Novel A,B,E-Ring-Modified Camptothecins Displaying High Lipophilicity and Markedly Improved Human Blood Stabilities

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Camptothecins are DNA topoisomerase I inhibitors that have recently emerged as a prominent class of anticancer agents.1–3 Topotecan and CPT-11 are water-soluble analogues of the natural product camptothecin and in 1996 were the first two members within the family to gain FDA approval (topotecan as second-line therapy for advanced epithelial ovarian cancer and CPT-11 as first-line therapy for colon cancer). Other camptothecin congeners currently under clinical evaluation include Lurtopotecan (GI147211), 9-aminocamptothecin, and DX-8951f, all of which display improved water solubility over camptothecin, and 9-nitrocamptothecin, which is a lipophilic analogue. Although widely used, camptothecins are known to undergo relatively rapid hydrolysis in the bloodstream resulting in a marked loss of therapeutic potential. It is the key α-hydroxy-β-lactone pharmacophore within the clinically relevant camptothecins that undergoes facile acyl cleavage at physiological pH4 to yield a biologically inactive5–7 carboxylate form. In this report we describe the rational design and total synthesis of highly lipophilic A,B,E-ring-modified camptothecins that are the most blood-stable camptothecins displaying intrinsic anticancer activity yet to be identified.

Our approach to the design of more blood-stable camptothecin-class topoisomerase I inhibitors was based upon three general considerations. First, structural modifications that eliminated the highly preferential binding of the carboxylate over the lactone form by human serum albumin (HSA)8–12 were sought. Previous research efforts in our laboratories have shown that 9-aminocamptothecin and camptothecin display extremely poor stabilities in human blood due to the high-affinity, noncovalent binding interactions of their carboxylate forms with HSA.11,12 For instance, frequency-domain lifetime fluorometry reveals that HSA preferentially binds camptothecin carboxylate with over a 100-fold higher affinity than camptothecin lactone;11 this selective binding of carboxylate over lactone results in a shifting of the equilibrium in favor of the carboxylate. In this manner, camptothecin opens more rapidly and completely in the presence of HSA than in the absence of the protein. In a solution containing HSA and in human plasma, pH 7.4 and 37 °C, camptothecin and 9-aminocamptothecin open rapidly and essentially completely, such that negligible 0.2% lactone levels remain at equilibrium.11,12 Time-resolved fluorescence spectroscopic investigations show that topotecan contains structural features which reduce binding of its carboxylate form to HSA,13 as a result, topotecan displays improved stabilities in human blood and plasma relative to camptothecin and 9-aminocamptothecin.

Second, our finding that lactone stabilization is achieved through lipid bilayer partitioning10,14,15 led us to pursue the design of more lipophilic camptothecin analogues. We have shown previously that lipid bilayer vesicles,14,15 as well as erythrocytes,10 promote camptothecin drug stability by preferentially binding electroneutral lactone over negatively charged carboxylate. Thus, the design of more lipophilic camptothecins would promote the reversible partitioning of the new agents into the lipid bilayers of erythrocytes, thereby protecting the active lactone forms from hydrolysis. Initial concerns about the loss of antitopoisomerase I activity through enhancing compound lipophilicity were lessened by the work of Pommier et al.,16 who in 1990 noted that several more lipophilic camptothecin analogues such as 10,11-(methyleneedioxy)camptothecin display superior intrinsic potencies against topoisomerase I.

Last, recent studies have shown that expansion of the camptothecin E-ring to a seven-membered system (by insertion of a methylene spacer between the 20-OH functionality and the carbonyl moiety) enhances the solution stability of the agent while maintaining anticancer activity.17,18 Whereas the 20-OH functionality in conventional camptothecins is thought to interact with the carbonyl oxygen and facilitate ring opening, inclusion of a methylene spacer decreases the interactions between the β-OH and carbonyl oxygen. This change diminishes hydrogen-bonding interactions between the two groups and is thought to result in a slower rate of lactone hydrolysis. Since our total synthetic approach allows for the E-ring to be readily modified, we included the expanded β-hydroxy-δ-lactone E-ring functionality in our drug design strategy.

The new agents, which we refer to as homosilatecan derivatives, were prepared using the cascade radical annulation approach, as summarized in Scheme 1. Enol ether 2, an intermediate in the synthesis of standard E-ring camptothecin analogues,19,20 was oxidatively cleaved to keto formate 3 by treatment with OsO4 followed by Pb(OAc)4. Chain extension by Reformatsky reaction followed by treatment with TFA provided an expanded lactone, which was then treated with ICl followed by TMSI to generate the pyridone lactone 4. This was then N-propargylated with trimethylsilyl- and tert-butyldimethylsilyl-substituted propargyl bromide to provide 5a,b. In the key cascade radical annulation,
these precursors were reacted under standard conditions\textsuperscript{19,20} with phenyl isonitrile or with p-NHBOc- and p-OAc-substituted phenyl isonitrile (followed by cleavage of the respective protecting groups) to provide four new homosilatecan analogues (\textsuperscript{1a–d}) in unoptimized yields of 13–27%. The new agents include 7-(trimethylsilyl)-10-aminohomocamptothecin (DB-38), 7-(\textit{tert}-butyl(dimethyl)silyl)homocamptothecin (DB-81), 7-(\textit{tert}-butyl(dimethyl)silyl)-10-aminohomocamptothecin (DB-90), and 7-(\textit{tert}-butyl(dimethyl)silyl)-10-hydroxyhomocamptothecin (DB-91). In addition to the expanded \(\beta\)-hydroxy-\(\alpha\)-lactone E-ring functionality, each of the new homosilatecans also contains a silylalkyl functionality at the 7-position. The silylalkyl functionality provides a convenient means of varying lipophilicity while concomitantly reducing the strength of carboxylate interactions with HSA. The DB-90 and DB-91 agents also contain amino and hydroxyl groups at the 10-position, respectively. These changes at position 10 were undertaken since previous studies had shown that 10-substitution in combination with 7-substitution decreased the binding of camptothecin carboxylate to HSA.\textsuperscript{9} In some cases (i.e. SN-38), the combination of 7- and 10-position substituents decreases carboxylate interactions while promoting lactone associations.\textsuperscript{9} A variety of analytical and biophysical methods were employed to compare the blood component interactions and blood stabilities of the new homosilatecans with those of camptothecin and its clinically relevant analogues.

The equilibrium associations of the new A,B,E-ring- and B,E-ring-modified homosilatecans with lipid bilayers were characterized by fluorescence spectroscopic methods. The intrinsic fluorescence from the new agents allowed us to directly monitor their interactions with small unilamellar vesicles (SUVs) composed of electroneutral L-\(\alpha\)-dimyristoylphosphatidylcholine (DMPC) and negatively charged L-\(\alpha\)-dimyristoylphosphatidylglycerol (DMPG). In the presence of model membranes,
Table 1. Overall Association Constants for Camptothecin Analogues Interacting with Unilamellar Vesicles of Electroneutral DMPC and Negatively Charged DMPG in PBS Buffer at pH 7.4 and 37 °C

| compound     | $K_{\text{DMPC}}$ (M$^{-1}$) | $K_{\text{DMPG}}$ (M$^{-1}$) |
|--------------|-------------------------------|------------------------------|
| DB-38        | 1400                          | 800                          |
| DB-81        | 14000                         | 19000                        |
| DB-90        | 9000                          | 9000                         |
| DB-91        | 8000                          | 4000                         |
| DB-90 carboxylate form | 800                  | 80                           |
| DB-91 carboxylate form | 700     | 100                          |
| topotecan    | 10                            | 50                           |
| camptothecin | 100                           | 100                          |

Equilibrium association constants were determined using the method of fluorescence anisotropy titration as described previously for camptothecins. Electroneutral, fluid-phase lipid bilayers were represented by SUVs comprised of DMPC. Negatively charged, fluid-phase bilayers were represented by DMPG SUVs. Binding isotherms were constructed by the method of fluorescence anisotropy titration, and K values were determined from the slopes of double-reciprocal plots. The K values are subject to 10% uncertainty.

Table 2. Summary of the Stability Parameters for Homosilatecans in Human Blood, Plasma, PBS, PBS with HSA, and PBS with RBCs

| drug name and fluid | % lactone after 3 h |
|---------------------|---------------------|
| DB-38               | whole blood         | 56.4 ± 0.6               |
|                     | HSA                 | 81.4 ± 0.3               |
|                     | PBS                 | 82.8 ± 0.7               |
|                     | plasma              | 40.3 ± 2.1               |
|                     | RBC                 | 84.8 ± 1.5               |
| DB-81               | whole blood         | 86.6 ± 0.5               |
|                     | HSA                 | 88.1 ± 0.2               |
|                     | PBS                 | 84.9 ± 0.3               |
|                     | plasma              | 85.0 ± 4.3               |
|                     | RBC                 | 92.0 ± 0.0               |
| DB-90               | whole blood         | 85.2 ± 0.7               |
|                     | HSA                 | 86.8 ± 0.2               |
|                     | PBS                 | 83.7 ± 0.5               |
|                     | plasma              | 71.1 ± 3.5               |
|                     | RBC                 | 85.5 ± 0.4               |
| DB-91               | whole blood         | 84.9 ± 0.3               |
|                     | HSA                 | 82.9 ± 0.3               |
|                     | PBS                 | 83.1 ± 0.3               |
|                     | plasma              | 61.5 ± 3.9               |
|                     | RBC                 | 88.5 ± 0.2               |

Hydrolysis parameters were determined using HPLC assays. Drug concentrations of 1 μM were employed. Drug samples were incubated in blood, plasma, or PBS (pH 7.4) either with or without physiologically relevant concentrations of HSA or RBC. Plasma samples were continuously aerated with "blood gas" (MEDIBLEND) in order to maintain constant pH (7.5 ± 0.1). All experiments were conducted at 37 °C.

In addition to the homosilatecans displaying markedly higher lipophilicities relative to the conventional camptothecins (i.e. camptothecin, topotecan), they exhibit improved aqueous stabilities as was observed previously for homocamptothecin. Table 2 summarizes the stabilities of 1 μM solutions of DB-38, DB-81, DB-90, and DB-91 in solutions of phosphate-buffered saline (PBS) at a pH value of 7.4. Whereas clinically relevant camptothecins typically show approximately 10–15% lactone remaining at equilibrium following 3 h of incubation in PBS, greater than 80% lactone remains for each of the homosilatecans under identical incubation conditions.

The most distinguishing stability considerations for our new homosilatecan agents are observed when they are incubated in whole human blood. Figure 2 depicts the improved human blood stabilities of our four novel homosilatecans, and the stability parameters are summarized in Table 2. In all cases, the DB-38, DB-81, DB-90, and DB-91 structures display markedly enhanced human blood stabilities relative to camptothecin analogues such as topotecan and SN-38; the human blood stability values noted for DB-81, DB-90, and DB-91 are the highest yet to be measured for an intrinsically potent camptothecin analogue. The greater than 80% lactone values following 3 h of incubation compare very favorably to the corresponding percent lactone levels in whole human blood for 9-aminocamptothecin (approximately 1%), camptothecin (approximately 7%), topotecan (approximately 12%), CPT-11 (approximately 21.0%), and SN-38 (approximately 20%).

Insight into the superior human blood stabilities of DB-81, DB-90, and DB-91 relative to DB-38 can be...
The increased lipophilicities of these agents correlate significantly enhanced lipophilicities relative to DB-38. Tables 1 and 2. DB-81, DB-90, and DB-91 all display gained through comparison of the data contained in blood stabilities for camptothecin and 9-aminocamptothecin. Table 3. Comparison of the Marked Interspecies Variations in Blood Stabilities for Camptothecin and 9-Aminocamptothecin Versus the Relatively Minor Differences Observed for Novel, Highly Lipophilic Camptothecin Analogues

| compound            | % lactone after 3 h of incubation |
|----------------------|----------------------------------|
|                      | in mouse blood | in human blood | ratio of lactone level mouse/human |
| 9-aminocamptothecin  | 38             | 1              | 38                          |
| camptothecin         | 20             | 7              | 3                           |
| DB-38                | 72             | 56             | 1.3                         |
| DB-81                | 80             | 87             | 0.9                         |
| DB-90                | 61             | 85             | 0.7                         |
| DB-91                | 70             | 85             | 0.8                         |

* Experiments were conducted at pH 7.4 and 37 °C and lactone levels determined using HPLC methods. Blood samples were drawn and kept at 5 °C prior to the initiation of an experiment.

In summary, the novel homosilatecans described here are potent topoisomerase I inhibitors that are stable not only in mouse blood but human blood as well. Three of the first four homosilatecans to be synthesized are the most blood-stable camptothecins yet to be identified that display intrinsic potency against the topoisomerase I target. Given the demonstrated scope and generality of the cascade radical annulation approach,19,20,23 the synthesis of homosilatecans described in Scheme 1 will prove useful for the future generation of a broad assortment of blood-stable and intrinsically potent homosilatecans.

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