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BN 80245: AN E-RING MODIFIED CAMPTOTHECIN WITH POTENT ANTIPROLIFERATIVE AND TOPOISOMERASE I INHIBITORY ACTIVITIES

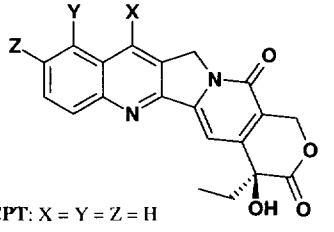
Olivier Lavergne, Laurence Lesueur-Ginot, Francesc Pla Rodas[†], Dennis C. H. Bigg*[§]

Institut Henri Beaufour, 5, avenue du Canada, F-91966 Les Ulis, France, and

[†]*Laboratorios Lasa, carretera Laureà Miró, 395, E-08980 San Feliu de Llobregat, Spain.*

Abstract: The crucial E-ring of camptothecin has been modified to afford the homologous β-hydroxylactone derivative BN 80245. This compound, which is more stable than camptothecin, remains a potent inhibitor of both cell growth and topoisomerase I. © 1997 Elsevier Science Ltd.

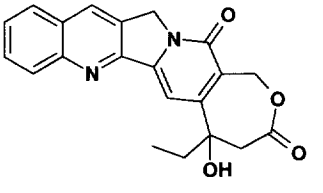
The discovery that the cytotoxicity of the natural product camptothecin (CPT) was due to a novel mechanism of action involving the stabilization of the complex formed by topoisomerase I and DNA[1] has led to the emergence of CPT analogs as a new class of antitumor agents[2] with impressive activity in xenograft models. Irinotecan[3] and topotecan[4] (TPT) are now established as clinically useful drugs for the treatment of chemoresistant cancers and several compounds such as 9-AC[5], GG-211[6] and DX-8951[7] are under clinical development.



CPT: X = Y = Z = H

TPT: X = H, Y = $CH_2N(CH_3)_2$, Z = OH

Irinotecan: X = C_2H_5 , Y = H, Z =



1: BN 80245

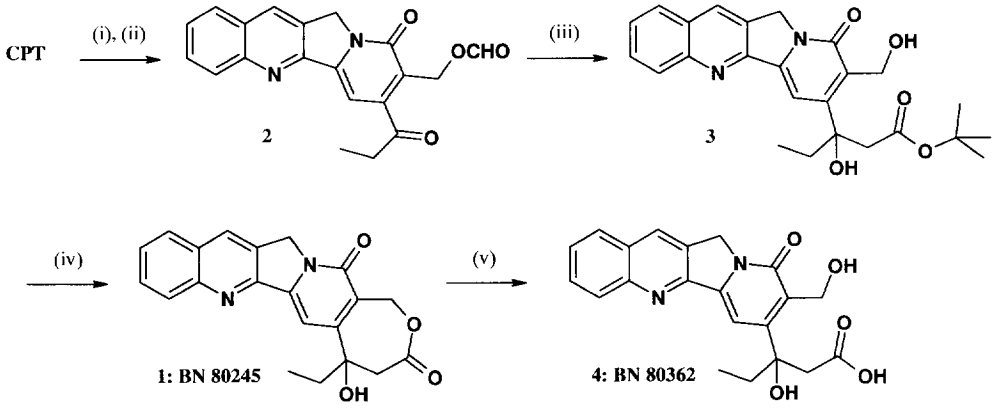
Although a variety of substituents on the quinoline moiety of CPT are tolerated,[8] the α–hydroxylactone ring is generally acknowledged to be an absolute requirement for high topoisomerase I mediated cytotoxicity,[9] due perhaps to a reversible covalent interaction between CPT and the enzyme-DNA complex.[10] The high

§ E-mail : dennis.bigg@beaufour-ipsen.com ; Fax : (33) 01 69 07 38 02

chemical reactivity of the α-hydroxycarbonyl group leads, however, to a rapid equilibration *in vivo* between the lactone and the biologically inactive open-chain hydroxyacid formed. We now report that the homologous, β-hydroxylactone compound, BN 80245, which is a much weaker electrophile than CPT, is both a potent inhibitor of topoisomerase I activity and a potent cytotoxic agent.

BN 80245 was prepared in racemic form by a convenient four-step semisynthetic sequence (Scheme 1) : CPT was readily reduced with sodium borohydride to the corresponding hydroxylactol whose 1,2-diol moiety was oxidatively cleaved with periodic acid to give formyloxy-mappicin-ketone (**2**).[11] A Reformatsky reaction with *t*-butyl bromoacetate and **2** gave, after aqueous work-up, the hydroxyester **3**[12] which on treatment with trifluoroacetic acid afforded BN 80245.[12]

Scheme 1 : Preparation of BN 80245 and BN 80362.



Reagents and conditions: (i) $NaBH_4$, MeOH, rt; (ii) $NaIO_4$, AcOH, rt, 84% overall; (iii) Zn, $BrCH_2COOt$-Bu, THF, reflux, 31%; (iv) CF_3COOH, rt, 73%; (v) 0.05M NaOH, 35°C, then pH 3, 89%.

The hydrolytic stability of this novel CPT homologue as a function of pH was determined by HPLC and the results are shown in Figure 1. It is apparent that the rate of hydrolysis increases with increasing pH, as might be expected. BN 80245 was found to be considerably more stable than compounds containing the α-hydroxylactone; thus at pH 7.4, 87% of the lactone is intact after 24h, whereas CPT, for example, reaches an equilibrium of *ca* 20% lactone within 1h,[13] and at pH 9.1 CPT ring-opens almost instantaneously. In addition, and in contrast to α-hydroxylactones, hydrolysis of BN 80245 leads to an isolable hydroxyacid BN 80362 (**4**).[12]

The difference in reactivity between an α-hydroxylactone and the homologous β-hydroxylactone is further illustrated by the fact that CPT, for example, is readily reduced to the lactol by sodium borohydride, whereas BN 80245 is inert to this reagent.

Figure 1 : HPLC determination of the hydrolytic stability of BN 80245 at room temperature.

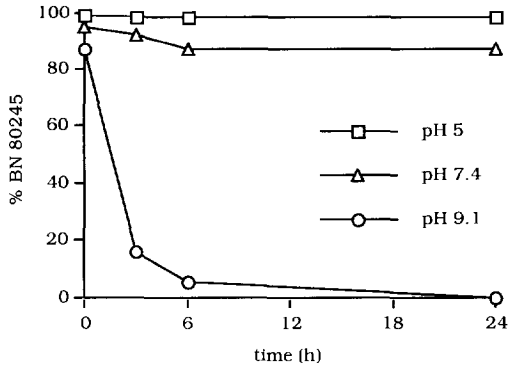


Table 1 presents the *in vitro* topoisomerase I inhibition[14] and the cytotoxic activity on L1210 murine leukemia cells.[15] BN 80245 was found to inhibit topoisomerase I mediated DNA relaxation with a potency comparable to that of CPT. Furthermore, BN 80245 also inhibited the growth of L1210 cells and, although racemic, proved to be considerably more potent than the reference compounds CPT and TPT in this assay. As observed by others[16] there was no strict correlation between topoisomerase I inhibition and antiproliferative activity.

Table 1 : Topoisomerase I inhibition and antiproliferative activity on L1210 cells.

Compound	Topoisomerase I inhibition (%)[a]	L1210 (IC_{50}, nM)[b]
CPT	36 ± 6	126
TPT	63 ± 7	601
BN 80245	40 ± 9	16.2

a) Determined by supercoiled DNA relaxation assay[14] with 100 μM compound.
b) Cell growth inhibition determined by MTT assay.[15]

The preservation of both cytotoxic and topoisomerase I inhibitory activities subsequent to a modification of the camptothecin E-ring is, to our knowledge, unprecedented.[17,18] Furthermore, the diminished electrophilicity of the homologous β-hydroxylactone ring, and the consequent improvement in stability may translate into differences in the kinetics of DNA-topoisomerase I complex stabilization, pharmacokinetics, or the spectrum of antitumor activity. Work is currently under progress to further evaluate the potential interest of homocamptothecin analogs.

Aknowledgements

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