JOC Notes

Effect of E-Ring Modifications in Camptothecin on Topoisomerase I Inhibition: A Quantum Mechanics Treatment

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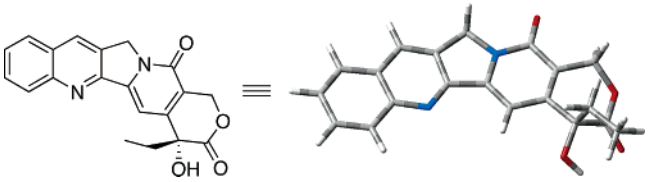
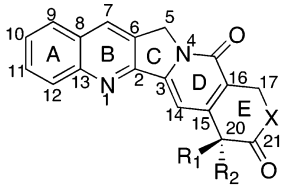


Camptothecins (CPTs) are the prototypical class of topoisomerase I (Top1) inhibitors with significant anticancer activities. Structure−activity relationship studies have demonstrated that inverting the stereochemistry at C-20 (*R*-CPT) or changing the E-ring lactone to a lactam (CPT-lactam) abolishes the Top1 inhibitory activity. The explanations that have been advanced for these effects are that there is either a failure of hydrogen bond formation involving the C-20 hydroxyl group of *R*-CPT or a failure of E-ring opening of the lactam, which have been proposed to be required for Top1 inhibition. We demonstrate here that the preferred conformation for the CPTs has the 20-Et pseudoaxial, while the 20-OH is pseudoequatorial, and therefore, the 20-OH groups in all the three CPT analogues (*S*-CPT, *R*-CPT, and CPT-lactam) are able to hydrogen bond with Asp533. The loss of the Top1 inhibitory activity by the latter two CPT analogues is attributed to the decreased $\pi-\pi$ stacking interaction energy with the neighboring base pairs compared to the natural *S*-CPT. The differences in $\pi-\pi$ stacking interaction energies are derived from the differential electrostatics on the E-ring.

Camptothecin (CPT, *S*-CPT) is a naturally occurring cytotoxic alkaloid isolated from *Camptotheca acuminata*.[1] The cytotoxicity observed with CPT is derived from cancer cell apoptosis resulting from the collision of the advancing replication fork with the CPT-trapped DNA-topoisomerase I (Top1) cleavage complex.[2,3] Trapping the metastable DNA-Top1 cleavage complex by CPTs is achieved by their intercalation between the DNA base pairs at the cleavage site, resulting in increased physical distance between the cleaved DNA termini, leading to inhibition of the DNA religation reaction catalyzed by Top1.[4,5]



***S*-CPT** R_1 = OH, R_2 = Et, X = O
***R*-CPT** R_1 = Et, R_2 = OH, X = O
CPT-lactam R_1 = OH, R_2 = Et, X = NH

Structure−activity relationship studies have demonstrated that the enantiomer of natural CPT, *R*-CPT, and camptothecin-lactam (CPT-lactam), are inactive as inhibitors of the DNA religation reaction and consequently do not poison Top1.[6−8] According to the X-ray crystal structure of the topotecan-stabilized DNA-Top1 cleavage complex,[4] the 20(*S*) hydroxyl group of *S*-CPT hydrogen bonds to Asp533 of Top1. It has been argued that the 20(*R*) hydroxyl group in *R*-CPT cannot interact with Asp533, which has been claimed to lead to the loss of Top1 inhibitory activity of *R*-CPT.[4] However, careful examination of all of the X-ray crystal structures of ternary complexes containing CPT or its derivatives reveals that the ethyl group in the six-membered lactone ring always adopts the pseudoaxial orientation, although it is sterically much larger than the hydroxyl group.[4,5,9] This is consistent with an earlier conformational analysis of a model of *S*-CPT, indicating that the stable conformer of *S*-CPT is the one with the ethyl group in the pseudoaxial conformation.[10] If the same conformational preference were true for bound *R*-CPT, one would expect that the pseudoequatorial 20(*R*) hydroxyl group could still interact with Asp533. To further investigate this point, the energies of two conformers of *R*-CPT with the ethyl group either pseudoaxial or pseudoequatorial were cal-

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(1) Wall, M. E.; Wani, M. C.; Cook, C. E.; Palmer, K. H.; McPhail, A. T.; Sim, G. A. *J. Am. Chem. Soc.* **1966**, *88*, 3888−3890.
(2) Li, T. K.; Liu, L. F. *Annu. Rev. Pharmacol. Toxicol.* **2001**, *41*, 53−77.
(3) Pommier, Y.; Redon, C.; Rao, V. A.; Seiler, J. A.; Sordet, O.; Takemura, H.; Antony, S.; Meng, L. H.; Liao, Z. Y.; Kohlhagen, G.; Zhang, H. L.; Kohn, K. W. *Mutat. Res.* **2003**, *532*, 173−203.
(4) Staker, B. L.; Hjerrild, K.; Feese, M. D.; Behnke, C. A.; Burgin, A. B., Jr.; Stewart, L. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15387−15392.
(5) Staker, B. L.; Feese, M. D.; Cushman, M.; Pommier, Y.; Zembower, D.; Stewart, L.; Burgin, A. B. *J. Med. Chem.* **2005**, *48*, 2336−2345.
(6) Jaxel, C.; Kohn, K. W.; Wani, M. C.; Wall, M. E.; Pommier, Y. *Cancer Res.* **1989**, *49*, 1465−1469.
(7) Hsiang, Y. H.; Liu, L. F.; Wall, M. E.; Wani, M. C.; Nicholas, A. W.; Manikumar, G.; Kirschenbaum, S.; Silber, R.; Potmesil, M. *Cancer Res.* **1989**, *49*, 4385−4389.
(8) Hertzberg, R. P.; Caranfa, M. J.; Holden, K. G.; Jakas, D. R.; Gallagher, G.; Mattern, M. R.; Mong, S. M.; Bartus, J. O.; Johnson, R. K.; Kingsbury, W. D. *J. Med. Chem.* **1989**, *32*, 715−720.
(9) Chrencik, J. E.; Staker, B. L.; Burgin, A. B.; Pourquier, P.; Pommier, Y.; Stewart, L.; Redinbo, M. R. *J. Mol. Biol.* **2004**, *339*, 773−784.
(10) Aiyama, R.; Satake, K.; Sawada, S. *Yakuruto Kenkyusho Kenkyu Hokokushu* **1988**, 13−17.

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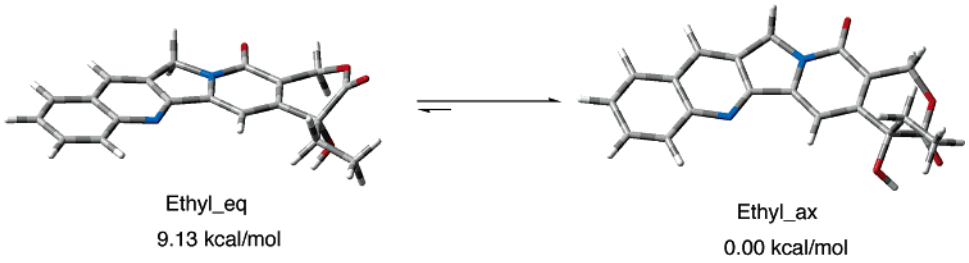

FIGURE 1. Two different conformations of *R*-CPT and their relative energies. Ethyl_eq represents the conformation where the ethyl group is pseudoequatorial, while Ethyl_ax denotes that the ethyl group is pseudoaxial.

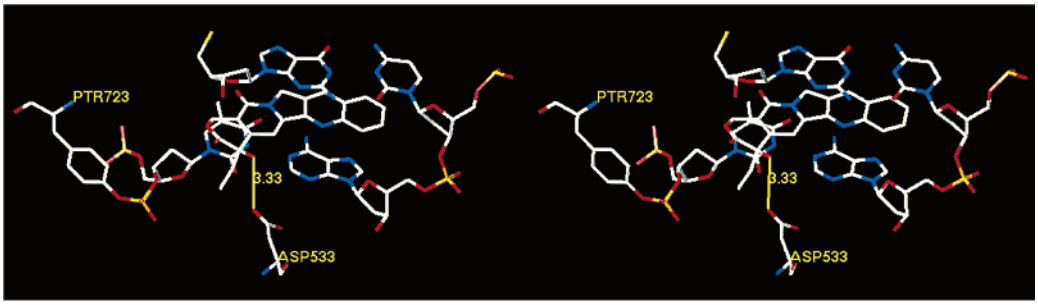

FIGURE 2. Hypothetical model of the binding of *R*-CPT in the ternary complex consisting of DNA, topoisomerase I, and *R*-CPT. The distance between the 20(*R*) hydroxyl group and the side chain of Asp533 is labeled. The diagram is programmed for wall-eyed viewing.

culated at the HF/6-31G** level of theory in Gaussian03 (Figure 1).[11] It is evident that the Ethyl_ax conformer is 9.13 kcal/mol more stable than the Ethyl_eq one. The reasons for the preferred pseudoaxial disposition of the ethyl group are 2-fold. First, the A strain between the ethyl group and the lactone carbonyl destabilizes the Ethyl_eq conformer.[12,13] Second, the hydrogen-bonding interaction between the hydroxyl and carbonyl observed in Ethyl_ax stabilizes this conformer. With this conformational preference in mind, the 20(*R*) hydroxyl group in *R*-CPT can still hydrogen bond with Asp533 (Figure 2). Since the stereochemistry in CPT-lactam is the same as that in *S*-CPT, the hydrogen-bonding interaction between the hydroxyl and Asp533 will therefore not be disrupted. One of the proposals for the loss of activity of CPT-lactam is that E-ring opening is required for Top1 inhibition.[4,6,14,15] But this is not the case for homocamptothecin and other nonlactone CPT analogues, whose rate for ring opening is much slower than CPT while retaining potent Top1 inhibitory activity.[16,17] Neither lack of hydrogen bonding nor failure to ring open provide convincing reasons for the inactivity of *R*-CPT and CPT-lactam, respectively.

Since the $\pi-\pi$ stacking interaction between CPT and the neighboring base pairs is the major contributor to the intercalation of CPT into the DNA-Top1 cleavage complex,[18] we hypothesized that the differences in $\pi-\pi$ stacking between different CPT analogues and their flanking base pairs determine the biological outcomes of different CPT analogues. To this end, the interaction energies of *S*-CPT, *R*-CPT, and CPT-lactam with the preferred −1 T, +1 G base pair step were computed as done previously (Table 1).[18]

From the interaction energies presented in Table 1, the large negative interaction energy (−6.69 kcal/mol) associated with *S*-CPT correlates with its potent Top1 inhibitory potency.[6] The interaction energy significantly decreased to −2.84 kcal/mol for *R*-CPT by simply inverting the stereochemistry at C-20, consistent with its reduced Top1 inhibition.[6] Change of the E-ring lactone to a lactam actually results in unfavorable binding as evidenced by the positive interaction energy, correlating

(11) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J., J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, Revision B.05 ed.; Gaussian, Inc: Pittsburgh, PA, 2003.
(12) Johnson, F.; Malhotra, S. K. *J. Am. Chem. Soc.* **1965**, *87*, 5492−5493.
(13) Johnson, F. *Chem. Rev.* **1968**, *68*, 375−413.
(14) Crow, R. T.; Crothers, D. M. *J. Med. Chem.* **1992**, *35*, 4160−4164.
(15) Hertzberg, R. P.; Caranfa, M. J.; Hecht, S. M. *Biochemistry* **1989**, *28*, 4629−4638.
(16) Laco, G. S.; Du, W.; Kohlhagen, G.; Sayer, J. M.; Jerina, D. M.; Burke, T. G.; Curran, D. P.; Pommier, Y. *Bioorg. Med. Chem.* **2004**, *12*, 5225−5235.
(17) Hautefaye, P.; Cimetière, B.; Pierré, A.; Léonce, S.; Hickman, J.; Laine, W.; Bailly, C.; Lavielle, G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2731−2735.
(18) Xiao, X.; Cushman, M. *J. Am. Chem. Soc.* **2005**, *127*, 9960−9961.

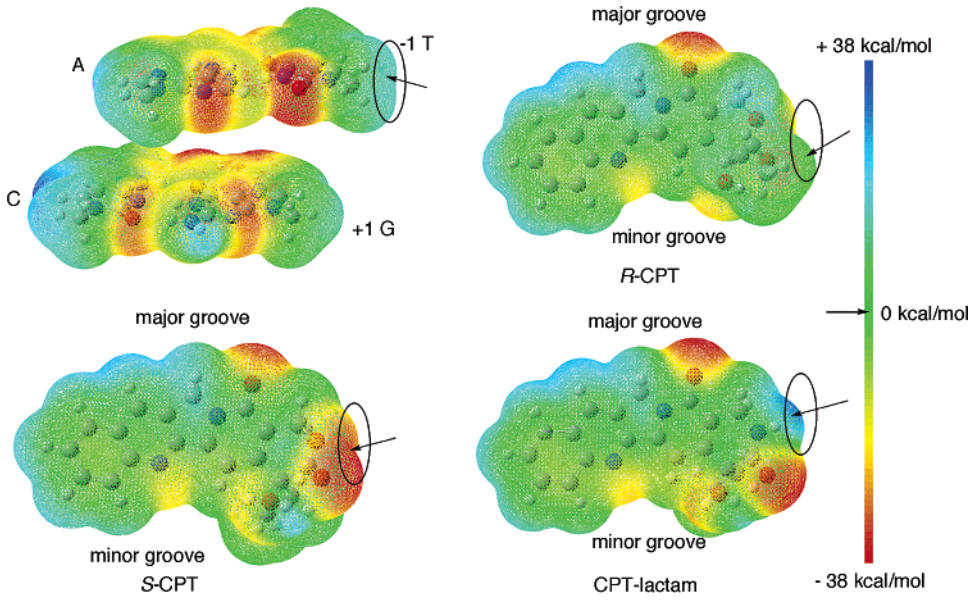

FIGURE 3. Electrostatic potential surface maps of TG base pair step, *S*-CPT, *R*-CPT, and CPT-lactam mapped on the total electron density calculated at the HF/6-31G** level of theory. The TG step and the individual camptothecins are oriented in a way that is similar to their orientations at the cleavage site. Thus, the scissile DNA strand is to the right of all the structures. The minor groove is below and the major groove is above each individual camptothecin. For the TG base pair step, the minor groove is pointing out of the page and the major groove is pointing behind the page. The interaction areas between the E-ring of CPTs and base pair step are highlighted with both circles and arrows. All of the electrostatic surfaces are scaled to a range of −38 to +38 kcal/mol.

TABLE 1. Interaction Energies (MP2/6-31G*) of *S*-CPT, *R*-CPT, and CPT-lactam with the −1 T, +1 G Base Pair Step

compd	E_{int}(MP2) (kcal/mol)	% Top1 inhibition[a]
S-CPT	−6.69	41
R-CPT	−2.84	9
CPT-lactam	+2.90	2

[a] Data from ref 6.

with its barely detectable Top1 inhibitory activity.[6] During the present calculations, the solvation and entropic effects were not considered by assuming they are similar and not important for comparison purposes since the structures of all three compounds are very similar to each other.[18,19] Therefore, loss of Top1 inhibitory activity by *R*-CPT and CPT-lactam is due to the differences in stacking interaction energies.

To trace the origin of the striking differences in the stacking interaction energies due to the subtle structural changes, the electrostatic potential surfaces of a TG base pair step and all the three CPT compounds were generated because it has been shown that electrostatic interaction plays an important role in the stacking interaction of a number of different intercalators.[19,20] Since the only differences among these three CPTs are in the E-ring, only the interaction areas involving the E-ring were focused on (Figure 3). Specifically, the E-ring mainly stacks with the edge of −1 T due to the helical nature of DNA (Figure 3).[4,5] The edge of −1 T (circled) is electrostatically moderately positively charged (+15−20 kcal/mol) (Figure 3). The corresponding area in *S*-CPT is negatively charged (−25−30 kcal/mol), indicating electrostatic attraction with −1 T. Consistent with the reduced calculated interaction energy of *R*-CPT, the corresponding area in *R*-CPT is neutral, resulting in little attraction. This reduction in the interaction energy may also come from the differences in the surface areas of CPTs stacked by base pairs. As can be seen in Figure 2, half of the E-ring in *R*-CPT is not covered by −1 T, which is in strong contrast with *S*-CPT.[4,5] As a matter of fact, there is a 9 Å^2 difference in the solvent-accessible surface areas between the two CPTs in the intercalation complex. On the other hand, the lactam area is highly positively charged (+30−35 kcal/mol), which will definitely encounter electrostatic repulsion with −1 T. This is why the interaction energy between CPT-lactam and TG base pair step has a positive value.

Although this analysis does not consider other interaction areas in the molecules, it is by no means underestimating the importance of other areas. It is known, for example, that the A-ring-truncated CPT analogues do not show any Top1 inhibition.[21] The analysis presented here assumes that all CPTs are binding in the same orientation as *S*-CPT as revealed by X-ray crystallography[4,5] and that suboptimal interactions were observed in *R*-CPT and CPT-lactam. On the other hand, if the CPT analogues assumed other binding orientations, the interaction energy would be decreased as well.[18]

In conclusion, the loss of Top1 inhibitory activity of *R*-CPT and CPT-lactam is related to their differences in $\pi-\pi$ stacking interaction energies with the neighboring base pairs. The hydrogen-bonding interaction between

(19) Xiao, X.; Antony, S.; Pommier, Y.; Cushman, M. *J. Med. Chem.* **2005**, *48*, 3231−3238.
(20) Medhi, C.; Mitchell, J. B. O.; Price, S. L.; Tabor, A. B. *Biopolymers* **1999**, *52*, 84−93.
(21) Wall, M. E.; Wani, M. C.; Nicholas, A. W.; Manikumar, G.; Tele, C.; Moore, L.; Truesdale, A.; Leitner, P.; Besterman, J. M. *J. Med. Chem.* **1993**, *36*, 2689−2700.

the C-20 hydroxyl group and Asp533 can still be present in all three CPTs when the preferred conformations of the CPTs are considered and, therefore, is not the reason for the loss of Top1 inhibitory activity by *R*-CPT and CPT-lactam.

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Supporting Information Available: A list of Cartesian coordinates and their corresponding electronic energies. This material is available free of charge via the Internet at http://pubs.acs.org.

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