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Synthesis and evaluation of a thio analogue of duocarmycin SA

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ABSTRACT

The design, synthesis, and preliminary evaluation of methyl 1,2,8,8a-tetrahydrocyclopropa[*c*]thieno[3,2-*e*]indol-4-one-6-carboxylate (CTI) derivatives are detailed representing a single atom change (N to S) embedded in the duocarmycin SA alkylation subunit.

Duocarmycin SA (**1**, IC_{50} = 10 pM)[1] is among the most potent members of a class of antitumor antibiotics that also include duocarmycin A (**2**),[2] yatakemycin (**3**),[3] and CC-1065 (**4**, Fig. 1).[4] Each derives its properties from a characteristic sequence-selective alkylation of duplex DNA,[5–9] in which a stereoelectronically controlled adenine N3 addition to the least substituted carbon of the activated cyclopropane occurs within selected minor-groove AT-rich sites.

Extensive investigations on the natural products in this class as well as their synthetic analogues have defined key and subtle features that contribute to their properties.[9,10] Most notable of these are the structural features that contribute to the AT-rich noncovalent binding selectivity dominating the alkylation selectivity,[11] those that are responsible for the source of catalysis for the DNA alkylation reaction,[12,13] and those that subtly impact the unusual and intrinsic stability of their alkylation subunits.[9,13–16]

In a recent study,[17] the rational design of a modified CC-1065 alkylation subunit was conducted with the goal of increasing its chemical stability and resulting biological potency and provided more potent analogues of the natural product. Thus, the replacement of the pyrrole NH in the CC-1065 alkylation subunit with a larger, more electron-withdrawing sulfur atom had the predicted and desired effect of reducing the solvolytic reactivity of the alkylation subunit, and ultimately increasing the cytotoxic potency of the corresponding CC-1065 analogue (IC_{50} = 7 pM vs 20 pM) to levels on par with duocarmycin SA (10 pM). Herein, we report the extension of these studies to an analogous alkylation subunit pyrrole NH to S modification of the duocarmycin SA alkylation subunit,[18] replacing a single atom in **1** to generate **7** (Fig. 2).

The synthesis of the modified alkylation subunit[19,20] began with treatment of 2-hydroxy-3-methoxy-5-nitrobenzaldehyde (**8**) with dimethylthiocarbamoyl chloride to afford the desired carbamothioate **9** (2 equiv DABCO, 1.5 equiv dimethylthiocarbamoyl

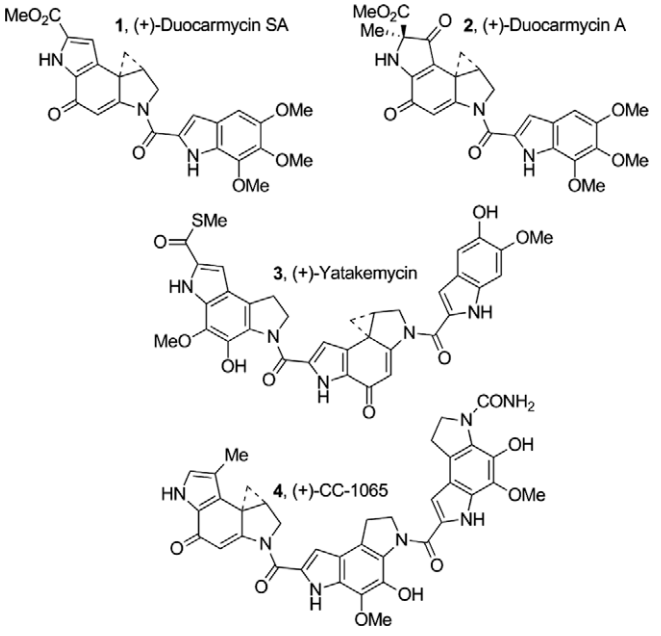

Figure 1. Natural products.

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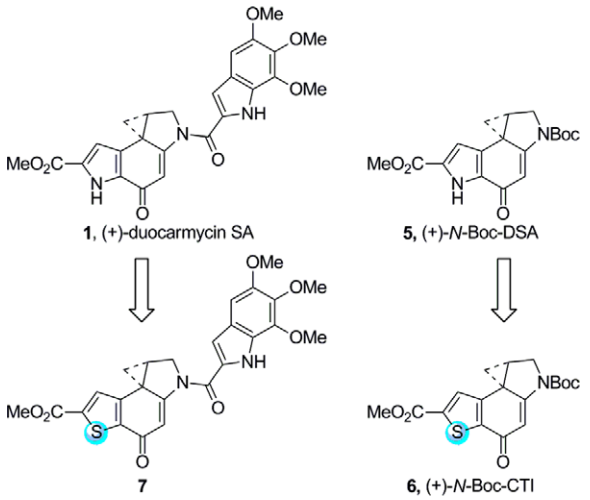
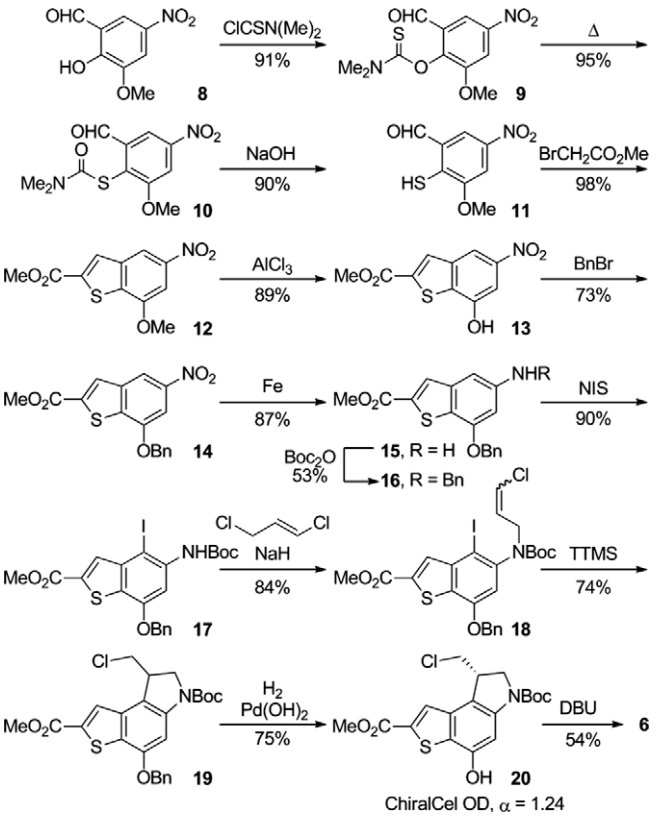
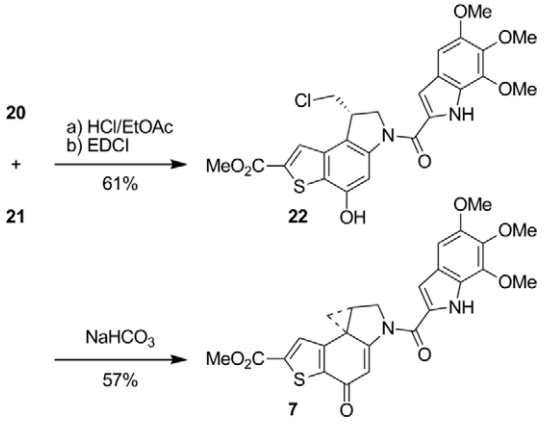


Figure 2. Duocarmycin SA analogues.

chloride, DMF, 50–60 °C, 2 h, 91%). Compound **9** was thermally rearranged to provide **10** (toluene, 110 °C, 12 h, 95%) and the dimethylformamide group was removed (4 M NaOH, then 20% HCl, MeOH, 100 °C, 5 h, 90%) to afford the free thiol **11**. Alkylation of **11** with methyl bromoacetate followed by cyclization afforded benzothiophene **12** (1.5 equiv $BrCH_2CO_2Me$, 1.3 equiv K_2CO_3, 60 °C, 12 h, 98%). Deprotection of the methyl ether enlisting $AlCl_3$ (**13**, 0.14 equiv, CH_2Cl_2, 40 °C, 32 h, 89%) was followed by protection of the free phenol as its benzyl ether **14** (1.2 equiv benzyl bromide, 1.2 equiv K_2CO_3, acetone, 60 °C, 3.5 h, 73%). The aryl nitro group was reduced to the corresponding amine **15** (3 equiv Fe, 0.6 equiv NH_4Cl, MeOH/H_2O, 100 °C, 4 h, 87%) and the resulting aniline was protected to provide **16** using Boc_2O (5 equiv, THF, 23 °C, 12 h, 53%). Regioselective C4-iodination using *N*-iodosuccinimide (1.1 equiv, 1.1 equiv H_2SO_4, MeOH/THF, 23 °C, 4 h, 90%) afforded **17**, which was alkylated with 1,3-dichloropropene (2 equiv, 3 equiv NaH, DMF, 0 °C, 4 h, 84%) to afford **18**. The final ring of the alkylation subunit was constructed using a tris(trimethylsilyl)silane-mediated 5-*exo*-*trig* aryl radical–alkene cyclization (0.9 equiv TTMS, 0.2 equiv AIBN, benzene, 80 °C, 4 h, 74%) to afford **19**.[21] Treatment of **19** with $Pd(OH)_2$ under an atmosphere of hydrogen gas ($Pd(OH)_2$, 1 atm H_2 gas, THF, 23 °C, 24 h, 75%) effectively cleaved the benzyl ether protecting group, affording **20**. Resolution of **20** into its enantiomers was possible at this stage using a ChiralCel OD semi-preparative HPLC column (2 × 25 cm, 2% *i*-PrOH/hexane, 7 mL/min, $\alpha = 1.24$).[22] Spirocyclization was accomplished upon treatment of **20** with DBU (1.3 equiv DBU, MeCN, 23 °C, 2.5 h, 54%) to afford **6** (Scheme 1, natural enantiomer shown).



Scheme 1.



Scheme 2.

Boc deprotection of **20** (4 N HCl/EtOAc, 23 °C, 1.5 h) followed by direct coupling of the resulting indoline hydrochloride salt with 5,6,7-trimethoxyindole-2-carboxylic acid[23] (**21**, 1.1 equiv, 3 equiv EDCI, DMF, 23 °C, 1.5 h, 61%) afforded **22** (Scheme 2, natural enantiomer shown) that was spirocyclized under mild conditions (saturated aqueous $NaHCO_3$, DMF, 57%) to afford **7**.

The results of the examination of *N*-Boc-CTI and the corresponding duocarmycin SA analogue in an L1210 cytotoxic assay are summarized in Table 1 along with the results of the comparison duocarmycin SA derivatives. Both enantiomers of the CTI-based analogues displayed cytotoxic activity (L1210) nearly identical to or slightly more potent than their DSA counterparts.

The natural enantiomer of CTI-TMI (**7**, $IC_{50} = 7$ pM) exhibited activity slightly more potent than that of duocarmycin SA (**1**, $IC_{50} = 10$ pM). The unnatural enantiomer of **7** exhibited the 10-fold loss of activity ($IC_{50} = 60$ pM) compared to the natural enantiomer and characteristic of duocarmycin SA itself. The Boc derivatives **6** exhibited nearly identical activity to that of *N*-Boc-DSA (**5**), with the unnatural enantiomers being 10-fold less potent than the natural enantiomers.

Table 1
In vitro cytotoxic activity, L1210

Compound	IC_{50} (pM)
(+)-**5**, (+)-*N*-Boc-DSA	6000
(−)-**5**, *ent*-(−)-*N*-Boc-DSA	60,000
(+)-**6**, (+)-*N*-Boc-CTI	6000
(−)-**6**, *ent*-(−)-*N*-Boc-CTI	55,000
(+)-**1**, (+)-duocarmycin SA	10
(−)-**1**, *ent*-(−)-duocarmycin SA	100
(+)-**7**, (+)-CTI-TMI	7
(−)-**7**, *ent*-(−)-CTI-TMI	60

The DNA alkylation selectivity of the new analogues was examined within a 150 base-pair segment of DNA utilized and described previously (w794).[24] The alkylation site identification and the assessment of the relative selectivity among the available sites were obtained by thermally-induced strand cleavage of the singly 5′-end-labeled duplex DNA after exposure to the compounds as detailed.[5–8] Figure 3 illustrates the alkylation selectivity of both (+)- and *ent*-(−)-CTI-TMI (**7**) alongside (+)- and *ent*-(−)-duocarmycin SA. Satisfyingly, each enantiomer of **7** alkylated the same site as its duocarmycin SA counterpart, displaying the same characteristic and enantiomerically distinguishable selectivity. The natural enantiomer, (+)-**7**, alkylated DNA with an efficiency not distinguishable from (+)-duocarmycin SA, (+)-**1**, and appreciable alkylation was seen at concentrations of 10^{-6} M (not shown). Like duocarmycin SA, the unnatural enantiomer of **7** proved less efficient at alkylating DNA than its natural enantiomer, and both unnatural enantiomers alkylated the same major site.

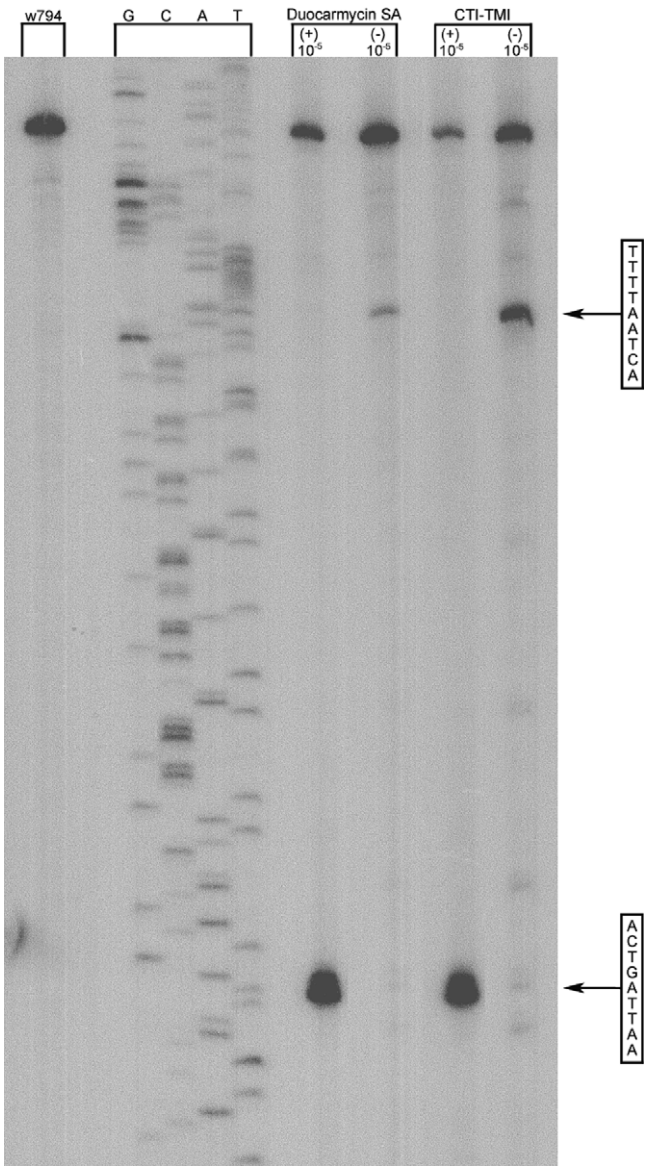


Figure 3. Thermally-induced strand cleavage of w794 DNA (144 bp, nucleotide no. 5238–138) after DNA–agent incubation with duocarmycin SA and CTI-TMI (24 h, 23 °C), removal of unbound agent by EtOH precipitation and 30 min thermolysis (100 °C), followed by denaturing 8% PAGE and autoradiography. Lane 1, control DNA; lanes 2–5, Sanger G, C, A, and T sequencing standards; lane 6, (+)-duocarmycin SA (**1**, 1×10^{-5} M); lane 7, (−)-duocarmycin SA (1×10^{-5} M); lanes 8 and 9, (+)-CTI-TMI and *ent*-(−)-CTI-TMI (**7**, 1×10^{-5} M).

The CTI alkylation subunit was examined to establish whether the magnitude of the effects observed with the MeCTI alkylation subunit derived from a single atom replacement in the alkylation subunit of CC-1065 would be similarly observed with duocarmycin SA. The work presented herein demonstrates that replacing a pyrrole NH of the alkylation subunit of duocarmycin SA with a sulfur atom maintains or slightly enhances the biological potency of the natural product, but not to the extent observed with MeCTI. Additionally, CTI-TMI alkylated DNA in a fashion identical to duocarmycin SA, exhibiting the characteristic enantiomeric selectivities and distinguishable enantiomeric efficiencies.

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Supplementary data

Full details of the synthesis of **6** and **7** and their experimental examination are provided. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.10.063.

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