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HPLC AND CIRCULAR DICHROISM STUDIES ON THE HYDROLYSIS OF CAMPTOTHECIN AND THIOCAMPOTHECIN: THE EFFECT OF SULPHUR ON LACTONE STABILITY

M. Pistolozzi ¹, D. Tedesco ¹, A. Barretta ¹, G. Varchi ², A. Guerrini ², C. Bertucci ¹
¹Department of Pharmaceutical Sciences, University of Bologna, Italy. ²Institute for Organic Synthesis and Photoreactivity, National Research Council, Bologna, Italy
daniele.tedesco@unibo.it

20-(S)-camptothecin (CPT) is a naturally-occurring alkaloid isolated from the Tibetan tree *Xi Shu* (*Camptotheca acuminata*), which displays a strong inhibitory activity towards type I topoisomerase (topo I). [1] Severe adverse effects and the limited stability of the lactone moiety, which plays a key role in the inhibition mechanism, limited the clinical potential of CPT as an antitumor agent and prompted the development of new CPT analogues with reduced toxicity and improved pharmacokinetics. A thiopyridone isostere of CPT, 20-(S)-thiocamptothecin (TCPT), was recently developed for this purpose and showed an increase in inhibitory activity in H460, HT29 and IGROV-1 cell lines, [2] probably due to the greater stability of the lactone ring.

In the present study, the kinetics of the reversible hydrolysis reactions of CPT and TCPT (**Figure 1**) from the closed lactone forms (**a**) to the open carboxylate forms (**b**) were investigated by high-performance liquid chromatography (HPLC) and electronic circular dichroism (ECD), both in the presence and absence of human serum albumin (HSA).

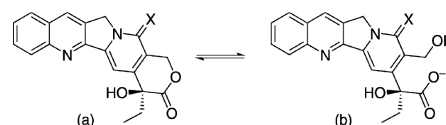


Figure 1. Hydrolysis reaction of CPT (X=O) and TCPT (X=S).

HPLC analysis was performed on CPT/HSA and TCPT/HSA mixtures using UV and fluorescence detection and a restricted access media (RAM) column, which allowed the direct separation of the lactone and carboxylate forms without any additional purification steps for the exclusion of HSA. [3] ECD analysis was performed at fixed wavelength directly on CPT/HSA and TCPT/HSA mixtures, after characterisation of the induced ECD of the HSA/analyte complexes.

The amount of closed lactone form in buffered solutions at equilibrium (24 h) was found to be significantly higher for TCPT than for CPT, and the same trend was observed in the presence of equimolar and excess quantities of HSA. Moreover, the presence of HSA caused a shift in the hydrolysis equilibrium of CPT and TCPT towards the carboxylate form, due to its higher affinity towards HSA with respect to the lactone form. Nevertheless, the fraction of closed lactone form resulted higher for TCPT than for CPT under the same experimental conditions, confirming the improved stability of the lactone moiety. The present study provides further insights into the stabilising effects of oxygen/sulphur replacement in the CPT pyridone ring, and represents a valid protocol for the analysis of new CPT derivatives as potential drug candidates.

References

- [1] R.P. Verma, C. Hansch, *Chem. Rev.*, **2009**, 109 (1), 213-235.
- [2] C. Samori, A. Guerrini, G. Varchi et al., *J. Med. Chem.*, **2008**, 51 (10), 3040-3044.
- [3] M. Pistolozzi, G. Varchi, A. Degli Esposti et al., *ChemMedChem*, **2011**, 6 (9), 1706-1714.