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RESOLUTION OF A CPzI PRECURSOR, SYNTHESIS AND BIOLOGICAL EVALUATION OF (+) AND (-)-N-Boc-CPzI: A FURTHER VALIDATION OF THE RELATIONSHIP BETWEEN CHEMICAL SOLVOLYTIC STABILITY AND CYTOTOXICITY

Pier Giovanni Baraldi*[1], Barbara Cacciari[1], Romeo Romagnoli[1], Giampiero Spalluto[2], Christopher W. Boyce[3], and Dale L. Boger[3]

[1]*Dipartimento di Scienze Farmaceutiche, Università di Ferrara, 44100 Ferrara, Italy*

[2]*Dipartimento di Scienze Farmaceutiche, Università di Trieste, 34127 Trieste, Italy*

[3]*Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA*

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Abstract: The chemical resolution, using N-tosyl-L-proline as a chiral auxiliary, of a racemate of the pyrazole analog (±)-N-Boc-CPzI of the left hand segment (CPI) of the antitumor agent CC-1065, and the cytotoxic evaluation of both enantiomers are described. The reported results further validate the direct relationship between chemical solvolytic stability of the cyclopropane ring and cytotoxicity proposed by Boger and coworkers.

Introduction

(+)-CC-1065 (**1**), an antitumor-antibiotic isolated from *Streptomyces zelensis*[1] posseses exceptionally potent *in vitro* and *in vivo* antitumor activity. It posseses a unique DNA alkylation capability in its cyclopropylpyrroloindole (CPI) left hand segment, with an extraordinary affinity and specificity for binding the B-DNA minor groove in AT rich sequences. In particular, **1** reacts with DNA within two preferred sequences, constituted by five base pairs, identified as 5'-PuNTTA* and 5'-AAAAA* where the compound in each sequence reacts at the 3' (asterisked) adenine, Pu is purine (adenine or guanine) and N any base.[2,3] This high specificity could be attributed to the 1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole dimer ($CDPI_2$) portion, since the CPI unit itself can alkylate DNA in a similar manner, but it does not show a good sequence selectivity.[4,5]

e-mail : pgb@ifeuniv.unife.it fax : + 39-0532-29-1270

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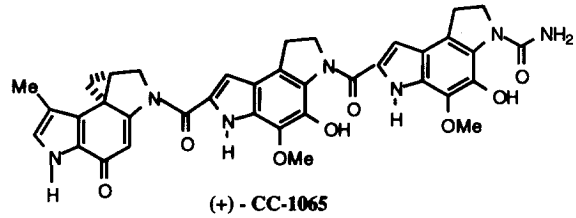
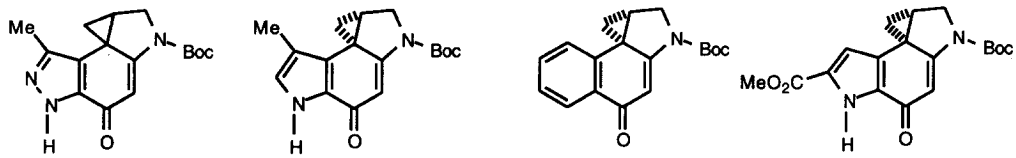
Despite its potency, CC-1065 cannot be used in humans, because it induces delayed death.[6] This effect is accompanied by dramatic changes in the morphology of hepatic mitochondria. For this reason many scientists have focused their attention on this class of compounds, modifying both CPI and $CDPI_2$ portions, in order to obtain new derivatives with equal *in vitro* potency but a better profile in *in vivo* models. In studies regarding structural modifications of CPI, Boger *et al.* have demonstrated an interesting correlation between the solvolytic stability of the cyclopropane ring and cytotoxicity.[7] They have studied a series of natural or unnatural agents containing structural modifications in the alkylation subunit and observed that the agents possessing the greatest solvolytic stability exhibit the most potent cytotoxic activity.



(+) - CC-1065

1

This relationship has been further validated with a series of N-2 substituted cyclopropylbenz[*e*]indole (CBI) derivatives.[7] Predictable linear relationships between solvolysis stability (-log *k*), cytotoxic potency (log $1/IC_{50}$ L1210 murine leukemia) and the electron-withdrawing properties of the N-2 substituent (Hammet σ_p constant) were observed.[8] Taking into account these findings and in order to further validate these relationships, we have recently designed and synthesized a pyrazole analog (**2**) of the CPI left hand segment of CC-1065 named N-Boc-CPzI (Figure 1).[9-11]



2 (±)-N-Boc-CPzI — $t_{1/2}$ **pH 3** 110 h; **IC_{50} (L1210)** 370 nM

3 (+)-N-Boc-CPI — $t_{1/2}$ **pH 3** 37 h; **IC_{50} (L1210)** 330 nM

4 (+)-N-Boc-CBI — $t_{1/2}$ **pH 3** 133 h; **IC_{50} (L1210)** 80 nM

5 (+)-N-Boc-DSA — $t_{1/2}$ **pH 3** 177 h; **IC_{50} (L1210)** 6 nM

Figure 1. Structures, chemical solvolytic stability and cytotoxicity of N-Boc-CPI and some its derivatives

The synthesized pyrazole analog (±)-N-Boc-CPzI (**2**) ($t_{1/2}$ = 110 h, k = 1.75 x 10^{-6} s^{-1}; r = 0.994) proved to be substantially more stable than N-Boc-CPI (**3**) ($t_{1/2}$ = 37 h, k = 5.26 x 10^{-6} s^{-1}) to solvolysis at a pH of 3,[10] with a cytotoxicity against L1210 cell lines of 370 nM. On this basis, N-Boc-CPzI (**2**) proved to be three times more stable than N-Boc-CPI (**3**) to solvolysis at pH 3 (110 h vs 37 h). However, the same comparison could not be

made for the cytotoxicity due to the different degree of steric bulk surrounding the C-7 center that produce substantial enantiomeric distinctions in terms of cytotoxicity (2-10 fold between natural and unnatural enantiomers).[7]

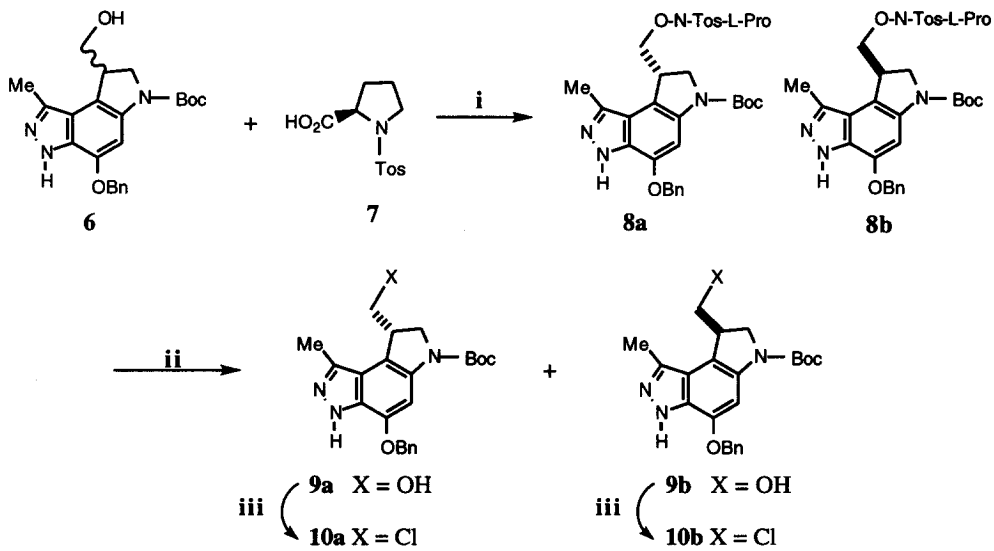
In order to validate the hypothesis proposed by Boger and coworkers we investigated the possibility of separating the racemic mixture for evaluation of the cytotoxicity of the two enantiomers of **2**.

Several attempts to resolve **2** by esterification of its precursor **6** with different chiral auxiliaries, including O-acetyl mandelic acid[12] or N-Boc-L-tryptophan,[13] were unsuccessful. Also enantiomer separation using a semi-preparative ChiralCel OD column (2 x 25 cm, 7% i-PrOH/hexane α = 1.10) gave only modest results on a preparative scale.[14] Finally, we decided to utilize N-Tosyl-L-proline **7** as chiral auxiliary, which gave good results in our previous work (Scheme 1).[15]

Chemistry

Esterification of **6**[10] with **7**, in presence of EDCI, gave a separable diastereomeric mixture of esters **8a,b** which can be easily separated by flash chromatography (CCl_4-EtOH 99.5 : 0.5).[16] The latter compounds were in turn hydrolysed to the corresponding alcohols **9a,b**, which were transformed into the primary chloride (**10a,b**) by treatment with triphenylphosphine and CCl_4 (Scheme 1).[17]

Scheme 1

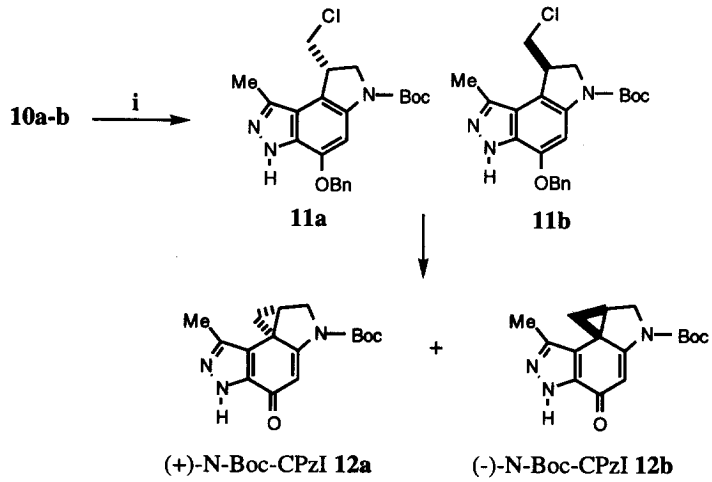


Reagents: i: EDCI, DMAP, CH_2Cl_2, rt, 24 h, (98%); **ii:** THF/MeOH/H_2O, LiOH, rt, 18 h, (99%); **iii:** CCl_4, CH_2Cl_2, PPh_3, rt, 18 h, (95%)

Optical purity of these derivative was confirmed by comparison of optical rotation of **10a,b** with analytical samples of the same compounds obtained by resolution on a semi-preparative ChiralCel OD column (2 x 25 cm, 5% i-PrOH/hexane $\alpha = 1.27$, ee 99%).

Optically pure **10a,b** were subjected to transfer hydrogenolysis[18] to afford the seco-precursors **11a,b** which were in turn converted into the spiroderivatives **12a** ((+)-N-Boc-CPzI) and **12b** ((-)-N-Boc-CPzI) by treatment with a mixture (1:1:1) of Et_3N, H_2O and MeCN (Scheme 2).[19,20]

Scheme 2



Reagents: i: HCO_2NH_4, 10% Pd-C, Me_2CO, reflux, (96%); **ii:** Et_3N/MeCN/H_2O 1:1;1, rt, (85%)

The assignment of absolute configuration was based on the relative cytotoxic potencies of natural (+) and ent-(-)-N-Boc-CPzI consistent with that reported for related analogs, in which the natural enantiomer exhibits the more potent activity.[7]

Results and discussion

The activity of both enantiomers of **12a,b** were tested *in vitro* on L1210 murine leukemia cells (obtained from NCI, Bethesda, USA) as previously described[21] and the drug sensitivity was determined by counting cells after 72 h of exposure to at least four concentrations of each compound.

All the values have been expressed as 50% inhibitory concentration (IC_{50}) and represent the mean ± SE from dose response curves of at least three experiments. As expected, a significant difference in terms of cytotoxicity has been observed for the compounds. While the compound with the natural configuration (**12a**)

showed an IC_{50} value of 206 ± 23 nM, the corresponding enantiomer (**12b**) proved to be about 5 times less active (923 ± 174 nM). These results confirm the well known enantiomer distinctions related CPI analogs, attributable to the steric bulk at the C7 position, and more importantly, further validate the direct relationship between chemical solvolytic stability and cytotoxic potency proposed by Boger and coworkers.[7] In fact, the cytotoxicity of (+)-N-Boc-CPzI (206 nM) is very close to the predicted value, based on its solvolysis $t_{1/2}$ at pH 3, derived from the above mentioned relationship (predicted value 185 nM).[7,10]

In addition, these results seem to confirm our rational design of this derivative,[10] based on the fact that the presence of an electron-withdrawing nitrogen on the pyrazole nucleus should slow down C-4 carbonyl protonation, a crucial step for catalysis of solvolysis and cyclopropyl ring cleavage, exactly as observed for the carbomethoxy function present in N-Boc-DSA (**5**), one of the most stable and consequently cytotoxic agent in this series of analogs.

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16. Characterization of compound **8a**. white solid, mp (Et_2O-light petroleum) 197 °C; $[\alpha]^{20}_D$ = -39.1 (*c* 0.24, $CHCl_3$); IR (KBr) cm^{-1}, 3150, 1735, 1710, 1620, 1345, 1135; ^1H NMR ($CDCl_3$) δ: 1.58 (s, 9H); 1.61-2.11 (m, 4H); 2.42 (s, 3H); 2.67 (s, 3H); 3.15-3.31 (m, 1H); 3.42-3.58 (m, 1H); 3.71-4.12 (m, 3H); 4.18-4.31 (m, 2H); 4.43-4.52 (m, 1H); 5.20 (s, 2H); 7.31 (d, 2H, *J* = 9 Hz); 7.36-7.44 (m, 5H); 7.74 (d, 2H, *J* = 9 Hz); 7.82 (s, 1H); 12.3 (bs, 1H). Characterization of compound **8b**: white solid, mp (Et_2O-light petroleum) 186-187 °C; $[\alpha]^{20}_D$ = -58 (*c* 0.23, $CHCl_3$); IR (KBr) cm^{-1}, 3145, 1730, 1715, 1620, 1343, 1130; ^1H NMR ($CDCl_3$) δ: 1.58 (s, 9H); 1.55-2.09 (m, 4H); 2.42 (s, 3H); 2.69 (s, 3H); 3.18-3.34 (m, 1H); 3.40-3.55 (m, 1H); 3.82-4.11 (m, 3H); 4.11-4.29 (m, 2H); 4.45-4.58 (m, 1H); 5.21 (s, 2H); 7.31 (d, 2H, *J* = 9 Hz); 7.39-7.45 (m, 5H); 7.73 (d, 2H, *J* = 9 Hz); 7.81 (s, 1H); 12.43 (bs, 1H).
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19. Optical rotation for: **9a** $[\alpha]^{20}_D$ = + 11.61 (*c* 0.31, $CHCl_3$); **9b** $[\alpha]^{20}_D$ = - 11.57 (*c* 0.35, $CHCl_3$); **10a** $[\alpha]^{20}_D$ = - 10.50 (*c* 0.3, $CHCl_3$); **10b** $[\alpha]^{20}_D$ = + 10.60 (*c* 0.35, $CHCl_3$), (the samples purified by ChiralCel OD column (ee 99%) showed the following optical rotations **10a** $[\alpha]^{20}_D$ = - 10.64 (*c* 0.4 $CHCl_3$), **10b** $[\alpha]^{20}_D$ = + 10.64 (*c* 0.4 $CHCl_3$)); **11a** $[\alpha]^{20}_D$ = + 240 (*c* 0.25, $CHCl_3$); **11b** $[\alpha]^{20}_D$ = - 238 (*c* 0.30, $CHCl_3$); **12a** $[\alpha]^{20}_D$ = + 100 (*c* 0.12, $CHCl_3$); **12b** $[\alpha]^{20}_D$ = - 99 (*c* 0.10, $CHCl_3$).
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