ECRONICON OPEN ACCESS

CHEMISTRY
Research Article

# Carboxylate Forms of Camptothecins -CPT, SN22 and Karenitecin® Bind Specifically to Site II (Diazepam site) of Human Serum Albumin

**Shijie Yao[1], Pavankumar Petluru[1], Aulma Parker[1], Daoyuan Ding[1], Xinghai Chen[1], Qiuli Huang[1], Harry Kochat[1], and Frederick Hausheer[1]***

*[1]BioNumerik Pharmaceuticals, USA*

***Corresponding Author:** Frederick Hausheer, BioNumerik Pharmaceuticals, Inc., 8122 Datapoint Drive, Suite 1250, San Antonio, Texas 78229, USA.

**Received:** July 21, 2015; **Published:** December 03, 2015

## Abstract

Camptothecins are an important class of anti-cancer agents and under physiological conditions exist in equilibrium between the lactone and carboxylate forms. The lactone form of camptothecins is crucial for their anti-tumor effect. Camptothecins, especially carboxylate forms, bind strongly to the plasma protein, human serum albumin (HSA). This binding of the carboxylate form alters the hydrolysis kinetics of the lactone form and may play a significant role in the mechanism of action of this class of anticancer drugs. High-performance affinity chromatography with an immobilized human serum albumin (HSA) stationary phase and a ligand competitive assay were employed to study the binding interactions between camptothecin (CPT), SN22 and a novel highly lipophilic camptothecin, Karenitecin®, with HSA. Employing known Site I (warfarin) and Site II (ibuprofen) binders of human serum albumin in a competitive HPLC assay, we show here that the carboxylate forms of CPT, SN22 and Karenitecin® bind to Site II on HSA but not to Site I on HSA. No direct experimental evidence was obtained to indicate that the lactone forms of these camptothecins bind to either Site I or Site II of HSA. This is the first report of binding interactions for Karentecin® with HSA.

***Keywords:*** *Camptothecin; SN22; Karenitecin®; Human Serum Albumin; HPLC; Diazepam Binding Site; and Warfarin Binding Site*

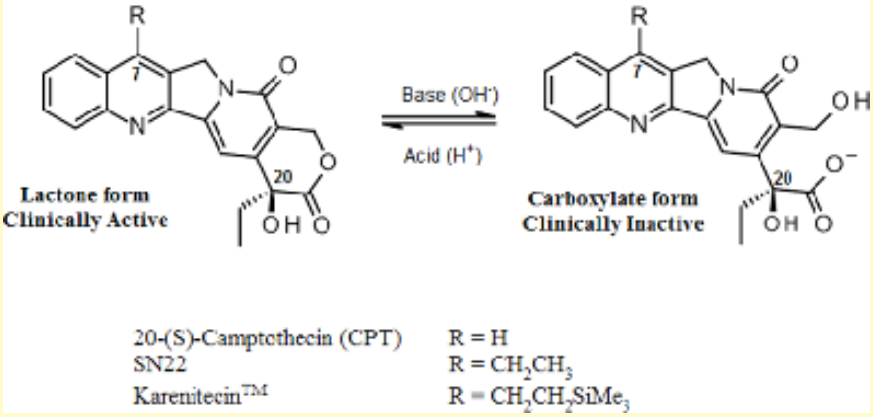
**Abbreviations:** HSA (Human serum albumin); CPT (Camptothecin); and HPLC (High-Performance Liquid Chromatography)

## Introduction

20(S)-Camptothecin (CPT) derivatives have become an important class of antitumor agents [1]. Currently two CPT analogues have been approved by the FDA for use in the treatment of patients with cancer - Camptosar® (irinotecan HCl, CPT-11) and Hycamtin® (topotecan HCl). A number of novel CPT derivatives, including Karenitecin® are under preclinical and clinical development [1, 2]. The α-hydroxyl δ-lactone ring in CPT and its derivatives (Scheme 1) is believed to be the principal structural requirement for the biological action, while the hydrolyzed carboxylate form of CPT and certain derivatives has been observed to be associated with severe and unpredictable toxicities such as hemorrhagic cystitis, diarrhea, and myelosuppression [3,4]. Results from our laboratory indicate that the lactone form of topotecan, a water soluble derivative of CPT, is the only form that binds to DNA sequences in the absence of topoisomerase I [5].

CPT and its derivatives, especially carboxylate forms, bind strongly to the plasma protein, human serum albumin (HSA). This HSA interaction alters the hydrolysis kinetics of the lactone form and may play a significant role in the mechanism of action of this class of anticancer drugs [6-10]. HSA is the most abundant plasma protein with a concentration of about 40 mg/mL [11] and binds to an enormous variety of ligands. HSA is highly helical with three domains and displays two main binding sites, Site I (the warfarin site in domain II) and Site II (the diazepam site in domain III) [12]. These two sites have been characterized structurally from X-ray crystallographic studies of the protein co-crystallized with different ligands [12]. There are also several other less well characterized ligand binding sites on HSA

such as the hemin site in domain I of HSA [13]. Although, the structural details of the binding site(s) for CPT on HSA are not clearly established, one study using CD and Raman spectroscopy methods suggested that CPT binds to Site I on HSA [14, 15]. A recent crystal structure of HSA with several camptothecins indicated that the carboxylate forms bind to the hemin site in domain I of HSA [16].




***Scheme 1:*** *The lactone and carboxylate forms of camptothecin and the camptothecin derivatives, SN22 and Karenitecin®.*

HPLC methods using an immobilized HSA column and in silico methods have been used by researchers to evaluate the relative HSA binding affinities of small molecules [17-21]. A change in the retention time of an HSA ligand when the HSA column is saturated by a second ligand is often observed and is interpreted to be the result of either direct competition for binding to the same HSA site, or an allosteric effect due to two ligands binding to different HSA sites [22]. In the studies reported herein, a similar HPLC method was used to identify the binding site(s) on HSA that directly interact with Camptothecins. Herein experimental results are presented consistent with the idea that the CPT Carboxylate binds to Site II on HSA.

## Materials and Methods

### Reagents and Chemicals

Karenitecin® and SN22 were synthesized at BioNumerik Pharmaceuticals, Inc. Diazepam in USP injection form formulation (5 mg/mL) was purchased from Cancer Therapy and Research Center Pharmacy (San Antonio, Texas). 20(S)-CPT and other HSA ligands, $Na_2HPO_4$, $KH_2PO_4$, DMSO and acetonitrile (HPLC grade) were purchased from Sigma-Aldrich Chemicals.

### Mobile Phase Solutions

Three types of premixed mobile phase solutions were prepared and used for the work described herein. The first was Mobile Phase A and contained 80% phosphate buffer (10 mM $KH_2PO_4$, 10 mM $Na_2HPO_4$, pH 7.0) and 20% acetonitrile. The second mobile phase solution was Mobile Phase B and was prepared by dissolving Phenylbutazone to a final concentration of 200 µM in Mobile Phase A. The third mobile phase was prepared by dissolving ibuprofen to a final concentration of 200 µM in Mobile Phase A and was called Mobile Phase C.

### HPLC Assay

The analytical HPLC system consisted of a Waters™ 717 plus auto sampler with temperature control capability, a 200 µL injection loop, a solvent delivery system with Waters™ 600 controller, and a Waters™ 474 scanning fluorescence detector. The analytical HSA protein column (4.0 x 150 mm, 5 µm, analytical) was from Chrom Tech Inc. Waters' Millennium32 (version 4) software was used for data acquisition and processing.

All the injection samples of the CPT derivatives were prepared in Mobile Phase A (1 μM final concentration) and were incubated for 30 min prior to injection. Five microliters of each injection sample was injected through the Waters 717 auto sampler. At least two injections per sample were made to ensure chromatographic reproducibility.

All HPLC experiments were carried out in three consecutive steps. In step one; samples were injected onto a Mobile Phase A equilibrated system. In step two, the system was equilibrated to Mobile Phase B or Mobile Phase C. Phenylbutazone in Mobile Phase B and ibuprofen in Mobile Phase C were monitored by UV detection (Waters 2996 Photo Diode Array Detector with a flow rate of 0.8 mL/min), at wavelengths of 240 nm and 264 nm, respectively, to assure that the system achieved equilibration. Samples were injected onto the fully equilibrated system with the new mobile phases. In the third and final step, the system was equilibrated back to original ligand-free Mobile Phase A. UV detection was used to monitor any residual phenylbutazone or ibuprofen and to determine the completion of clearance. Samples were injected again and the retention times were recorded. For each injected sample, the same retention time was obtained in the first and last steps of the assay.

## Results and Discussion

## Results

### The Effects of a Known Site I Ligand, Phenylbutazone, On the Retention Time of CPT and Derivatives

Phenylbutazone and warfarin, two well known Site I ligands [12], were used to demonstrate that our HPLC assay could sensitively detect the competition for binding to a single, specific HSA site. The HPLC chromatograms (Figure 1) of three injections of warfarin, with and without phenylbutazone saturating the HSA column, demonstrated that warfarin and phenylbutazone compete for the same HSA binding site. Panel A is the HPLC chromatogram obtained when warfarin was injected in the Mobile Phase A equilibrated system. The R and S forms of warfarin were observed at 8.3 and 10.5 minutes, respectively. The assignment of R and S warfarin is made based upon results from similar studies in the literature [17]. Panel B is the HPLC chromatogram obtained when warfarin was injected in the Mobile Phase B equilibrated system (Mobile Phase B contains 200 μM phenylbutazone). The presence of phenylbutazone in the mobile phase resulted in a reduced retention time for both the R and S forms of warfarin by 3.5 and 5.1 minutes, respectively. Since it is well established that both warfarin and phenylbutazone bind to Site I on HSA [12], the reduction in the retention time of warfarin when the system was equilibrated with phenylbutazone was inferred to mean that warfarin and phenylbutazone competed for binding to the same site. To emphasize the reversible and competitive nature of the binding to HSA by warfarin and phenylbutazone, panel C shows the elution profile of warfarin after the column was regenerated by switching to Mobile Phase A and after all phenylbutazone had cleared the system. As can been seen in Figure 1 (panel C), the original retention time for both the R and S forms of warfarin was restored after the phenylbutazone was removed completely from the mobile phase and the column. These data indicate that warfarin and phenylbutazone reversibly and competitively bind to the same site on HSA.

The results displayed in Figure 1 demonstrate that for the two ligands with the same HSA binding site, saturating the HSA column with one of the ligands will cause a reduction in the retention time of the other ligand. Diazepam is a small molecule that has been well characterized to bind to HSA at Site II (the diazepam site). As indicated in Figure 2, the presence of 200 μM Phenylbutazone (Site I binder), in the mobile phase did not have a noticeable effect on the retention time (~ 4.3 min) of diazepam. This observation suggested that the saturation of Site I on HSA with phenylbutazone did not interfere with the binding of another ligand to Site II.

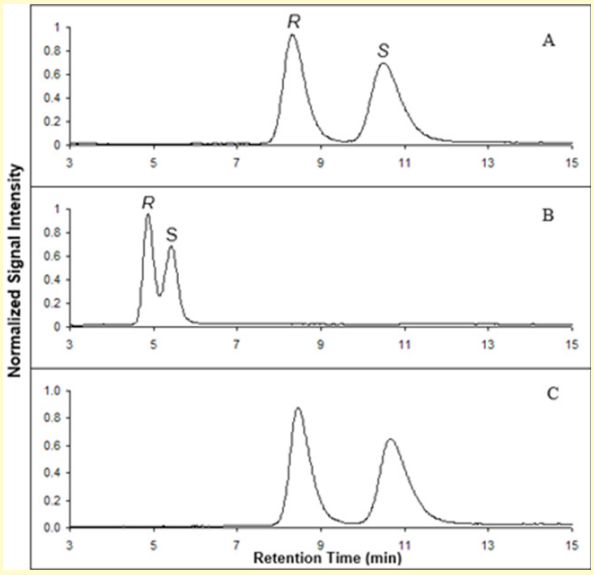



***Figure 1:*** *Equilibrating the HSA column with phenylbutazone (200 μM) drastically affected the retention time of warfarin. (A) The two isomers of warfarin in the ligand-free (no phenylbutazone) Mobile Phase A. (B) The change in retention time of R- and S-warfarin in the presence of phenylbutazone in the mobile phase (Mobile Phase B). (C) The restoration R- and S-warfarin retention times after the column was regenerated to ligand-free Mobile Phase A.*

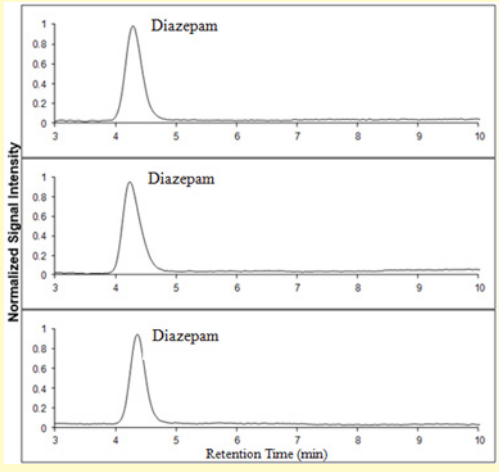



***Figure 2:*** *Equilibrating the HSA column with phenylbutazone (200 μM) did not have a notable effect on the retention time of diazepam. (A) Diazepam in the ligand-free Mobile Phase A. (B) Diazepam retention in Mobile Phase B. (C) Diazepam retention after the column was re-equilibrated to ligand-free Mobile Phase A.*

Karenitecin® is a novel investigational highly lipophilic CPT derivative (Scheme 1) that has been evaluated in oncology-related clinical studies, including studies in patients with advanced ovarian cancer [23]. Karenitecin® was engineered with the objective of achieving more consistent plasma lactone levels and reduced unfavorable metabolism (e.g., glucuronidation) that may lead to toxicity [24]. The HPLC chromatogram for Karenitecin® (injection sample was equilibrated for the lactone ring hydrolysis) in the Mobile Phase A equilibrated HPLC system is shown in panel A of Figure 3. The two well-separated peaks at 5.7 and 8.7 minutes correspond to the lactone and carboxylate forms of Karenitecin®, respectively. This assignment was made by injecting a freshly prepared Karenitecin® (pure lactone) sample and a Karenitecin® sample prepared in high pH phosphate buffer where formation of the carboxylate form is favored. The longer retention time of the carboxylate form suggests that the carboxylate form of Karenitecin® has a higher binding affinity for HSA than the corresponding lactone form. As was observed with diazepam, the presence of 200 μM phenylbutazone in the mobile phase had very little effect, if any, on the retention times of the lactone or carboxylate forms of Karenitecin® (compare panels A and B in Figure 3). There was no change in the retention time of the lactone form of Karenitecin® and only a 0.4 minute reduction in the retention time of the carboxylate form.

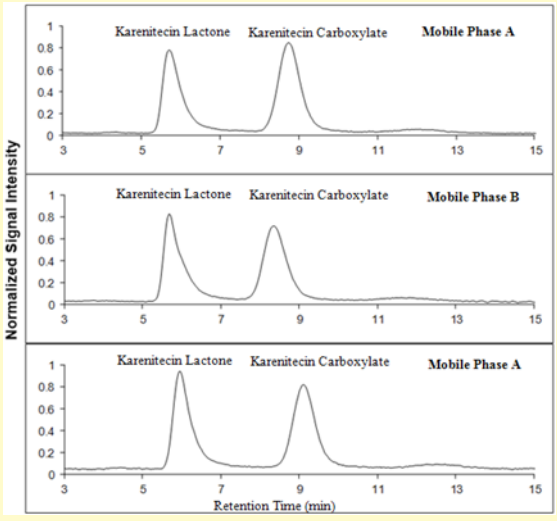



***Figure 3:*** *Equilibrating the HSA column with phenylbutazone (200 μM) did not have a notable effect on the retention times of Karenitecin®. (A) Karenitecin® in the ligand-free Mobile Phase A. (B) Karenitecin® retention in Mobile Phase B. (C) Karenitecin® retention after the column was re-equilibrated to ligand-free Mobile Phase A.*

SN22 is a CPT derivative that has a $-CH_2CH_3$ substitution at the C7 position (Scheme 1). HPLC chromatograms of SN22, with and without 200 μM phenylbutazone in the mobile phase, are shown in Figure 4. The retention times for the lactone and carboxylate forms of SN22 were assigned using the technique described above for Karenitecin®. The carboxylate form of SN22 displayed a much higher retention time relative to the corresponding retention times seen for the lactone form of SN22 and the carboxylate form of Karenitecin®, suggesting that the SN22 carboxylate binds to HSA more avidly. The presence of 200 μM phenylbutazone in the mobile phase did not change the lactone form retention time. While the retention time of SN22 carboxylate form was reduced in the Mobile Phase B equilibrated system by about 1.2 minutes, the magnitude of this reduction is much smaller than the reductions observed in experiments with warfarin and phenylbutazone.

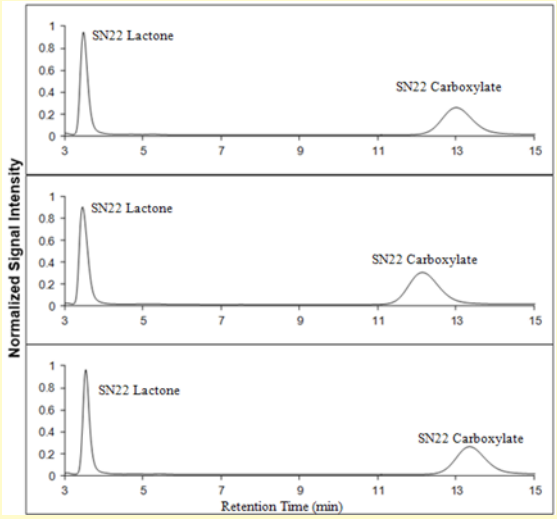



***Figure 4:*** *Equilibrating the HSA column with phenylbutazone (200 μM) did not have a notable effect on the retention times of SN22. (A) The retention of SN22 in the ligand-free Mobile Phase A. (B) SN22 retention in Mobile Phase B. (C) SN22 retention after the column was re-equilibrated to ligand-free Mobile Phase A.*

The phenylbutazone induced changes in retention times for all the selected compounds are summarized in Table 1. Figure 5 shows the correlation between the phenylbutazone induced retention time changes and the corresponding retention times in the ligand-free Mobile Phase A. From these data, compounds can be grouped into two distinct categories. Both warfarin (S and R isomers) and phenylbutazone are in one category, with large changes in retention time and a sensitive response (characterized by the steep slope of the linear correlation) to the presence of 200 μM phenylbutazone in the mobile phase. This is consistent with the fact that these ligands compete for the same HSA binding site that phenylbutazone binds to in the phenylbutazone equilibrated column. In contrast, diazepam and the selected CPTs (both lactone and carboxylate) comprise another category, with negligible phenylbutazone induced changes in retention times and an insensitive response (characterized by the flat slope of the linear correlation) to the presence of phenylbutazone in the mobile phase. These data are consistent with the other studies that characterize diazepam as a Site II ligand of HSA [22]. It is worth noting that, although the retention times in the ligand-free Mobile Phase A for the carboxylate forms of Karenitecin® and SN22 are similar to that of warfarin and phenylbutazone, the phenylbutazone induced changes in their retention times are small. These results suggest that Karenitecin® and SN22 do not compete with phenylbutazone for binding to Site I HSA. Therefore, we conclude that the CPT derivatives, in general, do not bind to Site I of HSA or bind with low affinity relative to phenylbutazone.

**The Effects of the Site Ii Ligand, Ibuprofen, On the Retention of CPT and Derivatives**

Mobile Phase C contained 200 μM ibuprofen, a Site II ligand, and was used to equilibrate the HPLC system for studies focusing on the Site II binding site on HSA. The retention times of the selected compounds were evaluated under the ligand-free (Mobile Phase A equilibrated) and ibuprofen pre-bound (Mobile Phase C equilibrated) HPLC conditions. Figure 6 shows that ibuprofen (200 μM) in the mobile phase reduced diazepam's retention time to 3.5 minutes compared to 4.1 minutes for the original value in the ligand-free Mobile Phase A, and this observation is consistent with the fact that both diazepam and ibuprofen are well known HSA Site II binders.

| Compound | Mobile Phase without Phenylbutazone | Mobile Phase with Phenylbutazone | Δrt (min) |
|---|---|---|---|
| R-warfarin | 8.3 | 4.8 | -3.5 |
| S-warfarin | 10.5 | 5.4 | -5.1 |
| Phenylbutazone | 13.3 | 5.1 | -8.2 |
| Diazepam | 4.3 | 4.2 | -0.1 |
| SN22 lactone | 3.5 | 3.5 | 0 |
| SN22 carboxylate | 13.3 | 12.1 | -1.2 |
| Karenitecin® lactone | 5.7 | 5.7 | 0 |
| Karenitecin® carboxylate | 8.7 | 8.3 | -0.4 |
| CPT carboxylate | ND | ND | |
| CPT lactone | ND | ND | |
| | **Mobile Phase without Ibuprofen** | **Mobile Phase with** | **Δrt (min)** |
| R-warfarin | 7.3 | 5.7 | -1.6 |
| S-warfarin | 10 | 6.9 | -3.1 |
| Phenylbutazone | 14.1 | 10.5 | -3.6 |
| Diazepam | 4.1 | 3.5 | -0.6 |
| SN22 carboxylate | 11.9 | 7.4 | -4.5 |
| Karenitecin® carboxylate | 8.5 | 6 | -2.5 |
| CPT carboxylate | 106.4 | 53.7 | -52.7 |
| SN22 lactone | 3.5 | 3.2 | -0.3 |
| Karenitecin® lactone | 5.9 | 5.6 | -0.3 |
| CPT lactone | 2.8 | 2.7 | -0.1 |

*Δrt = change in retention time*

*ND = not determined*

***Table 1:*** *Changes in Retention Time (in minutes) Induced by Phenylbutazone (200 μM) and Ibuprofen (200 μM) Equilibrated HSA column.*

The presence of ibuprofen in the HPLC mobile phase resulted in a retention time reduction of 2.5 minutes for the carboxylate form of Karenitecin® but only minimal changes in the retention time of the Karenitecin® lactone form (Figure 7). As a consequence there was reduced spectral resolution of the two species and a 100% lactone standard was needed to clearly differentiate between the two species under these conditions (Figure 7).

Similarly, the presence of 200 μM ibuprofen in the mobile phase resulted in a large change in the retention time of the SN22 carboxylate of 4.5 minutes, while the change in the retention time of the lactone form was negligible (Figure 8). The carboxylate form of 20(S) Camptothecin (CPT) was also evaluated and had an unusually long retention time of approximately 106 minutes in the ligand-free Mobile Phase A; however, the retention time for the CPT lactone under the same chromatographic conditions was only 2.8 minutes. The long retention time for the carboxylate and the large gap between the retention times for the carboxylate and lactone forms of CPT are consistent with studies that show that the carboxylate form of CPT binds to HSA with a much higher affinity than the lactone form [8]. When the HSA column was equilibrated with 200 μM ibuprofen, the retention time of the carboxylate form CPT was reduced to about 54 minutes, a net change of approximately 53 minutes (Figure 9).

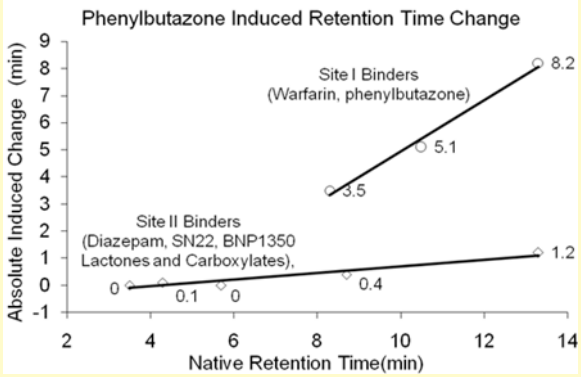



***Figure 5:*** *The linear correlation between the phenylbutazone induced retention time change and the retention time in the ligand-free Mobile Phase A. Large net changes in the retention time and steep slope of the linear correlation were obtained for direct site competitive ligands (i.e., Site I binders in this case). ( Note: Karenitecin® is also known as BNP1350).*

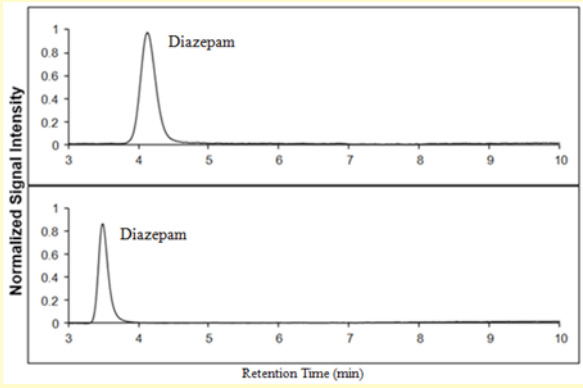



***Figure 6:*** *Equilibrating the HSA column with ibuprofen (200 μM) drastically affected the retention time of diazepam. (A) The retention of diazepam in the ligand-free Mobile Phase A. (B) The retention time changes in the presence of ibuprofen in the mobile phase (Mobile Phase C).*

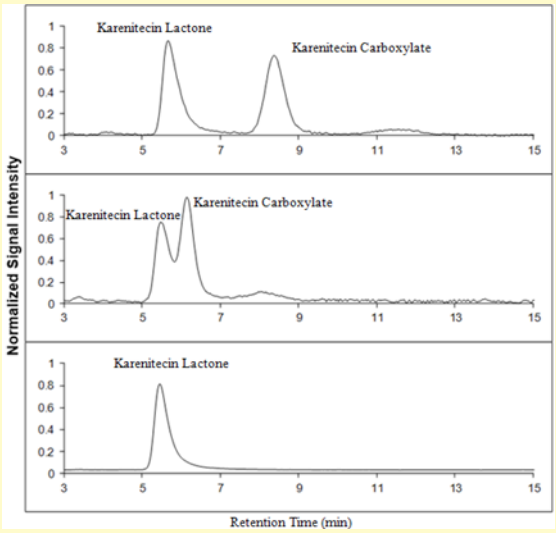



***Figure 7:*** *Equilibrating the HSA column with ibuprofen (200 μM) drastically affected the retention time of the Karenitecin® carboxylate. (A) The retention of Karenitecin® (carboxylate on the right and lactone on the left) in the ligand-free Mobile Phase A. (B) The change in retention time in the presence of ibuprofen in the mobile phase (Mobile Phase C). (C) The retention of 100% Karenitecin® lactone in Mobile Phase C.*

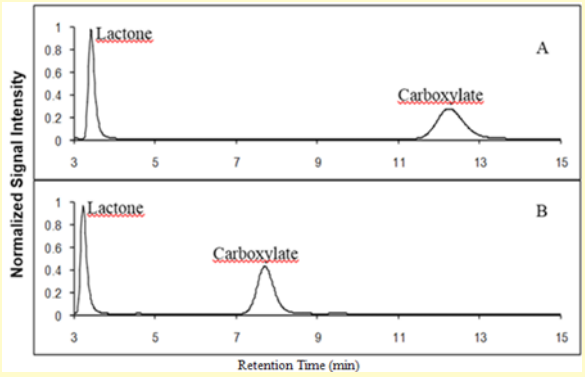



***Figure 8:*** *Equilibrating the HSA column with ibuprofen (200 μM) drastically affected the retention time of the SN22 carboxylate. (A) The retention of SN22 in the ligand-free Mobile Phase A. (B) The changes in retention time in the presence of ibuprofen in the mobile phase (Mobile Phase C).*

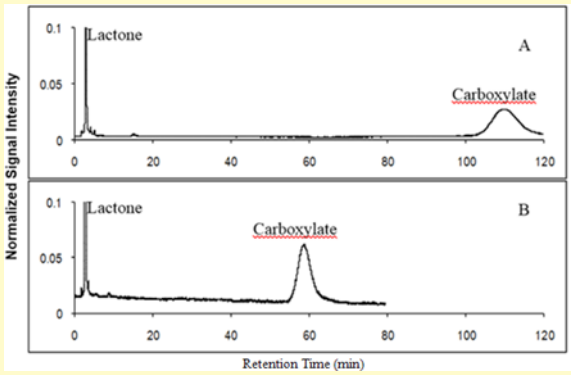



***Figure 9:*** *The effects of HSA saturation by 200 μM ibuprofen on the retention times of 20(S)-CPT (A) The native retention of 20(S)-CPT in Mobile A. (B) The retention time changes in the presence of 200 μM ibuprofen in the mobile phase.*

Although equilibrating the HSA column with the Site I binder phenylbutazone did not affect the retention time of compounds that bind to Site II, such as diazepam, the reverse was not true. Equilibrating the HSA column with ibuprofen, a Site II ligand, also noticeably reduced the retention times of warfarin and phenylbutazone, although the magnitude of the reduction was not as great as that produced by phenylbutazone (Table 1, Figure 10). Structure function studies have shown that the conserved residue Trp214 in HSA participates in a hydrophobic packing interaction between the Site I and Site II interface, and it has been observed that ligands that bind to Site II can affect or influence the binding affinity of ligands that bind to Site I [25]. The effects of ibuprofen on the retention times of the Site I ligands warfarin and phenylbutazone could be due to this structural feature of the Site I/Site II interface on HSA.

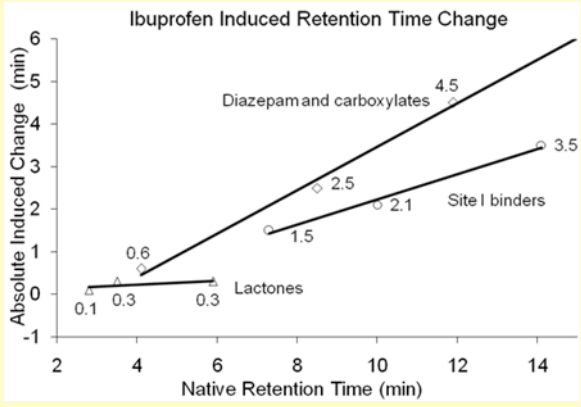



***Figure 10:*** *The linear relationship between the induced retention time change and the native retention. Large net changes in retention time and steep slope of the linear relationship are obtained for direct site competitive ligands, i.e. Site II binders in this case. Not as drastic but still noticeable changes for Site I binders may be due to the Trp214 mediated binding site coupling in HSA.*

The ibuprofen induced changes in retention times of the molecules studied herein are also summarized in Table 1 and the correlation between the changes in the retention times and the corresponding original retention times in the ligand-free Mobile Phase A is shown in Figure 10. Here, diazepam and the carboxylates of Karenitecin®, SN22 and CPT comprise one category, with a noticeable ibuprofen induced change in retention time. The retention time for compounds in this category were very sensitive to the presence of ibuprofen in mobile phase, evidenced by the steep slope of the linear correlation. This suggests that carboxylate forms of these CPT derivatives compete with ibuprofen for the Site II on HSA analogously to how diazepam competes with ibuprofen. Warfarin and phenylbutazone (both are Site I binders) form a second category that exhibited intermediate ibuprofen induced changes in retention times. And finally, Karenitecin®, SN22 and CPT lactones fall into a third category that displayed negligible changes in retention times when the HSA column was equilibrated with ibuprofen. We propose that the lactones either do not bind or bind with low affinity and/or specificity to Site II of HSA.

## Discussion

Camptothecins are bound by plasma proteins such as human serum albumin and alpha 1 acid glycoprotein (AGP). In a previous study, we reported that Karenitecin existed in the lactone form more than Topotecan and CPT owing to its binding to AGP [27]. In this study, using HPLC methods with an immobilized HSA affinity column and exploiting the competitive binding characteristics of HSA ligands, we have provided the first direct evidence that the carboxylate form of Karenitecin®, SN22 and CPT selectively compete with ibuprofen for binding to Site II on HSA. These results are consistent with observations that Site II on HSA is the primary binding site for small anionic aromatic compounds [25,26]. These results varied, although it was not a direct relative correlation using comparable methods, from results obtained in studies of CPT using CD and Raman spectroscopy that indicated CPT binds at or near Site I [14, 15]. The derivatives evaluated in this study contained substitutions at the C-7 position of CPT, and it is possible that these lipophilic substitutions influenced binding site preferences. Furthermore, the biophysical constraints associated with the varying analytical approaches (HPLC herein compared to CD and Raman spectroscopy by other researchers) may favor detection of one or another binding interaction. For example, it's possible that binding at multiple sites may occur but that each individual method cannot detect all possible binding interactions. In this regard, it is interesting to note that structural studies indicate that CPT (carboxylate form) binds to a site in domain IB [16] which is distinct from the binding site preference of CPT (carboxylate from) obtained from the CD or Raman spectroscopy data. The CPT derivatives selected in this study contain substitutions at C7 position of –H (CPT), $-CH_2CH_3$ (SN22), and $-CH_2CH_2SiMe_3$ (Karenitecin®) and all of these substituted molecules displayed specificity for binding to site II on HSA using the methods developed and employed herein (Scheme 1).

The lactone forms of all the CPT derivatives displayed weaker binding affinity to HSA, in general, relative to their carboxylate counterparts, but they did not exhibit distinguishable competitive binding to either Site I or Site II, using the methods described in this paper.

Preliminary computational binding studies indicated that the carboxylate forms of camptothecins have higher interaction energies with the HSA residues binding into the diazepam site than with the lactone forms binding to the same site in HSA [28]. The interaction map of camptothecins (carboxylate form based on the docked structure) in the diazepam binding site of HSA shows that Tyr 411 and Ser 489 have key stabilizing interactions with the open carboxylate form (Figure 11). Such stabilizing interactions are not possible with the closed lactone form of camptothecins.

It is possible that the lactones bind to a unique site other than Site I or Site II on HSA, as there are other potential binding sites for small molecules on HSA. Alternatively, the relatively weak affinity of the lactones for HSA may mean that this assay is unable to differentiate changes in the lactone retention times in the presence and absence of a competing molecule such as phenylbutazone or ibuprofen. Figures 5 and 10 show that the competitor induced change in retention time(s) corresponds linearly to the original retention time(s) for compounds in the same category. This linear correlation in Figure 10 enabled us to classify the lactones studied into a different category relative to the carboxylates and diazepam. This is consistent with the hypothesis that the lactones interact with HSA in a manner that is distinct from how diazepam interacts.

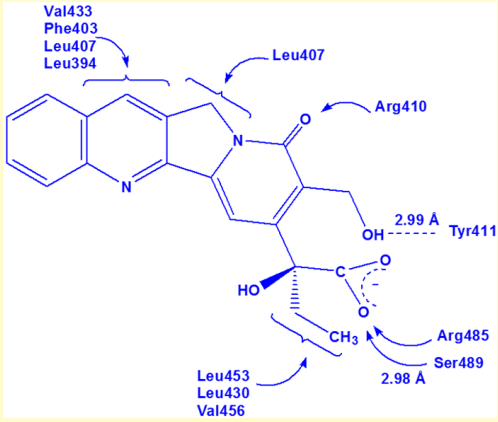



***Figure 11:*** *2-Dimensional interaction map showing the key interactions between Camptothecin and Human Serum Albumin Residues in Type II Site (Diazepam Site).*

## Conclusion

Camptothecins are an important class of anti-neoplastic agents. Camptothecins exist in two forms (carboxylate and lactone), based upon the pH conditions, with the closed lactone form being the clinically active moiety. The pharmacokinetics and/or pharmacodynamics of these drugs may be modified by binding to plasma proteins such as human serum albumin affecting the equilibrium between the lactone and the carboxylate forms. Using HPLC methods employing an immobilized HSA affinity column and using competitive assay conditions, data show that the carboxylate forms of Karenitecin® and other camptothecins (CPT and SN22) bind to the Site II binding site of HSA (diazepam binding site). Preliminary computational binding studies [28] indicated that Tyr 411 and Ser 489 residues of HSA in Site II binding site stabilize the carboxylate form and such stabilizing interactions are not possible for the lactone form of camptothecins.

Atomic level structural information is very valuable for the design of small molecules that have the desired HSA binding interactions [25]. In the absence of a high-resolution crystal or NMR structure of a CPT-HSA complex, the results presented in this report have provided indirect structural information for our ongoing experimental and theoretical studies of CPT-HSA interactions. A thorough understanding of the CPT-HSA interactions is crucial for elucidating the plasma protein binding mechanism(s) of this important class of antitumor agents and may facilitate development of more effective CPT analogues for use as antineoplastics.

## Acknowledgements

We thank David Margrave for careful review of the manuscript.

**Conflicts of Interest:** None

## Bibliography

1. Sparreboom A., *et al*. "Topoisomerase I-targeting drugs In: Cancer Chemotherapy and Biotherapy". 4 (2007): 371-413.
2. Daud A., *et al*. "Phase II trial of karenitecin in patients with malignant melanoma: clinical and translational study". *Clinical Cancer Research* 11.8 (2005): 3009-3016.
3. Muggia FM., *et al*. "Phase I clinical trial of weekly and daily treatment with camptothecin (NSC-100880): correlation with preclinical studies". *Cancer Chemotherapy Reports* 56.4 (1972): 515-521.
4. Hsiang Y-H., *et al*. "Identification of mammalian DNA topoisomerase I as an intracellular target of the anticancer drug camptothecin". *Cancer Research* 48.7 (1988): 1722-1726.

5. Yao S., *et al*. "Topotecan lactone selectively binds to double- and single-stranded DNA in the absence of topoisomerase I". *Cancer Research* 58.17 (1998): 3782-3786.
6. Burke TG., *et al*. "Ethyl substitution at the 7 position extends the half-life of 10-hydroxycamptothecin in the presence of human serum albumin". *Journal of Medicinal Chemistry* 36.17 (1993): 2580-2582.
7. Burke TG., *et al*. "Preferential binding of the carboxylate form of camptothecin by human serum albumin". *Analytical Biochemistry* 212.1 (1993): 285-287.
8. Burke TG., *et al*. "The structural basis of camptothecin interactions with human serum albumin: impact on drug stability". *Journal of Medicinal Chemistry* 37 (1994): 40-46.
9. Mi Z., *et al*. "Differential interactions of camptothecin lactone and carboxylate forms with human blood components". *Biochemistry* 33.34 (1994): 10325-10336.
10. Mi Z., *et al*. "Reduced albumin binding promotes the stability and activity of topotecan in human blood". *Biochemistry* 34.42 (1995): 13722-13728.
11. Rang HP., *et al*. "Pharmacology". (Livingstone, C., Ed.), Edinburgh, (1999).
12. Carter DC., *et al*. "Structure of serum albumin". *Advances in Protein Chemistry* 45 (1994): 153-203.
13. Zunszain PA., *et al*. "Crystal structural analysis of human serum albumin complexed with hemin and fatty acid". *BMC Structural Biology* 3 (2003): 6-14.
14. Fleury F., *et al*. "Camptothecin-binding site in human serum albumin and protein transformations induced by drug binding". *FEBS Letters* 411.2-3 (1997): 215-220.
15. Fleury F., *et al*. "Interactions of lactone, carboxylate and self-aggregated forms of camptothecin with human and bovine serum albumins". *FEBS Letters* 406.1-2 (1997): 151-156.
16. Wang ZM., *et al*. "Structural studies of several clinically important oncology drugs in complex with human serum albumin". *Biochimica Biophysica Acta* 1830.12 (2013): 5356-5374.
17. Loun B., *et al*. "Chiral separation mechanisms in protein-based HPLC columns. 1. Thermodynamic studies of (R)- and (S)-warfarin binding to immobilized human serum albumin". *Analytical Chemistry* 66.21 (1994): 3814-3822.
18. Hage DS. "High-performance affinity chromatography: a powerful tool for studying serum protein binding". *Journal of Chromatography B: Biomedical Sciences and Applications* 768.1 (2002): 3-30.
19. Hage DS., *et al*. "High-performance affinity chromatography and immobilized serum albumin as probes for drug- and hormone-protein binding". *Journal of Chromatography B: Biomedical Sciences and Applications* 739.1 (2000): 39-54.
20. Colmenarejo G. "In silico prediction of drug-binding strengths to human serum albumin". *Medicinal Research Reviews* 23.3 (2003): 275-301.
21. Colmenarejo G., *et al*. "Cheminformatic models to predict binding affinities to human serum albumin". *Journal of Medicinal Chemistry* 44.25 (2001): 4370-4378.
22. Fitos I., *et al*. "Stereoselective allosteric binding interaction on human serum albumin between ibuprofen and lorazepam acetate". *Chirality* 11.2 (1999): 115-120.
23. Kavanagh JJ., *et al*. "Phase II multicenter open-label study of karenitecin in previously treated epithelial ovarian and primary peritoneal cancer: a gynecologic oncology group study". *International Journal of Gynecological Cancer* 18.3 (2008): 460-464.
24. Brangi M., *et al*. "Camptothecin resistance: role of the ATP-binding cassette (ABC), mitoxantrone-resistance half-transporter (MXR), and potential for glucuronidation in MXR-expressing cells". *Cancer Research* 59.23 (1999): 5938-5946.
25. Peters T., Jr. "All About Albumin". Academic Press, San Diego CA (1996).
26. He XM., *et al*. "Atomic structure and chemistry of human serum albumin". *Nature* 358.6383 (1992): 209-215.
27. Yao S., *et al*. "Stabilization of the Karenitecin® lactone by alpha-1 acid glycoprotein". *Cancer Chemotherapy and Pharmacology* 75 (2015):719-728.

28. Yao S., *et al*. "Studies of the Protein Binding Properties of Karenitecin™ (BNP1350), A Novel Highly Lipophilic Camptothecin Analogue". 94th AACR National Meetings (2003) July 11-14, Washington, D.C (abs# 1786).

**Volume 2 Issue 1 December 2015**