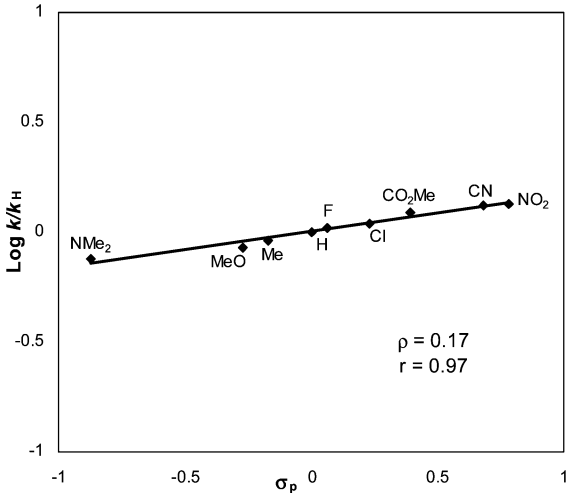

Figure 2. Log k/k_H (solvolysis, pH 2) vs σ_p.

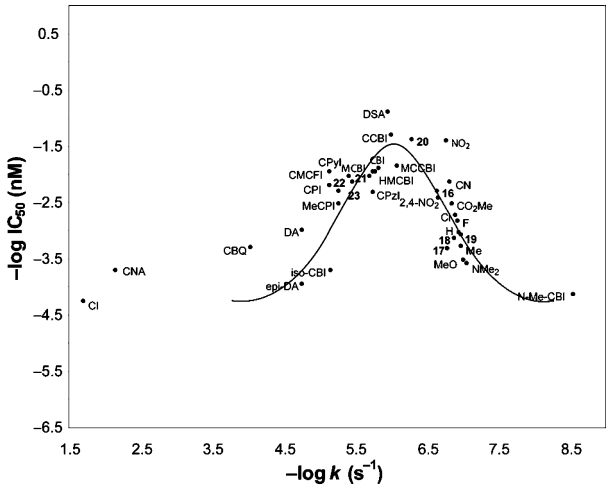

Figure 3. Relationship between reactivity (solvolysis *k*, pH 3) and cytotoxic potency (L1210).

= 90 nM). Moreover, when this correlation between reactivity (−log *k*, pH 3) and cytotoxic activity (−log IC_{50}, L1210) is plotted along with prior data for the more reactive *N*-acyl-CBI derivatives and its analogues, it established a well-defined parabolic relationship between reactivity and biological potency (Figure 3). This plot incorporates not only the *N*-aryl derivatives disclosed herein, but all *N*-Boc derivatives of the alkylation subunits that we have examined to date,[21] including a class of unusually stable C3 halogen CBI derivatives **16**−**19**[21,22] and a series of simple *N*-acyl CBI derivatives **20**−**23**.[21,11] The parabolic relationship establishes that the compounds should possess sufficient stability to reach their biological target (DNA), yet maintain sufficient reactivity to alkylate DNA upon reaching the biological target and, importantly, defines this optimal balance of stability and reactivity.[23]

The variations that appear in this correlation may be attributed not only to the inherent error in the cytotoxic assay especially with data that has been collected intermittently over a 15-year period but also to structural differences in the derivatives that impact features beyond reactivity (e.g., cell penetration and distribution, DNA binding affinity). Even without accounting for such variables, the adherence to the parabolic relationship is remarkable, consistent with a correlation of fundamental importance.

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Supporting Information Available: Full experimental details for the preparation of **6**−**15**; solvolysis experimental details; tables of first-order rate constants for **6**−**15**, *N*-Boc-CBI analogues, and *N*-aryl-CBI data; plot of log *k* vs log IC_{50} for **6**−**14**; and structure key (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) The X-ray structures have been deposited with the Cambridge Crystallographic Data Centre: **7** (CCDC 216387), **9** (CCDC 216392), and **13** (CCDC 216386).
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(23) The unnatural enantiomers follow an analogous parabolic relationship, albeit with 5−10-fold less potent cytotoxic activity. See Supporting Information Figure S2.

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