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NOVEL A-RING MODIFIED CAMPTOTHECINS AS TOPOISOMERASE I INHIBITORS

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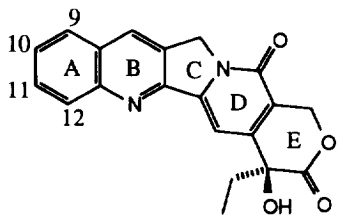
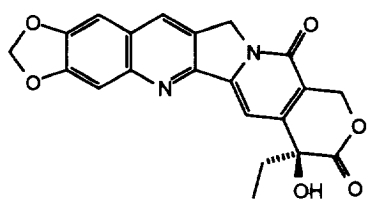
Abstract. A camptothecin derivative has been prepared wherein the A-ring is fused to an oxazole ring. The compound was prepared via a Friedlander condensation involving benzoxazole **8** and tricyclic ketone **9**. This derivative displays potent topoisomerase I inhibition (IC_{50} 150 nM) when assayed in the 'cleavable complex' assay.

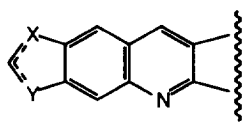
The camptothecin class of antitumor agents has recently re-emerged as a promising source of new anticancer agents due primarily to the demonstration that topoisomerase I inhibition is the mechanism by which camptothecin exerts its cytotoxic effects[1-4] and by the subsequent demonstration that new, water soluble derivatives of camptothecin show impressive activity in preclinical xenograft models and more importantly in early clinical trials.[5] Much of the emphasis of the recent work on camptothecin has been toward the preparation of compounds with improved aqueous solubility, and the current clinical evaluation of Topotecan,[6] Irinotecan,[7] and GI 147211C[8] attest to the success of these endeavours. While the structure-activity relationships of the camptothecin nucleus have been extensively investigated by a number of workers, most notably Wall and Wani,[9] the search for new derivatives which may show improved topoisomerase I inhibition and cytotoxicity, or improved selectivity toward tumor cells over normal cells continues to be a valuable goal.



Camptothecin (**1**)



2



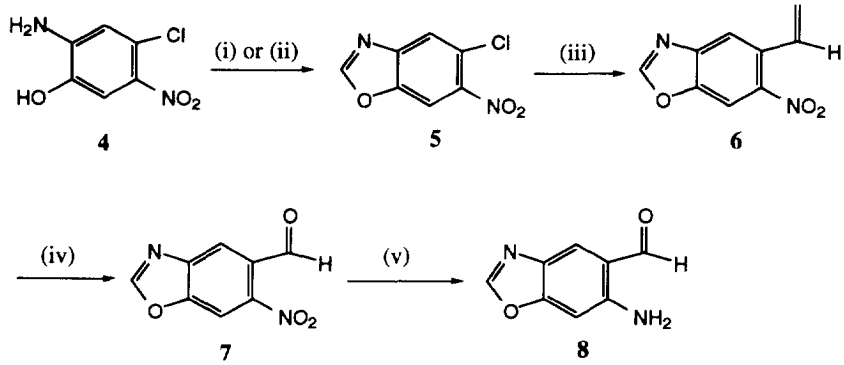
X = N, Y = O **3**

The functionalization of the A-ring of camptothecin has proven to be a particularly rich source of structure-activity relationships and several key features have emerged: (i) the 9- and 10- positions are amenable to substitution with a wide variety of groups, some of which show a dramatic increase in potency (e.g., 9-NH_2, 9-OMe, 10-OH); (ii) the 11-position is much less tolerant of substitution and most substitutions cause a decrease in potency; (iii) the 12-position is completely intolerant of substitution. One significant exception to these trends is the 10,11-methylenedioxy substitution which results in a compound which is some 20x more potent than camptothecin itself.[10]

We became interested in preparing compounds represented by **3** in which the A-ring is appended by a heterocyclic ring. Clearly X and Y could be selected from carbon, nitrogen and oxygen either singly or in combination to give new derivatives which may show improved potency. Moreover, when the appended ring is fully oxidised the position between the two heteroatoms may provide another, chemically tractable, position from which to add water solublizing groups. In view of the potent activity demonstrated by 10,11-methylenedioxycamptothecin (**2**) and the documented sensitivity of substitution in the 11- and 12-positions we initially chose to retain an oxygen substituent at the 11-position and so oxazole **3** was selected as the target.

One of the most obvious approaches to this compound would be to prepare 10-amino-11-hydroxycamptothecin and effect oxazole formation by treatment with a trialkylorthoformate. However, since our strategy would call for a Friedlander reaction to form the quinoline ring system then this would require selective protection/deprotection of a diamino-hydroxybenzaldehyde, so it was decided to essentially 'protect' the amino and hydroxy groups by formation of the oxazole ring, Scheme 1.

Scheme 1



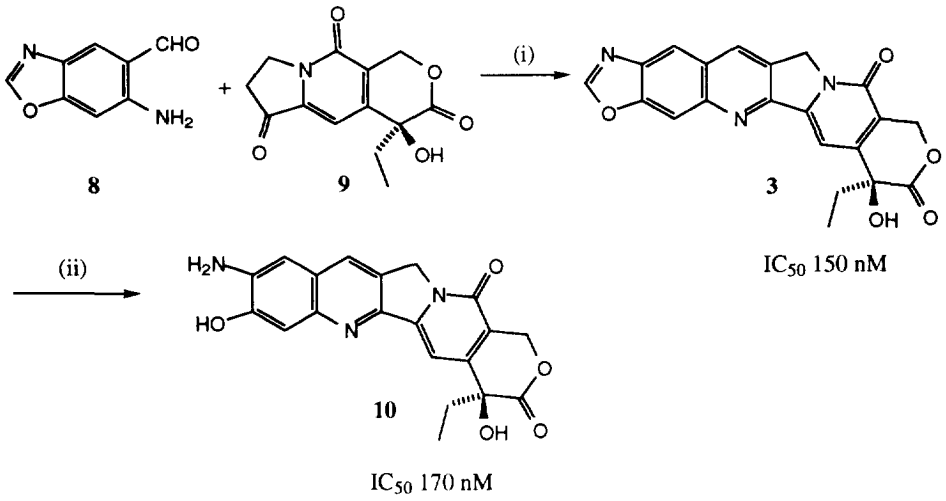
4 **5** **6** **7** **8**

Reagents: (i) HCO_2H then TsOH/toluene (85%), (ii) $HC(OMe)_3$/MeOH/HCl (83%), (iii) vinyltributyltin/$(Ph_3P)_2PdCl_2$/DMF/135 °C (34%), (iv) ozone/Me_2S (34%), (v) $FeSO_4/NH_4OH$/EtOH/60 °C (69%).

To this end the required benzoxazole **5** was readily available from commercially available **4** either by formylation of the amino group followed by dehydrative ring closure, or more directly by treatment of the hydroxyaniline with trimethylorthoformate. A latent aldehyde function was introduced via a palladium catalyzed coupling of vinyltributyltin to chloride **5** to give nitro olefin **6**. This coupling reaction proved to be somewhat

sensitive to reaction conditions in that no reaction was observed at temperatures below 120 °C, however prolonged heating at 135 °C led to extensive decomposition. Nevertheless, the use of aryl chlorides in palladium catalyzed coupling of organostannanes is not usually successful and is clearly a consequence of the activating nitro substituent.[11] The aldehyde function was unmasked by ozonolytic cleavage of the vinyl group followed by a reductive work-up to give **7** which was reduced to the desired amino aldehyde **8** using iron(II)sulfate. Treatment of a mixture of compound **8** and the optically pure tricyclic ketone **9**[12] with catalytic p-toluenesulfonic acid in refluxing toluene resulted in a Friedlander condensation to give the target compound **3**, Scheme 2. While a sample of compound **3** could be purified from residual traces of tricyclic ketone **9** by chromatography this proved to be a tedious task and so we attempted to purify **3** by reverse phase HPLC. Somewhat surprisingly it was found that the major peak which eluted from the column was found to be the aminohydroxycamptothecin **10** in which the oxazole ring had been hydrolyzed.[13]

Scheme 2



Reagents: (i) p-TsOH/H+/AcOH/130 °C, (ii) RP HPLC.

Compounds **3** and **10** were assayed for their ability to inhibit calf thymus topoisomerase I as determined by 'cleavable complex' formation.[14] Under the conditions of this assay camptothecin was found to have an IC_{50} of ca.700 nM. The two analogues tested were found to be significantly more potent than camptothecin as inhibitors of topoisomerase I and begin to approximate the activity of 10,11-methylenedioxycamptothecin (**2**) (IC_{50} 30 nM). While neither of these compounds solve the key problem of water solubility associated with 10,11-methylenedioxycamptothecin, the oxazole ring of compound **3** offers a good opportunity to append further substituents which may result in improved water solubility while retaining activity against topoisomerase I.

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(13) All new compounds gave ^{1}H NMR and high resolution mass spectra consistant with the proposed structures. RP HPLC conditions: Column: 8 μM C-8 Dynamax. Solvent: Isocratic 80:20 H_2O + 2% TFA:CH_3CN/THF (60/40) + 2% TFA.

(14) The details of the assay procedure can be found in ref. 8.

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