Brief Articles

CC-1065 Analogues Bearing Different DNA-Binding Subunits: Synthesis, Antitumor Activity, and Preliminary Toxicity Study

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CC-1065 analogues bearing different DNA-binding subunits were synthesized. A terminal C5−NO_2 and −F moiety at the DNA-binding subunit increased the drug's potency and antitumor efficacy. A C5−OCH_3 reduced the potency and antitumor efficacy. Compound (±)-**7**, bearing a trans double bond, had increased antitumor efficacy. A preliminary toxicity study indicated that terminal C5−OCH_3 and −acetamido moieties at the DNA-binding subunit caused delayed death in mice.

Introduction

CC-1065 (Figure 1) is one of the most potent antitumor agents discovered,[1] and has potent antitumor activity in vitro and in vivo.[1−4] CC-1065 binds to double-stranded B-DNA within the minor groove and alkylates the N3 position of the 3′-adenine.[5−7] CC-1065 also inhibits gene transcription by interfering with binding of the TATA box binding protein to its target DNA.[8] CC-1065 cannot be used in humans because it caused delayed death in experimental animals.[9]

CC-1065 comprises three indoles linked by amide bonds. The left-hand cyclopropylindole subunit alkylates DNA, and the middle and right-hand subunits enhance the DNA-binding affinity and selectivity. Substituents at the DNA-binding subunits have profound effects on the compound's antitumor potency and efficacy. A C5−acyl group was found to increase the agent's potency by more than 1000-fold in vitro.[10] A trans double bond linking the left-hand DNA-reactive and the right-hand DNA-binding subunits of CPI greatly increases the agent's potency in vitro.[11−14] Duocarmycin analogues bearing a trans double bond have increased antitumor efficacy and reduced myelosuppression.[15,16] We have previously reported that CC-1065 analogues bearing a C5−acetamido group had enhanced antitumor activity and reduced hematological effects.[17] Herein, we report the synthesis, antitumor activity, and preliminary toxicity study of new CC-1065 analogues bearing different terminal moieties at the DNA-binding subunits (Figure 2).

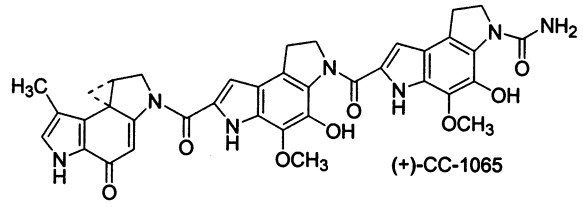

Figure 1. Structure of CC-1065.

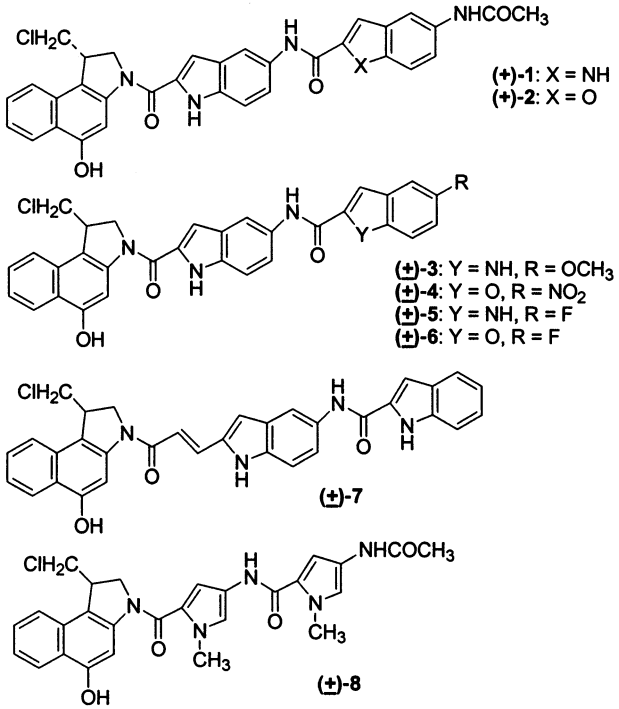

Figure 2. Structures of new CC-1065 analogues.

Chemistry

Compound **10**[17] was treated with concentrated hydrochloric acid followed by sodium nitrite and tetrafluo-

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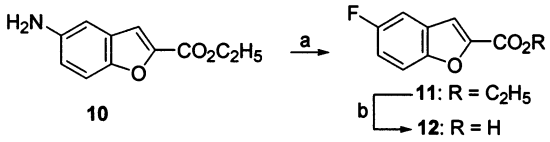
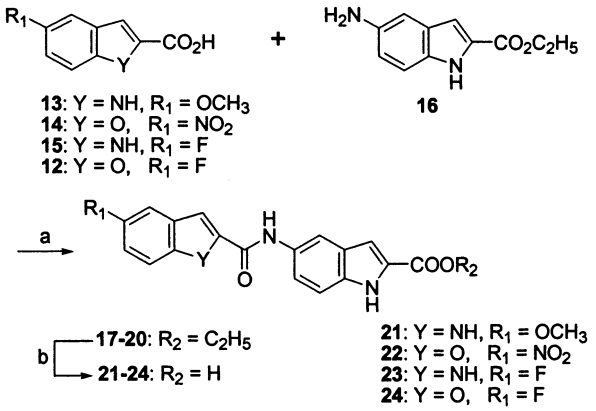
Scheme 1. Synthesis of Compound **12**[a]



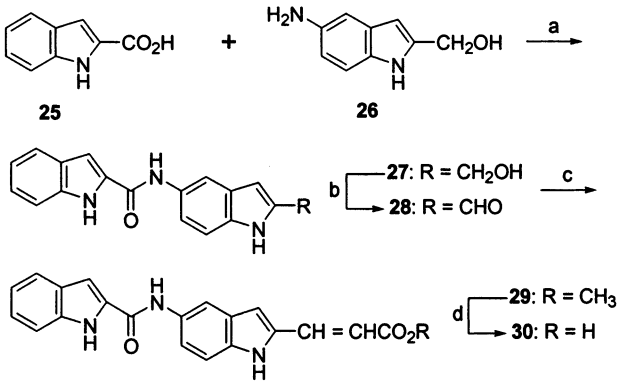
[a] (a) HNO_2, HBF_4; (b) NaOH, then HCl.

Scheme 2. Synthesis of Compounds **21**−**24**[a]



[a] (a) HBTU; (b) NaOH, then HCl.

Scheme 3. Synthesis of Compound **30**[a]



[a] (a) EDCI; (b) MnO_2; (c) $Ph_3P{=}CHCO_2CH_3$; (d) NaOH, then HCl.

roboric acid to afford an aryldiazonium tetrafluoroborate. Thermal decomposition of the latter produced the required ester **11** (Scheme 1), which was hydrolyzed to afford acid **12**. The commercially available **13**−**15** and **12** were coupled to amine **17**, respectively, in the presence of 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) to afford esters **18**−**21** (Scheme 2). Because of poor water solubility of the esters, a mixture of solvents had to be used for the subsequent saponification, and the yields varied from 40% to 80%. Acrylic acid **30** was synthesized using a strategy we previously developed (Scheme 3).[13,17] Aldehyde **28** was refluxed with methyl (triphenylphosphoranylidene)acetate in toluene for 4 days to afford ester **29** (83% yield), which was hydrolyzed to afford acid **30**. Acids **1**, **2**, and **8** were synthesized as we previously reported.[17] The targeted compounds **1**−**8** were synthesized using a method we previously developed.[17] CBI, synthesized according to a published procedure,[18] was treated with anhydrous hydrogen chloride in ethyl acetate to afford the *seco*-CBI, which was then coupled to the corresponding acids, respectively, in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI), affording **1**−**8**.

Table 1. Cytotoxicity against L1210 Leukemia Cells[a]

compd	IC_{50} (nM)	compd	IC_{50} (nM)
(+)-**1**	1.2 ± 0.73	(±)-**5**	0.29 ± 0.16
(±)-**1**	2.5 ± 1.3	(±)-**6**	0.85 ± 0.28
(+)-**2**	0.65 ± 0.35	(±)-**7**	1.0 ± 0.71
(±)-**2**	1.2 ± 0.28	(±)-**8**	44 ± 5.7
(±)-**3**	5.7 ± 1.6	doxorubicin	179 ± 94
(±)-**4**	0.44 ± 0.13		

[a] Drugs were incubated with cells for 48 h.

Table 2. Antitumor Activity in Mice Bearing L1210 Leukemia

compd	dose (μg /kg)	% weight change[a]	% ILS	30-day survivors
(+)-**1**	25	+7	173	1
	42	+5	133	0
	70	−6	80	0
(+)-**2**	25	+12	120	1
	42	−7	67	0
	70	−18	toxic	0
(±)-**3**	45	+2	93	0
	67	+3	93	0
	100	−15	53	0
(±)-**4**	45	+2	120	0
	67	−2	147	0
	100	−5	173	0
(±)-**5**	45	+2	137	0
	67	−2	160	0
	100	−7	158	0
(±)-**6**	30	+5	67	0
	45	+5	107	0
	67	0	147	0
(±)-**7**	298	0	157	0
	498	0	231	1
	620	−13	147	0
(±)-**8**	250	+7	31	0
	500	+5	31	0
	1000	−6	77	0
CP[b]	125 mg	−5	173	0

[a] Group body weight change between days 0 and 6. [b] CP = Cyclophosphamide.

Results and Discussion

Cytotoxicity. The compounds were tested in vitro against L1210 leukemia cells (Table 1). As expected, the chiral compounds (+)-**1** and (+)-**2**, with the same configuration as that of the natural CC-1065, are twice as potent as their corresponding racemic counterparts (±)-**1** and (±)-**2**. It appears that an electron-withdrawing moiety at the C5 position leads to an increased cytotoxicity. For example, compounds **4** (C5−NO_2), **5** (C5−F), and **6** (C5−F) are more potent than **1** (C5−amido), **2** (C5−amido), and **3** (C5−OCH_3). The pyrrole derivative (±)-**8** is much less potent than the indole and benzofuran derivatives **1**−**7**. This is in agreement with what has been reported before[17] and is probably due to the reduced binding between DNA and the compounds.

Antitumor Screening in Mice. Antitumor activity was tested against L1210 leukemia in mice (Table 2). The chiral (+)-**1** not only is more potent than its racemic counterpart but also has improved antitumor efficacy. For example, at an optimal dose (the dose at which the drug produces the best antitumor efficacy and the body weight loss is ≤15%), (+)-**1** (25 μg/kg) has an ILS of 173% and the racemic (±)-**1** (42 μg/kg) has only 67%.[17] The nitro analogue (±)-**4** and the fluorine agents (±)-**5** and (±)-**6** are not only more potent than the methoxy

Table 3. Time of Death in Mice Treated with Compounds **1**−**8**[a]

compd	dose (μg/kg)	days until first mouse died	no. of mice surviving on day 180
(+)-**1**	100	49	2/8
(+)-**2**	100	8	4/8
(±)-**3**	100	35	5/8
(±)-**4**	100	no death	8/8
(±)-**5**	100	no death	8/8
(±)-**6**	100	no death	7/7
(±)-**7**	100	no death	8/8
(±)-**8**	1000	60	1/6
	2000	29	0/6

[a] Drugs were administered on day 1, ip.

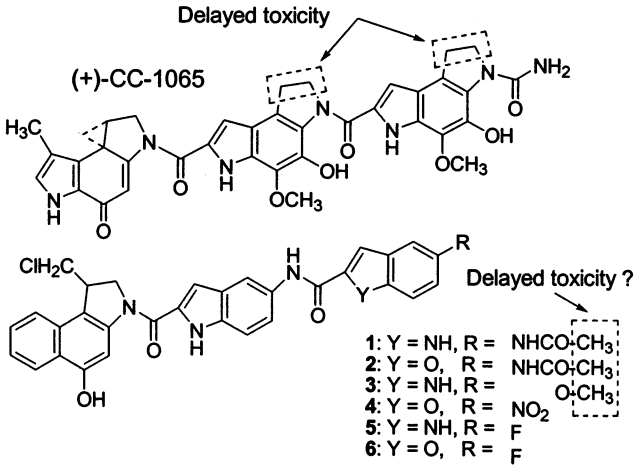


Figure 3. Structural features responsible for delayed toxicity.

analogue (±)-**3** in vitro but also much more efficacious than (±)-**3** against L1210 leukemia in mice. Compounds with two indoles such as (+)-**1** and (±)-**5** have better antitimor efficacy than their counterparts with one indole and one benzofuran such as (+)-**2** and (±)-**6**. Compound (±)-**7** is approximately 3 times less toxic than (±)-**5** in vitro but is 7 times less potent than the latter in mice. Despite the low potency, (±)-**7** has the highest therapeutic efficacy among **1**−**8**. The pyrrole derivative (±)-**8** has the lowest therapeutic efficacy.

Preliminary Toxicity Study. CC-1065 and some of its analogues have delayed toxicity in mice; i.e., mice die long after the normal observation period of 15 days for the acute toxicity study. To investigate delayed toxicity, **1**−**8** were given to non-tumor-bearing BDF_1 mice ip on day 0 (Table 3). These animals were observed for up to 180 days. Compounds (+)-**1**, (+)-**2**, and (±)-**8**, bearing a $NHCOCH_3$, and (±)-**3**, bearing a C5−OCH_3, caused delayed death, a characteristic of the phenomena observed for CC-1065.[9] The animals grew normally initially and began to lose weight 1 or 2 weeks before they died. Compounds (±)-**4**, (±)-**5**, and (±)-**6**, bearing a C5-substituted NO_2 and F, and (±)-**7**, bearing a trans double bond in the middle of the molecule, did not cause delayed toxicity.

The delayed toxicity of CC-1065 has been attributed to the presence of the ethylene groups in the molecule as indicated in Figure 3.[19] When the structures of CC-1065 and **1**−**8** are compared, it appears that compounds bearing a terminal methyl/methylene attached to a C5−indole/benzofuran- and C4−pyrrole-substituted moiety caused delayed toxicity. A C6 substituent bearing a terminal ethyl group in carzelesin did not cause delayed toxicity,[19,20] indicating that the C5 position of indole/benzofuran is particularly sensitive to substitution in terms of delayed toxicity. KW-2189, a duocarmycin derivative, has a C5 methoxy group but does not cause delayed toxicity.[21] It is important to point out that KW-2189 has only one DNA-binding subunit whereas CC-1065, **1**−**3**, and **8** have two. The delayed toxicity has no relationship to the drug's potency and therapeutic efficacy. For example, compounds **4**−**6** are more potent and more efficacious than **3** and the racemic **1**[17] and **2**;[17] however, **4**−**6** did not have delayed toxicity. Further structure−toxicity studies are needed to reveal the structural features responsible for the delayed toxicity.

Conclusions

The chiral compound (+)-**1** showed significant antitumor activity against L1210. However, it caused delayed toxicity in mice. A C5−OCH_3 resulted in reduced potency and antitumor efficacy as well as delayed toxicity. Compounds **4**−**6** had increased potency and antitumor efficacy in vivo without delayed toxicity. A trans double bond linking the DNA-reactive and -binding subunits led to an increased antitumor efficacy. A preliminary toxicity study suggests that the terminal C5 position of indole/benzofuran and the C4 position of pyrrole substituents of the DNA-binding subunit of CC-1065 analogues are very sensitive in terms of antitumor efficacy and delayed toxicity.

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Supporting Information Available: Chemical synthesis, spectral and analytical data for all new compounds, and experimental details of antitumor activity evaluations in vitro and in mice. This material is available free of charge via the Internet at http://pubs.acs.org.

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