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NITROGEN AND SULFUR ANALOGUES OF THE *seco*-CI ALKYLATING AGENT: SYNTHESIS AND CYTOTOXICITY

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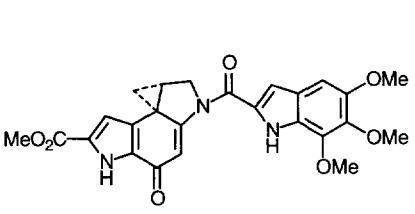
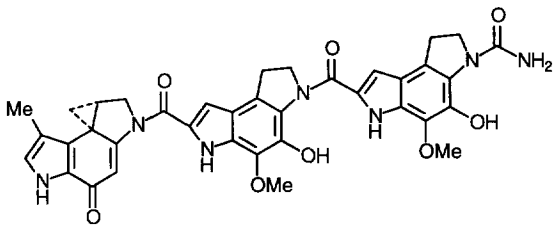
Abstract: The first synthesis of *seco*-CI alkylating agents bearing a nitrogen or sulfur substituent in place of the oxygen at C-6 is described. In comparison with a phenol, an amino substituent confers reduced but significant cytotoxicity, even when mono- or dimethylated, while sulfur analogues are considerably less potent.

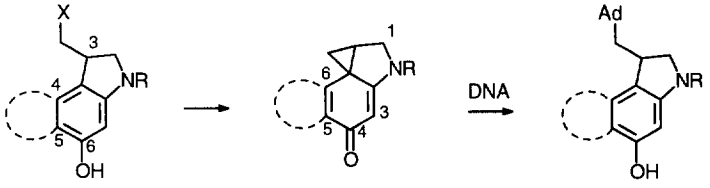
CC-1065 and the duocarmycins comprise a group of exceptionally potent antitumor antibiotics that bind to DNA in the minor groove and alkylate at N-3 of adenine in a sequence selective manner.[1] Extensive investigation of these natural products and related synthetic derivatives has identified **2** (1,2,7,7a-tetrahydrocycloprop[1,2-*c*]indol-4-one; CI) as the minimum potent pharmacophore, which can be formed by ring closure of a *seco*-CI parent **1**. Synthetic variations on the CI theme have included ring fusion at C-5,6[1,2] (CI-numbering) or other substitution at C-5,[3] alteration of the minor groove targeting unit R,[4] expansion[5] or deletion[6] of the N-containing ring, and variation of the leaving group X of the *seco*-CI precursor.[3,7] Important



(+)-duocarmycin SA

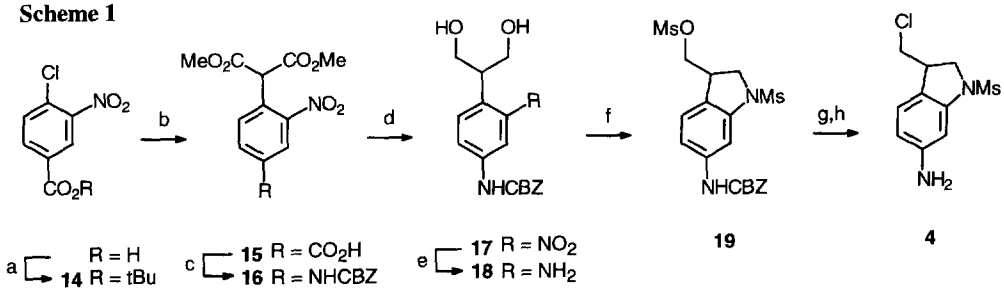


(+)-CC-1065



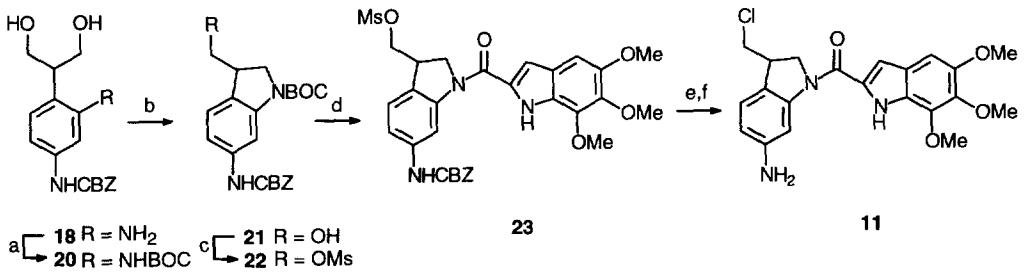
seco-CI **1** CI **2**

Scheme 1



Reagents: (a) $SOCl_2$, DMF then KOtBu (89%) (b) $CH_2(CO_2Me)_2$, NaH, then HCO_2H (87%) (c) $SOCl_2$ then NaN_3 then toluene reflux then $PhCH_2OH$ (81%) (d) iBu_2AlH, THF (50-61%) (e) H_2, PtO_2, EtOH (94%) (f) $MeSO_2Cl$, py (93%) (g) Pd/C, HCO_2NH_4 (89%) (h) LiCl, DMF (94%)

Scheme 2

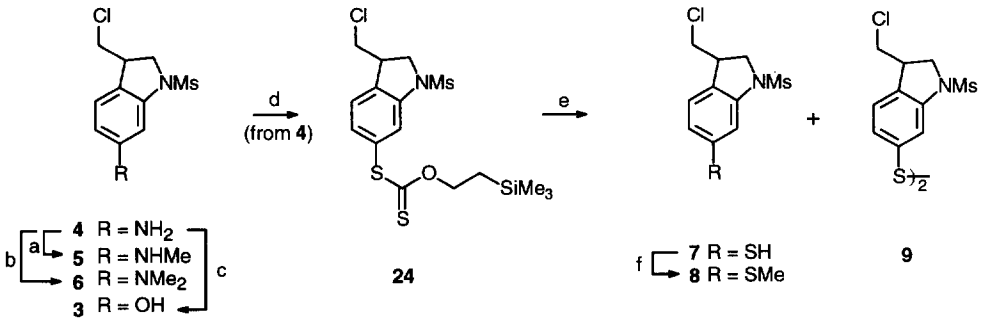


Reagents: (a) $(BOC)_2O$, Na_2CO_3 (85%) (b) DEAD, PPh_3 (79%) (c) $MeSO_2Cl$, Et_3N (97%) (d) HCl then EDCI.HCl, 5,6,7-trimethoxyindole-2-carboxylic acid (59%) (e) Pd/C, HCO_2NH_4 (f) LiCl, DMF (61% from **23**)

structure-activity relationships derived from these studies include the observation of an *inverse* relationship between CI reactivity (rate of solvolysis) and biological potency (IC_{50}).[1] In contrast, modification of the oxygen substituent has been surprisingly limited, involving the preparation of simple ethers, esters, and carbamates of **1**.[7-9] These studies have revealed that a free OH (i.e. the ability to ring close to a cyclopropane) is *not* obligatory for DNA alkylation. Rather, for those agents incapable of ring closure, both alkylating ability and cytotoxicity appear related to the electron-donating ability of the C-6 substituent (*seco*-CI numbering), at least for the limited series of compounds known.[7,9]

We report herein the synthesis of the first *seco*-CI compounds in which the oxygen functionality at C-6 is replaced by nitrogen or sulfur (see the Table for structures of the target compounds). Heteroatom substitution at this crucial position raises the questions of whether DNA alkylation will still occur, and if so whether by the

Scheme 3



Reagents: (a) CH_3CO_2CHO then BH_3.DMS (76%) (b) HCHO, $NaBH_3CN$, HCl (84%) (c) H_2SO_4, $NaNO_2$, then toluene reflux (52%) (d) HCl, $NaNO_2$ then $Me_3SiCH_2CH_2OCS_2K$ (e) TBAF (from **4**; **7** 10%, **9** 14%) (f) MeI (84%)

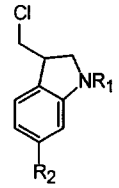
same mechanism and with the same relative intensity, sequence selectivity, and resulting cytotoxicity. As an initial investigation we present a comparison of the cytotoxicity of oxygen, nitrogen, and sulfur analogues, and the effect on cytotoxicity of minor groove targeting and methylation of the C-6 substituent.

Scheme 1 shows the synthesis of racemic[10] **4,** the anilino analogue of the known *seco*-CI **3**.[3] This synthesis is a derivation of early work on CPI compounds,[11] but begins with 4-chloro-3-nitrobenzoic acid in place of 4-chloro-3-nitrophenol. The extra electron-withdrawing carboxyl substituent (protected as the *t*-Bu ester **14**) not only facilitates nucleophilic displacement by the malonate anion, but after selective hydrolysis to the acid **15** is converted by Curtius rearrangement to **16**, thus introducing the required nitrogen substituent in a suitably protected form. Reduction of the esters and nitro group, and cyclisation to **19** proceeded as expected.[11] The CBZ protecting group was removed by hydrogenolysis and the labile mesylate displaced with LiCl to provide the first target compound **4**.

Scheme 2 shows a refinement of this synthesis that allows incorporation of various minor groove targeting units, illustrated in this case with the (5,6,7-trimethoxyindol-2-yl)carbonyl (TMI) group common to the duocarmycins. This modification required selective protection of the amino group of **18**; this was achieved with di-*t*-butyldicarbonate and Na_2CO_3 in aqueous THF, and the product cyclised under Mitsunobu conditions. The remaining alcohol was converted to its mesylate **22**, the BOC protecting group removed, and the amine hydrochloride coupled with 5,6,7-trimethoxyindole-2-carboxylic acid under standard conditions[7] to give **23**. Reactions as in Scheme 1 then provided **11**, the anilino analogue of the known *seco*-CI **10**.[7] This straightforward synthesis thus proceeds in 10 steps from 4-chloro-3-nitrobenzoic acid in an overall yield of 9%.

Scheme 3 shows various ways in which the amino *seco*-CI compounds can be derivatised. Mono- and

Table



Compound	R_1	R_2	σ_p of R_2[b]	IC_{50} (μM)[a]		
				EMT6	AA8	UV4
3	SO_2Me	OH	-0.37	7.7	20	1.9
4	SO_2Me	NH_2	-0.66	33	158	13
5	SO_2Me	NHMe	-0.84	13	50	4.1
6	SO_2Me	NMe_2	-0.83	20	72	15
7	SO_2Me	SH	0.15	>200[c]	>200[c]	124
8	SO_2Me	SMe	0.00	>200[c]	>200[c]	>200[c]
9	SO_2Me	$S)_2$		>50[c]	>50[c]	>50[c]
10	TMI[d]	OH		0.0022	0.0065	0.0010
11	TMI	NH_2		0.27	0.32	0.059
12	TMI	NHMe		0.11	0.22	0.055
13	TMI	NMe_2		0.11	0.28	0.088

(a) IC_{50} for 4 h drug exposure at pH 7.4. Stock solutions were prepared in DMSO and diluted into culture medium to give final DMSO concentrations <0.5%. Values are the average of 2-4 experiments, the SEM was on average 9% of the mean (b) ref. 12 (c) tested at solubility limit (d) TMI = (5,6,7-trimethoxyindol-2-yl)carbonyl

dimethylation resulted from formylation/borane reduction[13] and reductive amination respectively, giving **5** and **6**; similar reactions in the TMI series provided **12** and **13**. In an unoptimised procedure, **4** was treated with $H_2SO_4/NaNO_2$ at 0 °C and the diazonium solution heated in the presence of toluene. A 52% yield of **3** (identical to authentic material)[3,14] was obtained from the toluene layer. Diazonium chemistry was also used to prepare the thiophenol analogue **7**. One established route to thiophenols employs an *O*-ethyl dithiocarbonate intermediate, but requires a strongly basic hydrolysis step to yield the free thiophenol. Since **7** was not expected to be stable under these conditions, a new reagent, potassium *O*-[2-(trimethylsilyl)ethyl]dithiocarbonate was prepared. This gave the expected substituted dithiocarbonate **24**, which was readily cleaved under the mild

conditions of exposure to fluoride for a few minutes at room temperature. Unfortunately, the overall conversion from **4** to thiophenol **7** and disulfide **9** (obtained directly in the deprotection step) was poor. Low yields meant that the TMI analogues of **7** and **9** were not accessible by this route. Treatment of **7** with MeI/$NaHCO_3$ gave **8**, completing the synthesis of the target compounds.

Cytotoxicities were determined as IC_{50} values in three cell lines (the murine mammary carcinoma EMT6 and the CHO lines AA8 and UV4) for a 4 h drug exposure using a growth inhibition microassay, which has been described previously.[15] The UV4 cell line carries a mutation in the ERCC-1 gene[16] and is hypersensitive to agents whose cytotoxicity is due to bulky DNA adducts or cross-links.[17] In accord with previous results[3,7] the phenol **3** exhibited a cytotoxicity in the low micromolar range, while the minor groove targeted analogue **10** was approximately 1000-fold more potent (Table). The cytotoxicity ratio between AA8 and UV4 cell lines [hypersensitivity factor, HF = IC_{50}(AA8)/IC_{50}(UV4)] of 6-10 is within the range expected for a DNA mono-alkylating agent.[17]

The aniline compounds proved to be less potent than the corresponding phenols: not only is **4** 4 to 8 times less cytotoxic than **3**, but the potency gain on minor groove targeting is considerably reduced. This gives an overall loss of potency of 50- to 120-fold when comparing aniline **11** with phenol **10**. However, the HF values (12 for **4**, 5.5 for **11**) suggest a mechanism of action similar to that of the phenols. This has recently been confirmed both by DNA alkylation studies (showing **10** and **11** to have similar sequence selectivity) and by isolation and characterisation of the adenine N-3 adduct of **11**.[18] The observed potencies presumably reflect both the propensity of the *seco*-CI agent to ring close, and the reactivity of any cyclopropane species that is formed. If a strongly electron-donating NH_2 group (see Table for σ_p values) correlates with a reactive CI intermediate, then the observation of reduced potency would be consistent with the previously reported inverse relationship between CI reactivity and IC_{50}.[1] Other factors, such as relative solvolytic stability, reversibility of adduct formation, and especially the considerably reduced acidity of the amino substituent, may also play a role. Interestingly, both **3** and **10** are less cytotoxic when assayed under acidic conditions (5- to 7-fold loss of potency against the EMT6 cell line at pH 6.5) while **4** and **11** show no such loss of activity, an observation of relevance given the generally more acidic nature of solid tumors compared to normal tissues.[19] Monomethylation, and surprisingly also dimethylation of the amino group, gives analogues of similar or even slightly enhanced potency. This is in contrast to the relative cytotoxicities of phenol and methoxy compounds [IC_{50}(OMe)/IC_{50}(OH) = 10 nontargeted (R_1 = BOC); 150 targeted (R_1 = TMI)], where agents incapable of ring closure had significantly attenuated potency.[9] The relative rates of solvolysis of **11-13**, and characterisation of any CI intermediates, are currently under investigation.

Given the above observations it was hoped that sulfur analogues might prove to be highly cytotoxic, in that the electron-withdrawing SH or neutral SMe substituents might confer low reactivity and hence high potency. However, **7-9** are in fact surprisingly inactive.

Further studies on the stability, DNA interaction, and preparation of analogues and prodrugs of these new *seco*-CI alkylating agents are in progress.

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