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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,

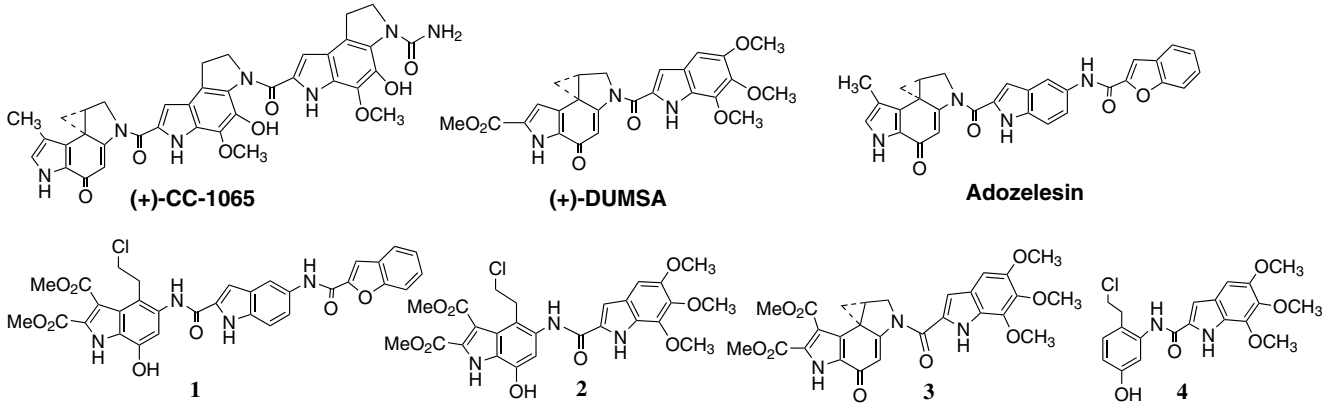

Figure 1. Structures of (+)-CC-1065, doucarmycin SA (DUMSA), adozelesin, and compounds **1**–**4**.

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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.

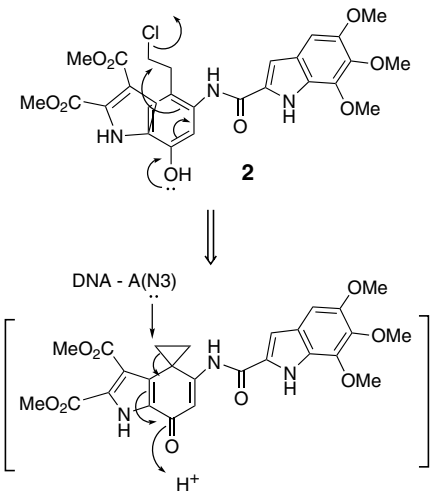

Figure 2. Proposed mechanism of activation and DNA alkylation by achiral duocarmycin analog **2**.

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).