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Design, synthesis, and biological evaluation of achiral analogs of duocarmycin SA

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Abstract—The design, synthesis, as well as biochemical and biological evaluation of two novel achiral analogs of duocarmycin SA (DUMSA), **1** and **2**, are described. Like CC-1065 and adozelesin, compounds **1** and **2** covalently reacted with adenine-N3 in AT-rich sequences and led to the formation of DNA strand breaks upon heating. The cytotoxicity of compounds **1** and **2** against human cancer cells (K562, LS174T) was determined using a MTT assay giving IC_{50} values in the low nanomolar. Further cytotoxicity screening of compound **2** conducted by the NCI against a panel of 60 different human cancer cell lines indicated that it was particularly active against several solid tumor cells lines derived from the lung, colon, CNS, skin, and breast.

CC-1065[1] and duocarmycin SA[2] (Fig. 1) are cyclopropanepyrroloindolone- or CPI-containing natural products isolated from *Streptomyces*. Both compounds exhibit potent anticancer activity, with IC_{50} values in the picomolar range against the growth of mouse L1210 leukemia cells in culture.[3] They derive their cytotoxic property through covalent reaction with adenine-N3 in the minor groove of AT-rich sequences.[3] Due to their potent cytotoxic properties, the CC-1065 and duocarmycin class of compounds have received significant attention, and four analogs were selected for clinical evaluation, including adozelesin (Fig. 1).[3b] Presently,

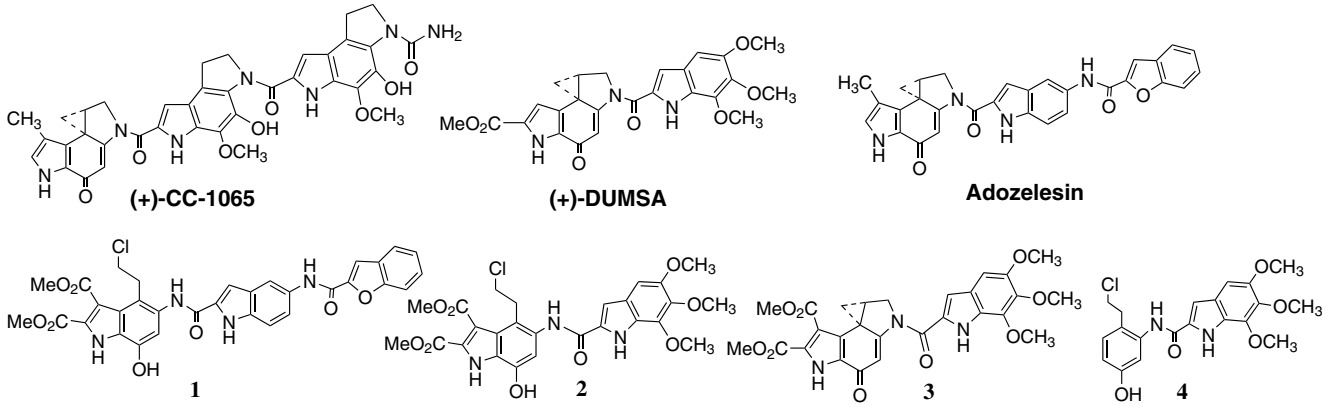

Figure 1. Structures of (+)-CC-1065, doucarmycin SA (DUMSA), adozelesin, and compounds **1**–**4**.

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only one of the four compounds, bizelesin, remains in clinical trial.[4] One of the severe dose limiting toxicities of these compounds is bone marrow suppression.[3b,4,5] Consequently, there is a strong interest in the design, synthesis, and testing of novel analogs that have comparable antitumor activity, but with reduced systemic toxicity. A wide range of analogs of CC-1065 and the duocarmycins, including modifications of the alkylating subunit, such as analog **3**,[6] *seco-iso*-cyclopropanefuranoindoline (*seco-iso*-CFI) analogs, and other heterocycles have been investigated.[7] Studies were also conducted in which the non-covalent binding subunit of the molecules was altered, which included the use of water soluble pyridyl systems.[8]

One aspect of the CPI structure that has not been investigated with respect to DNA interaction and anticancer properties is the chiral center present in CC-1065 and the duocarmycins. Studies have revealed that the optically active (+)-(*S*) enantiomers are generally more cytotoxic than their mirror images. For example, natural (+)-(*S*)-DUMSA has an IC_{50} of 10 pM, compared to 100 pM for the unnatural isomer. This is consistent with (+)-(*S*)-DUMSA being ten times more effective in reacting with DNA.[3b,3c,9] Further, the binding orientation of (+)-DUMSA is 3′–5′ over an AT-rich 3–5 base pair site, but the (−)-enantiomer orients in the 5′–3′ direction. Evidently, chirality can influence the biological properties of the duocarmycin compounds. This has led our laboratories to initiate a program to investigate whether the chiral effects could be eliminated while retaining DNA interaction and cytotoxicity. In an earlier study, we have shown that the hydroxyphenethyl chloride in compound **4**, an achiral *seco*-cyclopropaneindoline and the simplest analog of the duocarmycins was able to interact with DNA and inhibited the growth of cancer cells in vitro[10] Based on the structure–activity relationship that a DUMSA alkylating subunit, which is solvolytically one of the most stable analogs and the most cytotoxic,[3] compounds **1** and **2** (Fig. 1) were designed.

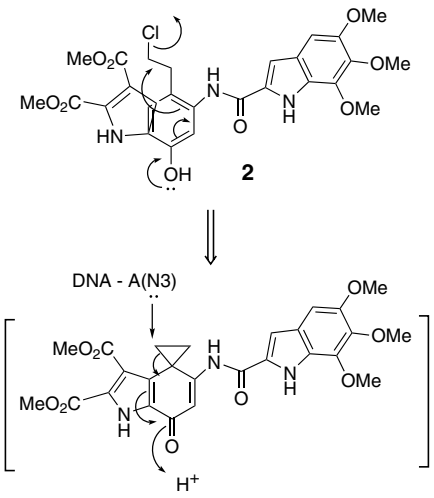
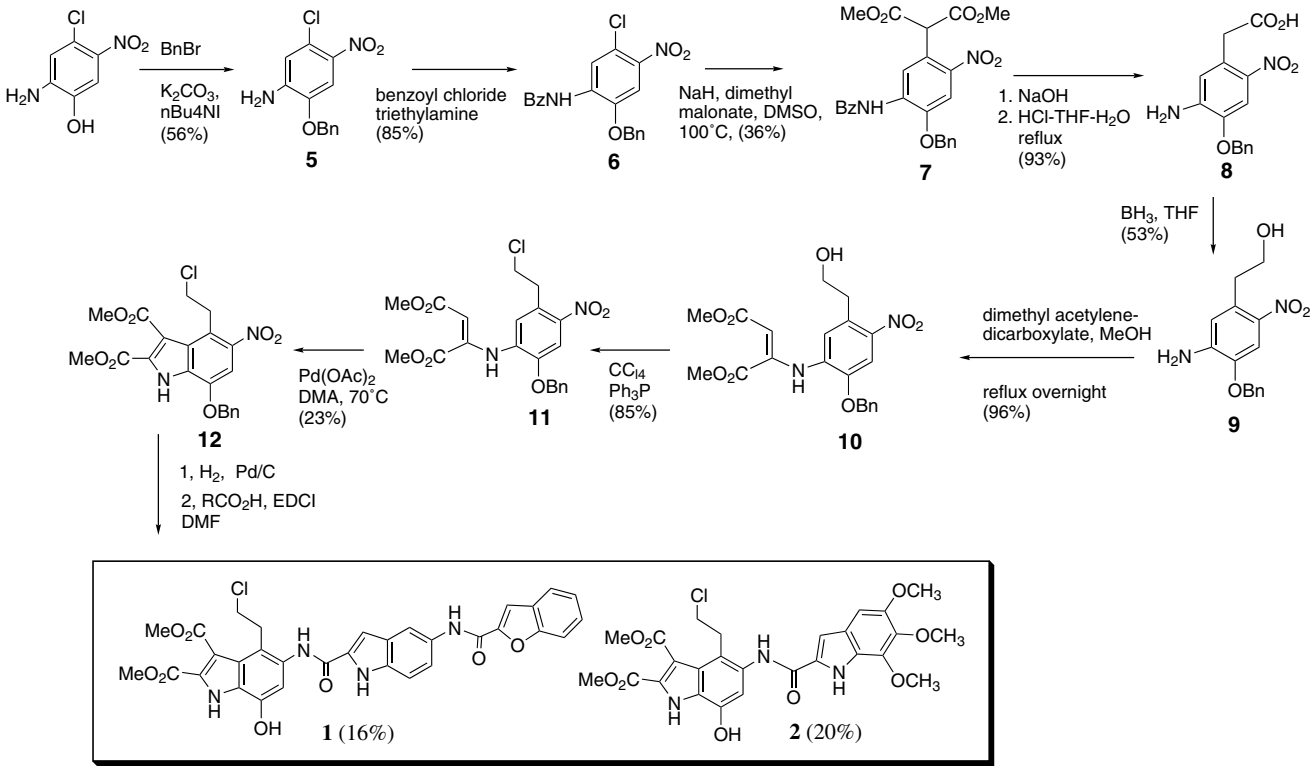


Figure 2. Proposed mechanism of activation and DNA alkylation by achiral duocarmycin analog **2**.

The achiral *seco*-duocarmycin analog **2** should lose HCl to generate the ultimate cyclopropane-containing drug, which should react with adenine-N3 (Fig. 2). Compound **2** is analogous to the previously reported DUMSA analog **3**, which has an IC_{50} value of 1.38 nM against P388 murine leukemia cancer cells in vitro[5] Along with the synthesis of compounds **1** and **2**, their biochemical and cytotoxic properties are described herein (Scheme 1).

Synthesis of compounds **1** and **2** began with the reaction of 2-amino-4-chloro-5-nitrophenol with benzyl bromide to give aniline **5** in 56% yield. Reaction of **5** with benzoyl chloride provided benzamide **6** in 85% yield, which was reacted with sodium dimethyl malonate to afford malonate **7** in 36% yield. Hydrolysis of the ester groups of compound **7**, followed by decarboxylation afforded acid **8** in 93% yield. The carboxylic acid group was selectively reduced with borane in THF to produce alcohol **9** in 53% yield. Reaction of compound **9** with dimethyl acetylenedicarboxylate in methanol gave compound **10** in 96% yield. The alcohol group in compound **10** was converted to a chloride **11** in 85% yield with carbon tetrachloride and triphenylphosphine. Reaction of compound **11** with palladium(II) acetate in DMA at 70 °C gave indole **12** in 23% yield.[6] Hydrogenation of compound **12** with 10% palladium-on-carbon in THF gave an amine intermediate, which was directly coupled with 5,6,7-trimethoxyindole-2-carboxylic acid[11] and 5-(benzofuran-2-carboxamido)-indole-2-carboxylic acid[12] in the presence of EDCI in DMF at room temperature for three days. The target achiral *seco*-duocarmycin analogs **1** and **2** were isolated in 16% and 20% yield, respectively. All compounds reported in this paper were characterized by NMR, IR, high resolution MS. Compounds **1** and **2** were further characterized by elemental analysis.

The cytotoxic and DNA binding properties of compounds **1** and **2** were assessed. The cytotoxicity studies were conducted with 3-day continuous exposure on two human cancer cell lines using a MTT based colorimetric assay.[13] The IC_{50} values given in Table 1 indicate that both compounds have activity in the nanomolar range and they are active against leukemia and solid tumors. The results showed that achiral duocarmycins **1** and **2** were 58–3450 times more cytotoxic than the achiral-CI compound **4**, and that was likely to be a result of enhanced stability of the duocarmycin alkylating subunit.[3b,3c] More significantly, compound **2** has comparable cytotoxic potency to its chiral counterpart **3**, albeit the latter was against P388 cells.[6] These results suggest that the chiral center present in the duocarmycins is not critical for cytotoxicity. Compound **2** was further tested by the NCI against their panel of 60 different human cancer cells. The agent has potent activity with 50% net growth inhibition conferred by 5.6–330 nM (95 nM mean). Compound **2** was found to exhibit selectivity against several solid tumor cells of the lung (NCI-H522, NCI-H226, EKVX), colon (HT29, KM12), CNS (SF-268, SF-539, SNB-75), melanoma (M14, SK-MEL-2, UACC-62), ovarian (OVCAR-8), and breast (HS-578T).



Scheme 1. Synthesis of achiral duocarmycin **1** and **2**.

Table 1. Cytotoxicity of compounds **1** and **2** determined using the MTT assay

Compound	IC_{50} (nM)	
	K562	LS174T
1	15	29
2	25	4.0
4	1470	13800

K562 = chronic human myeloid leukemia cells; LS174T = human colon carcinoma.

The ability of compounds **1** and **2** to covalently interact with DNA was studied using a thermal induced DNA cleavage assay.[7,10] Treatment of supercoiled pBR322 DNA with 6.6 μM of the compounds for 40 h at 40 °C produced 30 ± 3% and 23 ± 3% of Form I DNA, respectively. Control pBR322 DNA gave 5% spontaneous DNA cleavage under similar conditions. These results are consistent with the alkylation of purine-N3 positions in the minor groove of the DNA. DNA sequence specificity was ascertained using a Taq DNA polymerase stop assay.[14] The pBR322 DNA was linearized to provide a stop for the *Taq* downstream from the primer. The oligodeoxynucleotide primers were 5′-end labeled prior to amplification using T4 polynucleotide kinase and [γ^{32}P]-ATP (5000 Ci/mmol). The primer 5′-GCAGCAGATTACGCGCAGAA-3′ binds to the complementary strand at position 3090–3109 and was used to examine the alkylation on the bottom strand. The primer 5′-GCATTGGTAACTG-TCAGACC-3′ binds in the sequence 3303–3284 and was used to examine the top strand. From the gels shown in Fig. 3, achiral *seco*-DUMSA **2** generally showed similar covalent sequences selectivity to CC-1065 and adozelesin, at 5′-TTA*A*-3′ and AAA*AAA* sequences. However, the gel showed additional sites of alkylation at some adenine-N3 sites that were absent for adozelesin and CC-1065.

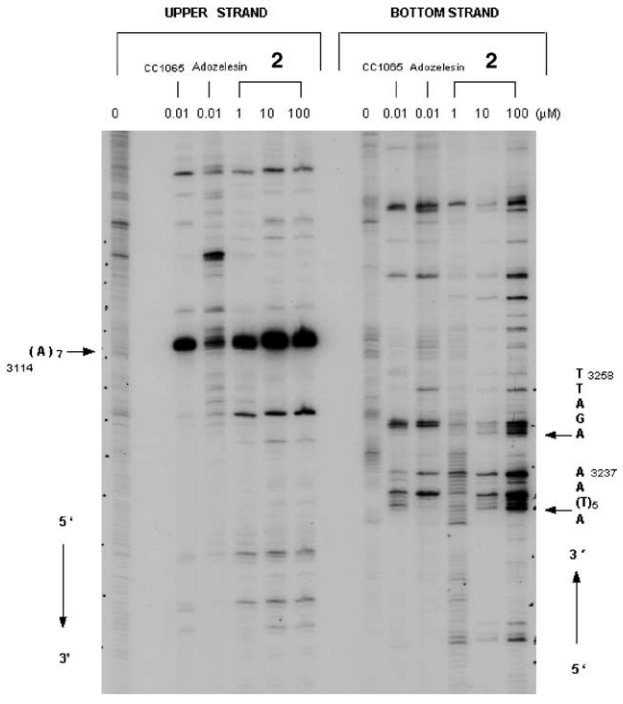


Figure 3. Taq DNA polymerase stop studies on compounds **2**.

The sequence preference of compound **1** was identical to that for adozelesin and CC-1065 (data not shown). These results further support our contention that the chiral center is not needed for this class of compounds to display potent biological activities. They also provide indication that, like the chiral compounds, improvement of the chemical stability of the achiral alkylating subunit can increase the cytotoxic potency of the compounds. In summary, achiral analogs of CC-1065 and the duocarmycins represent a novel class of agents with potential as anticancer drugs.

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