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BN 80927: A NOVEL HOMOCAMPTOTHECIN WITH INHIBITORY ACTIVITIES ON BOTH TOPOISOMERASE I AND TOPOISOMERASE II

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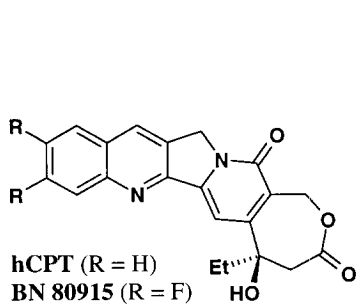
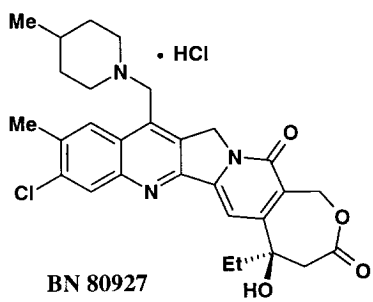
Abstract: BN 80927, a novel homocamptothecin derivative, inhibits both topoisomerase I and topoisomerase II mediated DNA relaxation and shows pronounced cytotoxicity against HT29, SKOV-3, DU145 and MCF7 human tumor cell lines.

Camptothecin (CPT) derivatives represent a promising class of anticancer agents which, by selective poisoning of the ubiquitous nuclear enzyme topoisomerase I (Topo I), exert potent cytotoxicity against a wide spectrum of tumor cell lines, including those which show multidrug resistance.[1] We have previously reported that homocamptothecin (hCPT), a semi-synthetic CPT analog with a modified E-ring,[2] conserved Topo I-mediated activity *in vitro* and *in vivo*, while showing increased plasma stablility.[3] This unexpected observation prompted us to synthesise and screen hCPT analogues in order to identify potential anticancer drugs.



hCPT (R = H)
BN 80915 (R = F)



BN 80927

Part of this program has been recently disclosed concerning racemic compounds[4] and enantiomerically pure fluorinated derivatives such as BN 80915, for which potent and specific Topo I inhibition is observed.[5] We now report preliminary results showing that BN 80927, a novel hCPT derivative, inhibits both Topo I and Topo II enzymes in DNA relaxation assays. Such dual activity, which has been previously observed with DNA

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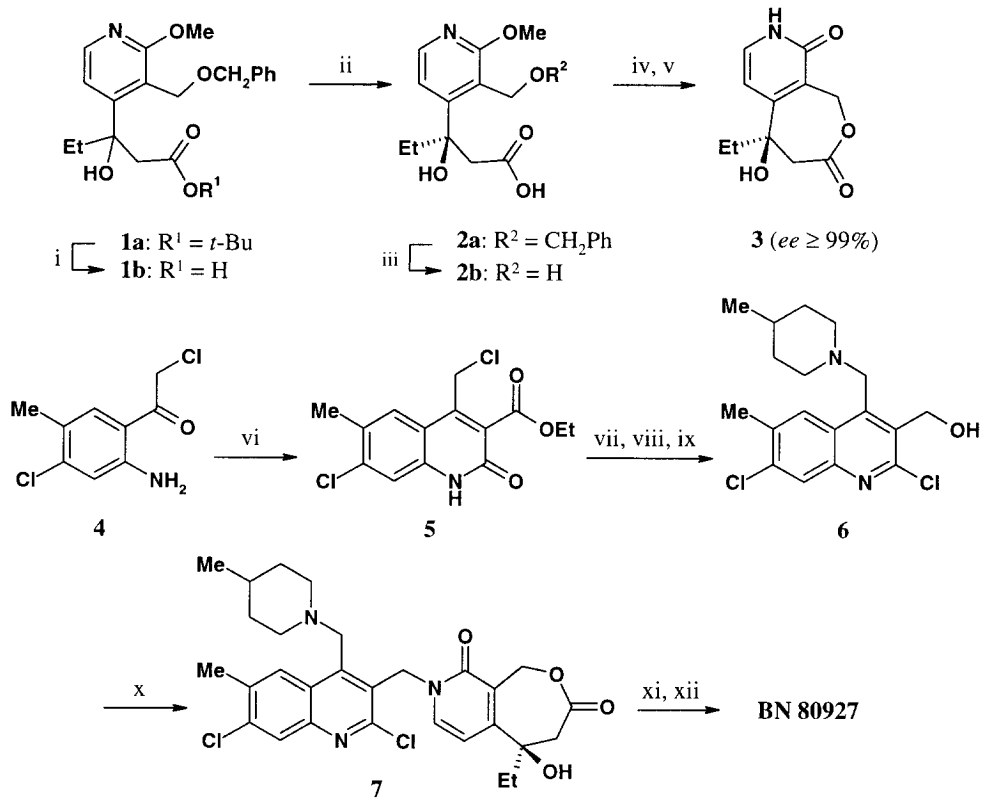
intercalators such as DACA[6] or intoplicine,[7] is unprecedented for a CPT derivative,[8,9] and may translate into a different antitumor profile with respect to "classical" CPTs.

The convergent synthesis of BN 80927 required the preparation, in high enantiomeric excess, of intermediate **3**, a 7-membered β-hydroxylactone fused to a pyridone (Scheme 1). Acid **1b**, obtained from its ester **1a**,[4] was partially resolved with quinidine. Subsequent debenzylation, lactonization, and treatment with TMSI afforded **3**,[10] whose enantiomeric purity was brought to 99% *ee* by recrystallization.

Scheme 1

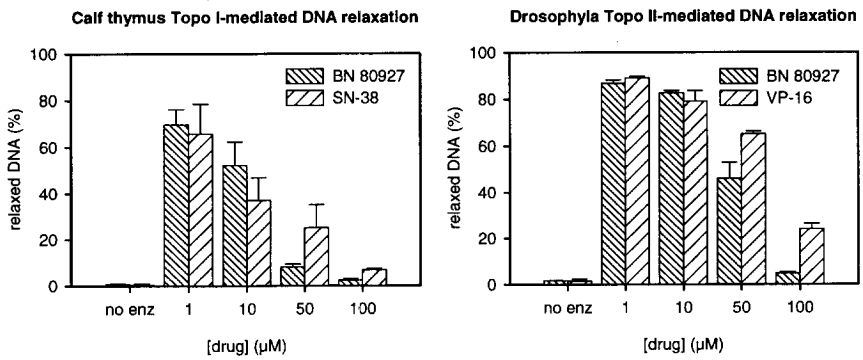


Reagents and conditions: (i) TFA, rt, 90%; (ii) resolution with quinidine, *i*-PrOH, rt, 40%; (iii) HCOOH, Pd/C, rt, 88%; (iv) DCC, THF, rt; (v) TMSI, MeCN, reflux, 34% from **2b**, then recrystallization from *i*-PrOH, 71%; (vi) $ClCOCH_2COOEt$, Et_3N, MeCN, rt, then EtONa, EtOH, rt, 83%; (vii) $POCl_3$, reflux, 85%; (viii) Dibal, CH_2Cl_2, rt, 74%; (ix) 4-Me-piperidine, THF, rt, 93%; (x) **3**, DIAD, Ph_3P, dioxane, rt, 50%; (xi) $Pd(OAc)_2$, KOAc, Ph_3P, Bu_4NBr, MeCN, reflux, 70%; (xii) 1*N* HCl, EtOH, rt, 85%.

The quinoline moiety of BN 80927 was obtained from aminoacetophenone **4**,[11] which was converted to quinolinone **5** by condensation with ethylmalonyl chloride. Further functional group manipulation gave quinoline **6**, suitable for coupling with compound **3** under Mitsunobu reaction conditions.[12] An intramolecular Heck reaction[13] provided the desired pentacyclic compound which was readily salified to afford BN 80927.

BN 80927 was compared to SN-38 (the active principle of CPT-11) or VP-16 (etoposide) in supercoiled pUK19 plasmid DNA relaxation assays[14] using Topo I or Topo II, respectively (Fig 1). Its inhibitory activity was dose-dependent and equivalent or superior to that of SN-38 on Topo I, and to that of VP-16 on Topo II.

Figure 1 : Inhibition of topoisomerases I and II.



BN 80927 was found to exhibit pronounced antiproliferative activity upon 72h incubation with human tumor cell lines (Table 1). The IC_{50} (50% inhibitory concentration) values of BN 80927 determined by WST assay[15] are lower than those of the above-mentioned specific Topo I or Topo II inhibitors, SN-38 and VP-16, respectively. The cytotoxicity of BN 80927 is also superior to that of DACA or Intoplicine, two dual Topo I and Topo II inhibitors currently undergoing clinical trials.[6,16]

Table 1 : Antiproliferative activities (IC_{50}, μM).

Drug	HT29 (colon)	SKOV-3 (ovarian)	DU145 (prostate)	MCF7 (breast)
BN 80927	0.0066	0.013	0.003	0.048
SN-38	0.022	0.72	0.013	0.37
VP-16	0.20	4.9	1.3	100
DACA	> 10	2.4	2.3	3.8
Intoplicine	0.89	0.45	0.56	1.0

The search for efficacious CPT analogs has generated an important body of structure-activity information[1,17] showing the CPT skeleton to be amenable to substitution which allows pharmacological modulation.[18] Early efforts were aimed at improving water-solubility and potency, while more recent studies have focused on compounds with improved plasma stability.[19-21] Other investigations have produced derivatives exhibiting dual activities involving DNA alkylation,[22] or minor-groove binding,[23] in addition to Topo1 inhibition, but observation of a dual Topo I and Topo II inhibitory activity is unprecedented for a compound structurally related to CPT.[9] Further testing in DNA cleavage assays shows Topo II poisoning only

at high concentration,[24] and therefore BN 80927 should be better considered as a catalytic inhibitor of Topo II, in addition to being a potent Topo I poison. Work is currently under progress to further characterize the interaction of BN 80927 with DNA topoisomerases, and to compare its spectrum of antitumor activity and in vivo efficacy with those of other relevant chemotherapeutic agents.

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