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SYNTHESIS AND CYTOTOXICITY OF AMINO ANALOGUES OF THE POTENT DNA ALKYLATING AGENT *seco*-CBI-TMI

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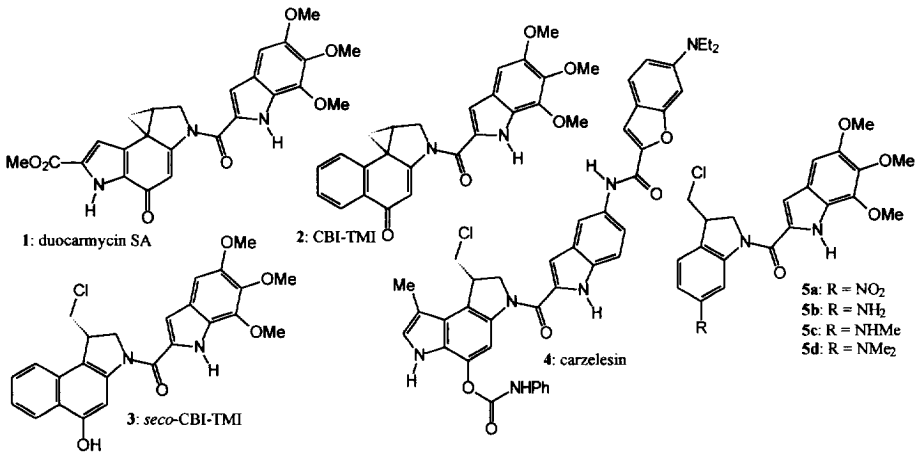
Abstract: The synthesis of racemic *seco*-CBI-TMI analogues containing nitrogen-based groups in place of the 5-OH is reported, employing a synthetic strategy where the incipient C-5 amino substituent is generated in the last step from a nitro precursor. The resulting amino *seco*-CBI analogues are up to 1000-fold more potent cytotoxins than the corresponding known amino *seco*-CI compounds, making them attractive candidates as effectors in prodrug strategies. © 1997 Elsevier Science Ltd.

The cyclopropylindole antitumor antibiotics, exemplified by CC-1065 and duocarmycin SA (**1**), have received much attention because of their high cytotoxicity (IC_{50}s in the low pM range) and unique mode of DNA alkylation at adenine N-3 sites in the minor groove.[1] The exact structure of the natural products is not required for high cytotoxicity, with synthetic analogues such as CBI-TMI[2] (**2**) being essentially as cytotoxic as **1** in cell culture (IC_{50}s 30 and 10 pM respectively in L1210 leukemia for a 72 h exposure).[3] The phenolic *seco* forms of these compounds (e.g., **3**) retain the full cytotoxicity of the corresponding cyclopropyldienones, indicating that ring closure to form the cyclopropane ring occurs rapidly in cell culture.[1,4]

The high potency of simpler *seco* analogues of the cyclopropylindole natural products makes them attractive for use in anticancer prodrugs, where release of a potent cytotoxin from a less toxic prodrug form in a tumour-specific manner can be expected to achieve improved therapeutic effects.[5,6] However, reported prodrug forms of the *seco* phenolic analogues of the natural cyclopropyldienones [e.g., the carbamate carzelesin (**4**), an anticancer drug in clinical trials], are very labile in plasma, rapidly and non-specifically releasing the corresponding phenol.[7] We have therefore been developing amino-substituted *seco* analogues, to allow the formation of a range of different and potentially more stable prodrug forms, and to compare the mechanisms of their cytotoxicity and interaction with DNA with those of the phenol analogues. Some possibilities are demonstrated in the *seco*-CI-TMI series by the analogues **5a** and **5b** (Table 1). The nitro compound **5a**[8] (a potential prodrug form) is much less cytotoxic than the amino analogue **5b**[9] (a cytotoxicity ratio of >125 in AA8 cells for a 4 h exposure; Table 1).

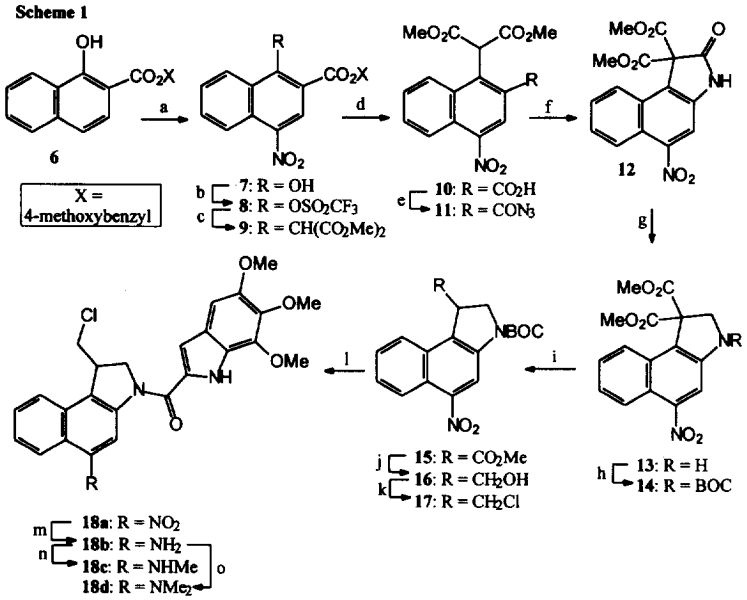
However, while these compounds show good differential cytotoxicity, the amino compound **5b** has only moderate potency, being much less cytotoxic than the corresponding phenol.[9] Guided by literature reports[3] that in the oxygen series the benzo[*e*]indoline (CBI) compounds show considerably higher potency than the corresponding indoline (CI) analogues, we report here the synthesis and cytotoxicities of amino analogues **18b-18d**, and the nitro derivative **18a**, of the related *seco* CBI-TMI series.



This synthesis used a basic strategy successfully employed[8] in our previous preparation of **5b**, with the required amino substituent of **18b** being carried through the process as a nitro group and generated by reduction in the last step (Scheme 1). 1-Hydroxynaphthalene-2-carboxylic acid was protected as the 4-methoxybenzyl ester **6**, and this was nitrated under mild conditions (70% HNO_3 in AcOH) to give a 61% yield of the 4-nitro isomer **7**. Conversion of this to the trifluoromethanesulfonate derivative **8**, followed by reaction with dimethyl malonate anion, gave the malonate derivative **9**. Selective cleavage of the 4-methoxybenzyl ester with TFA/anisole afforded the key acid **10**. Preparation of the desired nitrolactam **12** in an acceptable yield from **10** proved difficult. A number of different methods were tried, including the procedure (DPPA/Et_3N) used in our previous synthesis,[8] but were not successful. The best procedure involved formation of the intermediate carbonyl azide **11** by reaction of **10** with *N,N*-dimethyl(chlorosulfonyl)methaniminium chloride (the $SOCl_2$/DMF adduct) and NaN_3. Thermal Curtius rearrangement of purified **11** under neutral conditions (refluxing toluene) then gave an 81% yield of the desired lactam **12,** via trapping of the intermediate isocyanate. Selective reduction of **12** with BH_3.DMS, followed by reaction of the product indoline **13** with $(BOC)_2O$, gave the desired N-BOC derivative **14**. Reaction of **14** with anhydrous base (NaOMe/MeOH/THF) gave the monoester **15**, which was reduced (DIBAL-H) to the alcohol **16**. This was converted via the mesylate to the chloromethyl compound **17**. Deprotection of this with HCl/dioxane, and coupling with 5,6,7-trimethoxyindole-2-carboxylic acid, gave **18a**, which was reduced over platinum oxide to give the target (racemic) amino *seco*-

CBI analogue **18b**, in an overall yield of 4% (in 13 steps from the naphthoic acid). This route has been used to prepare **18b** on a 12 g scale. The mono- and dimethyl analogues **18c** and **18d** were prepared from **18b** using methods previously reported.[9]

Scheme 1



Reagents: (a) 70% HNO_3/AcOH/30 °C (61%). (b) $(Tf)_2O/Et_3N/CH_2Cl_2$/0 °C→25 °C (81%). (c) $CH_2(CO_2Me)_2/K_2CO_3$/DMF/-10 °C→25 °C (88%). (d) CF_3CO_2H/PhOMe/20 °C (93%). (e) Pyridine/NaN_3/[$SOCl_2$/DMF (1:1)]/CH_2Cl_2/0 °C→25 °C. (f) PhMe/reflux (81% for e+f). (g) BH_3.DMS/THF/heat (57%). (h) $(BOC)_2O$/1-methyimidazole/THF/40 °C (82%). (i) MeONa/MeOH/THF/20 °C, then CF_3CO_2H (98%). (j) DIBAL-H/THF/0 °C→5 °C (70%). (k) MsCl/pyridine, then LiCl/DMF (86%). (l) HCl/dioxane, then EDCI hydrochloride/5,6,7-trimethoxyindole-2-carboxylic acid/DMA/20 °C (77%). (m) H_2/PtO_2/THF (94%). (n) CH_3CO_2CHO/THF/0 °C, then BH_3.DMS/THF/reflux (42%). (o) $NaBH_3CN$/HCHO/THF/aqueous HCl/20 °C (68%).

The cytotoxicities of compounds **18a-18d** were evaluated in AA8 cells (4 h drug exposure), and compared with those of the related *seco*-amino-CI analogues **5a-5d** reported previously (Table 1). Two main points are apparent from these results. Firstly, as shown previously for the amino *seco*-CI compounds,[8,9] the nature of the substituent group has a very large influence on cytotoxicity, with a difference of ca. 4000-fold between the nitro and amino compounds **18a** and **18b**. This suggests that other electron-withdrawing amine-protecting groups will provide relatively non-toxic prodrug forms. Secondly, as has been shown previously[1] with the analogous oxygen derivatives (albeit in different systems), the amino *seco*-CBI analogues are much more potent than the corresponding CI derivatives (as high as 1300-fold in the case of the methylamino compounds **5c** and **18c**). The dimethylamino *seco*-CBI analogue **18d** did not show quite the same differential with respect to the CI analogue **5d** (50-fold), possibly because of a significantly out-of-plane dimethylamino group in the CBI series. Despite this, **5d** retains relatively high cytotoxicity (IC_{50} 5.6 nM) for a compound that

is unable to ring close to form a cyclopropyl intermediate. The high cytotoxicity of the amino *seco*-CBI-TMI analogues **18b-18d**, and the substantial influence of the electronic properties of groups at the 5-position on potency (compare **18a**), make this class of compounds attractive as potential effectors in prodrug strategies, and such work is in progress.

Table 1. Growth inhibition (IC_{50} values in nanomolar ± SEM) of AA8 cells, following a 4 h drug exposure.

	CI series		CBI series	
R	**compd.**	**IC_{50} (nM)**	**compd.**	**IC_{50} (nM)**
NO_2	**5a**	>40,000	**18a**	1600 ± 500
NH_2	**5b**	320 ± 30[a]	**18b**	0.43 ± 0.05
NHMe	**5c**	220 ± 13[a]	**18c**	0.17 ± 0.02
NMe_2	**5d**	280 ± 30[a]	**18d**	5.6 ± 0.6

[a]Reported in ref 9.

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