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Substituent Effects Within the DNA Binding Subunit of CBI Analogues of the Duocarmycins and CC-1065

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Abstract—A series of CBI analogues of the duocarmycins and CC-1065 exploring substituent effects within the first indole DNA binding subunit are detailed. Substitution at the indole C5 position led to cytotoxic potency enhancements that are $\geq$1000-fold, providing simplified analogues containing a single DNA binding subunit that are more potent (IC_{50} = 2–3 pM) than CBI-TMI, duocarmycin SA, or CC-1065.

CC-1065 (**1**),[1] duocarmycin A (**2**)[2] and duocarmycin SA (**3**)[3] constitute the parent members of a class of potent antitumor antibiotics[4] that derive their properties through a sequence-selective alkylation of duplex DNA.[5] Recent studies have established that the catalysis of the DNA alkylation reaction is derived at least in part from a DNA binding-induced conformational change in the agents which activate them for nucleophilic attack.[6,7] The conformational change twists the amide linking the alkylation subunit and attached DNA binding domain which disrupts the cross conjugated vinylogous amide stabilization of the alkylation subunit activating the cyclopropane for nucleophilic attack. This ground-state destabilization of the cyclopropane upon DNA binding is consistent with the proposal that the DNA alkylation sequence selectivity originates in the noncovalent binding selectivity of the agents.

Recent studies have highlighted that the role of attached DNA binding domain goes beyond that of simply providing DNA binding affinity and selectivity, but that it contributes to and is largely responsible for the DNA alkylation catalysis.[6,7] Minor groove bound substituents on both the alkylation subunit[8–11] and the first DNA binding subunit[12,13] have been shown to have a pronounced effect on the rate and efficiency of DNA alkylation and the resulting biological potency of the compounds. These effects proved to be independent of the electronic properties of the substituent and their inherent effects on reactivity, but could be attributed to their simple presence and the fact that they extend the rigid length of the agent. In doing so, they increase the extent of the DNA binding-induced conformational change, increase the degree of vinylogous amide disruption, and increase the rate of DNA alkylation (Fig. 1).

For example, the contribution of each of the three methoxy groups of 5,6,7-trimethoxyindole (TMI) was

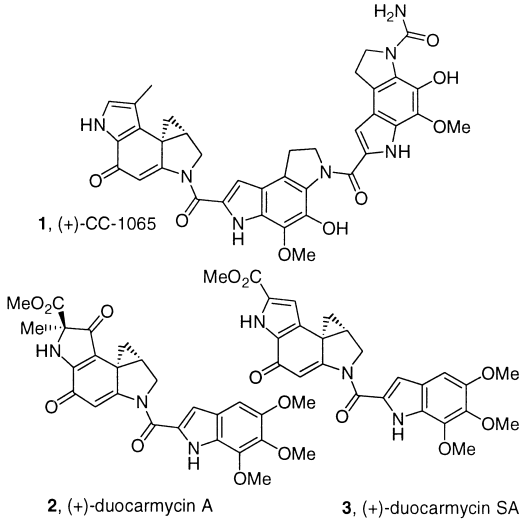

Figure 1.

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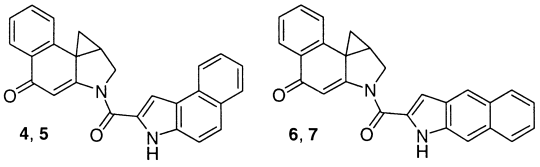
established using the DNA alkylation subunits DSA[12,13] and CPI.[10] These studies demonstrated the predominant importance of the C5 methoxy substituent, which alone provided a fully active agent, with little or no contribution derived from the C6 and C7 methoxy groups. The cytotoxic potency of these agents nicely correlated with their DNA alkylation rate and efficiency, the conclusion being that the agents bearing a C5 methoxy substituent were more effective and that this was due to the extended rigid length provided by the minor groove bound substituent. Subsequently, this was found to be consistent with the high resolution NMR structures of (+)-duocarmycin SA[14] and its derivative DSI, lacking the three methoxy groups, bound to DNA which confirmed that the presence of C5 methoxy group increased the twist in the DNA bound agent.[15] Despite these studies and reports of limited series of agents,[16,17] no systematic examination of the DNA binding subunit C5 substituent has been disclosed. Utilizing the CBI alkylation subunit (1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one),[18–32] herein was described such a study of the impact of extending the length of the DNA binding subunit. The data for **4–7** with extension by attaching a fused benzene ring are shown in Table 1. The data for **8–27** with a substituent extension at the C5 position of the indole and the comparison data for **28–30** with a C7 substituent are shown in Table 2. As previously disclosed, the *seco*-CBI precursors displayed cytotoxic activity that was not distinguishable from that of the final cyclopropanes.

Synthesis[33]

The compounds **4–30** were prepared by acid-catalyzed deprotection of *seco*-*N*-BOC-CBI (natural or unnatural enantiomer) followed by coupling of the resulting hydrochloride salt with the appropriate indole-2-carboxylic acid (3 equiv EDCI, DMF, 25 °C, 14 h) in the absence of added base.[11] Spirocyclization was effected by treatment with DBU[34] or NaH[18,19] providing **4–30** in the yields reported in Tables 1 and 2 for the two steps. In the case of **4–7**, both enantiomers of the compounds were examined, whereas in the case of **8–30** only the more potent natural enantiomers were examined. The noncommercially available indole-2-carboxylic acids[33] were obtained by saponification of the corresponding methyl or ethyl ester using method A: 4 equiv KOH, EtOH, 80 °C, 30–45 min, 68–99%; method B: 4 equiv LiOH, dioxane/H_2O, 25 °C, 36–48 h, 38–100%; or method C: 3 equiv Cs_2CO_3, EtOH/H_2O, 80 °C, 2.5 h, 56–83%.

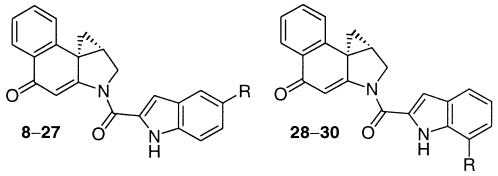
Table 1.



Subunits	Compd	Yield (%)	MALDIFT HRMS	Cytotoxicity[a] IC_{50} (pM)
(+)-CBI-benz[*e*]indole	**4**	31	MH^+ 391.1454	5000
(−)-CBI-benz[*e*]indole	**5**	45	calcd 391.1441	3×10^5
(+)-CBI-benz[*f*]indole	**6**	20	MH^+ 391.1430	500
(−)-CBI-benz[*f*]indole	**7**	41	calcd 391.1441	4×10^5

[a]L1210 cytotoxic activity, average of 2–7 determinations run in triplicate.

Table 2.



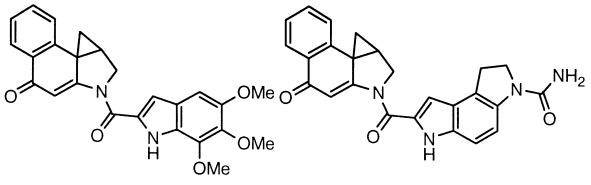
R	Compd	Yield (%)	MALDIFT HRMS	Cytotoxicity[a] IC_{50} (pM)
5-H	**8**	50	MH^+ 341.1276 calcd 341.1284	2700
5-Me	**9**	46	MH^+ 355.1441 calcd 355.1441	2000
5-Cl	**10**	57	MH^+ 375.0901 calcd 375.0895	400
5-Br	**11**	21	MH^+ 419.0408 calcd 419.0390	500
5-CN	**12**	15	MH^+ 366.1235 calcd 366.1237	30
5-C≡CH	**13**	28	MH^+ 365.1278 calcd 365.1284	300
5-C≡CMe	**14**	43	MH^+ 378.1362 calcd 378.1368	1000
5-N_3	**15**	50	MH^+ 382 (ESI(+)) $M-H^-$ 380 (ESI(−))	300
5-OMe	**16**	55	MH^+ 371.1405 calcd 371.1390	50
5-OEt	**17**	51	MH^+ 384.1483 calcd 384.1474	10
5-OPr	**18**	44	MH^+ 398.1624 calcd 398.1625	40
5-OBu	**19**	40	MH^+ 413.1855 calcd 413.1860	50
5-OBn	**20**	28	MH^+ 447.1693 calcd 447.1703	50
5-COMe	**21**	22	MH^+ 383.1391 calcd 383.1390	2
5-COEt	**22**	29	MH^+ 397.1546 calcd 397.1547	3
5-COPr	**23**	20	MH^+ 411.1708 calcd 411.1703	20
5-NHCOMe	**24**	21	MH^+ 398.1512 calcd 398.1499	30
5-NHCOEt	**25**	20	MH^+ 412.1658 calcd 412.1656	30
5-NHCOPr	**26**	44	MH^+ 426.1819 calcd 426.1812	30
5-NMeAc	**27**	46	MH^+ 412.1664 calcd 412.1656	30
7-COMe	**28**	34	MH^+ 383.1392 calcd 383.1390	300
7-CN	**29**	22	M^+ 365.1169 calcd 365.1164	300
7-OMe	**30**	49	MH^+ 371.1395 calcd 371.1390	300

[a]L1210 cytotoxic activity, average of 2–7 determinations run in triplicate.

Discussion

The CBI-based agents proved to be more sensitive to the removal of the TMI subunit methoxy groups than the DSA- or CPI-based agents. Comparing the cytotoxic potency of CBI-TMI (**31**) ($IC_{50} = 30$ pM[9]) with **8**, **30**, and **16**, decreases of 90×, 10× and 1.6× were observed. Thus, maintenance of the C5 methoxy group with removal of the C6 and C7 methoxy groups with analogue **16** maintained the cytotoxic potency, whereas its removal in **8** and **30** led to a $\geq$10× reduction in activity. Similar, but less pronounced, reductions in potency with the removal of the TMI methoxy substituents were observed with duocarmycin SA (6.5–10×) and CPI (7×). In the case of duocarmycin SA, the C7 methoxy substituent did not provide a contribution to cytotoxic potency, whereas with CBI (**8** vs **30**) the C7 methoxy group was found to improve potency significantly, but not nearly of the magnitude observed with the C5 methoxy group. Though interesting, this latter effect was found to be insensitive to the nature of the C7 substituent (**28**–**30**) and was not examined further.

Extending the rigid length of the DNA binding indole by adding a linear or angular fused benzene ring did not substantially alter the cytotoxic potency. The linear extension with **6** resulted in a modest 5-fold increase in potency whereas the angular extension with **4** resulted in a modest 2-fold reduction. Consistent with expectations and past observations, the corresponding unnatural enantiomers **5** and **7** were found to be approximately 100–1000× less potent. The behavior of the angular derivatives **4** and **5** is especially striking in comparison with CBI-$CDPI_1$ (**32**) which bears an angular fused saturated five-membered heterocycle. (+)-CBI-$CDPI_1$ was found to be exceptionally potent exceeding the activity of CBI-TMI (**31**). Presumably the increased size of the angularly fused benzene ring found in **4** and **5** hinders rather than facilitates minor groove binding and penetration required to observe DNA alkylation.



31, (+)-CBI-TMI IC_{50} = 30 pM (–)-CBI-TMI IC_{50} = 2000 pM

32, (+)-CBI-$CDPI_1$ IC_{50} = 5 pM (–)-CBI-$CDPI_1$ IC_{50} = ≥380 pM

The examination of a range of C5 substituents revealed the most interesting trends. Addition of a single heavy atom substituent (**9**–**11**) resulted in a modest 1.3–7× increase in potency. Although other factors may contribute to the distinctions, the further extension of the rigid length of the C5 substituent smoothly follows the trend of $0 < 1 < 2 > 3$ atoms (**8** vs **9**–**11** vs **12** vs **13**–**15**) indicating an optimal rigid length provided by the C5 nitrile (**12**) which surpassed the potency of **16**.

Alterations in the C5 methoxy group providing longer, flexible, and more hydrophobic aryl ethers had little effect on the cytotoxic potency although a modest and optimal additional 5-fold increase was observed with the C5 ethoxy derivative **17**, $IC_{50} = 10$ pM. An analogous effect was observed with C5 amide derivatives ($IC_{50} = 30$ pM), although no additional effect was observed upon extending the amide with flexible, hydrophobic substitutions (**24** vs **25** and **26**). This latter result is in contrast to the observations of Lown with amide substituted (+)-CPI-*N*-methylpyrrole agents constituting hybrid structures of CPI linked with the DNA binding subunit of distamycin.[35] In these studies, the amide substitution is reported to exhibit a more substantial effect. Most likely, this may be attributed to the intrinsically poorer properties of the CPI–pyrrole conjugates reported by Lown and the greater influence such amide substitutions may have. The tertiary amide **27**, in which the H-bond donor capability of **24** was removed, maintained potency. Notably, this derivative **27** proved to be only 6× less potent than the constrained analogue (+)-CBI-$CDPI_1$ (**32**).

Most significant of the observations was the behavior of the C5 acyl derivatives **21**–**23**. A 1000-fold increase in potency over (+)-CBI-$indole_1$ (**8**) was observed with **21** and **22** representing an additional $\geq$10-fold increase in potency beyond most C5 substituted derivatives described above. Analogues **21** and **22** are exceptionally potent cytotoxic compounds ($IC_{50} = 2$–3 pM) exceeding the activity of CBI-TMI (**31**, 30 pM), CC-1065 (**1**, 20 pM), duocarmycin SA (**3**, 6–10 pM), and CBI-$CDPI_1$ (**32**, 5 pM). Interestingly, the analogue **23** containing a propyl chain extension reverted to the potency characteristic of the C5 substituted analogues ($IC_{50} = 20$ pM), but did not show the further enhancement observed with **21** and **22**. Although speculative, perhaps this reflects the adoption of a DNA bound conformation for **21** and **22** analogous to the angular fusion of a five-membered ring (see **32**) with the methyl or ethyl group of **21** and **22** deeply embedded in the minor groove. This bound conformation would not be accessible to **23** because of the extended chain length and its behavior reverts back to that of an extended, flexible C5 substituent. The analogous, but less pronounced, enhancement observed with **17** may reflect a similar behavior. Notably, the methyl group of the C5 methoxy substituent of duocarmycin SA has been shown to extend into the minor groove[14,15] consistent with such a bound conformation.

Conclusion

C5 substituents on the first indole DNA binding subunit have a pronounced effect on the cytotoxic potency of CBI analogues of the duocarmycins and CC-1065. This effect, which provides as large as a 1000-fold increase in potency with **21** and **22**, is more pronounced with the CBI versus DSA- or CPI-based analogues. Moreover, this effect is largely insensitive to the electronic character of the C5 substituent but is sensitive to the size, rigid length, and shape (sp, sp^2, sp^3 hybridization) of this substituent consistent with expectation that the impact is due simply to its presence. With these

substitutions, simplified CBI analogues were identified which surpass the potency of duocarmycin SA, CC-1065, and CBI-TMI.

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