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Analysis of Stereoelectronic Properties of Camptothecin Analogues in Relation to Biological Activity

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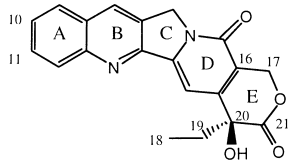
Abstract—Camptothecin and four of its 10,11-methylenedioxy analogues were examined for their activity against the pathogenic protozoan *Leishmania donovani* in vitro. The methylenedioxy analogues were 36- to 180-fold more potent than the parent camptothecin, possessing IC_{50} values ranging from 160 to 32 nM against the parasite. Our finding that the methylenedioxy camptothecins possess greater activity than camptothecin, which is also the case for other cell types and for the generation of cleavable complex in the presence of DNA and purified mammalian topoisomerase I, prompted us to examine the molecular features of camptothecin and methylenedioxy camptothecin analogues. A delocalization of positive potential was observed in the methylenedioxy camptothecin analogues, which could increase the affinity of these molecules for DNA. In addition, geometrical and electronic differences between the E ring of camptothecin and its methylenedioxy analogues were noted. One or both of these factors may contribute to the superior biological activity of the methylenedioxy camptothecin analogues. Published by Elsevier Science Ltd.

Introduction

Camptothecin (**1**), a pentacyclic natural product from *Camptotheca acuminata*, is a powerful antitumor alkaloid.[1] The cytotoxicity of camptothecin is most likely due to its ability to stabilize cleavage complexes between DNA and topoisomerase I, an enzyme that changes the linking number of DNA in increments of one.[2] Camptothecin and its analogues must possess an intact lactone ring in order to maintain biological activity.[3,4] At physiological pH, the carboxylate form predominates. A recent study showed that the active lactone form of camptothecin and two of its analogues is stabilized through interactions with double stranded DNA.[5] Such an interaction could have a direct influence on the biological activity of camptothecin and its analogues by increasing the cellular concentration of active drug, by directing the molecule to its eventual target, or both.

Over the past decades, a substantial effort has been focused on the synthesis of camptothecin analogues and the evaluation of their anticancer properties.[6] Camptothecin and its analogues also have significant activity against *Trypanosoma brucei*, the causative parasite of African trypanosomiasis,[7] and camptothecin possesses activity against the malaria parasite as well.[8] Leishmaniasis, a spectrum of disease caused by the *Leishmania* parasite, is treated by antimony-based drugs.[9] Antimonials reportedly inhibit a partially purified leishmanial topoisomerase I[10] and produce protein-DNA cleavage complexes in antimony-treated *Leishmania*.[11] These data indicate that topoisomerase I inhibitors merit investigation as antileishmanial agents and prompted us to study the activity of a set of camptothecin analogues against *Leishmania donovani*, the causative agent of Old World visceral leishmaniasis. Our results in turn led us to examine stereoelectronic features of the camptothecin analogues in an attempt to explain the order of potency of these molecules.



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Results and Discussion

Antiparasitic activity

We synthesized four camptothecin analogues that incorporated a 10,11-methylenedioxy substituent for

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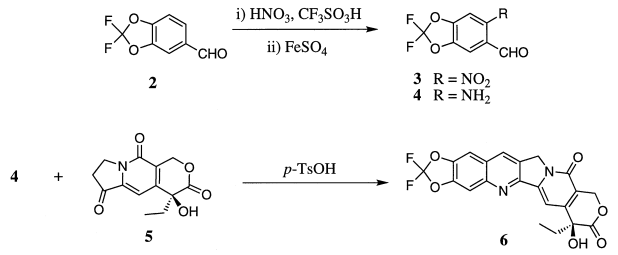
antileishmanial testing because methylenedioxy-containing camptothecins possessed potent activity against the related *T. brucei* parasite.[7] One of these analogues, compound **6**, has not been previously reported. The synthesis of this difluoromethylenedioxy camptothecin is shown in Scheme 1. The key step in the reaction sequence involved Friedlander condensation of amino aldehyde **4** with the previously reported chiral tricyclic intermediate **5** as described in the preparation of several other camptothecin analogues.[3,12,13]

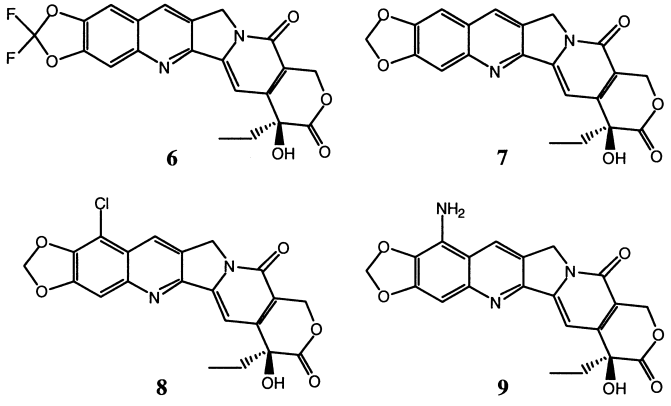


Scheme 1.

These 10,11-methylenedioxy camptothecin analogues, along with camptothecin, were evaluated for activity against *Leishmania donovani* axenic amastigotes (Table 1). To provide a basis for comparison, we have also included data previously reported by Bodley et al. for compounds **1** and **7**–**9** against trypanosomes.[7] The antileishmanial activities of two standard antileishmanial drugs employed in clinical practice were also determined. Camptothecin is nearly 60-fold more active against *L. donovani* than the first-line drug, Pentostam, but is less active than the second line antileishmanial drug amphotericin B. The antiparasitic effect of camptothecin against *L. donovani* was comparable to the effect reported against *Trypanosoma brucei*.

Derivatives of camptothecin (compounds **6**–**9**) compare quite favorably with amphotericin B. Incorporation of a methylenedioxy moiety into the pentacyclic ring system to give 10,11-methylenedioxycamptothecin (**7**) substantially enhances antileishmanial activity: compound **7** is 90-fold more active than the parent compound. A similar effect was noted in the structure–activity relationship for antitrypanosomal activity where this transformation produces a 10-fold enhancement of activity. The antileishmanial activity of 9-chloro-10,11-methylenedioxycamptothecin (**8**) and 9-amino-10,11-methylenedioxycamptothecin (**9**) were essentially identical, having IC_{50} values which were 180-fold less than camptothecin. 10,11-Difluoromethylenedioxycamptothecin (**6**) was less active than the corresponding methylenedioxy analogue (**7**): compound **6** was nonetheless 36-fold more active than camptothecin itself. This compound was synthesized on the basis of molecular modeling calculations which predicted that electronic effects should enhance the activity of this structure over parent **7**. It is difficult to determine whether to ascribe this lack of correlation to a shortcoming in the proposed relationship between electronic structure and antiparasitic efficacy or whether the compounds achieved different concentrations within the parasite. The ability of a compound to cross biological membranes is frequently related to its octanol/water partition coefficient, with biological activity in a series associated with an optimal value of this parameter. In this regard, the octanol/water partition coefficients (log P) for the analogues were calculated using the Villar's scale as in SPARTAN (Table 1). Considering the large difference between the calculated log P values for compounds **8** and **9**, it seems unlikely that there is a simple relationship between the partition coefficient and antileishmanial activity for the camptothecin analogues.

Table 1. Antileishmanial activity of camptothecin analogues against *Leishmania donovani* axenic amastigotes and log P values of these compounds



Compound	EC_{50} (μM) versus *L. donovani*[a]	EC_{50} (μM) versus *T. brucei*[b]	Log P
Pentostam	330±8	—	—
Amphotericin B	0.45±0.03	—	—
1 (camptothecin)	5.8±1.9	1.6	1.48
6	0.16±0.050	—	1.49
7	0.064±0.014	0.16	0.64
8	0.033±0.001	0.041	1.10
9	0.032±0.002	0.074	−0.32

[a]EC_{50} values are presented as the mean ± standard deviation of two independent experiments.
[b]EC_{50} values are taken from Bodley et al.[7]

Steric attributes

Molecular level information obtained from semi-empirical (AM1) quantum chemical calculations on the electronic structure of camptothecin and its analogues were investigated to explain the differential activity the compounds. The stereoelectronic parameters are calculated on the minimum energy and the most abundant conformer by further optimization of the geometry of the compounds. The optimized geometry of the compounds indicates that rings A, B, C, and D are planar and superimposable (data not shown). However, the structural parameters of ring E, particularly the dihedral angles involving this ring, differ significantly between camptothecin and its methylenedioxy analogues. In particular, the three dihedral angles defined by $O_{18}C_{19}C_{20}O_{OH}$, $O_{CO}C_{19}C_{20}O_{OH}$, and $O_{18}C_{19}C_{20}C_{C2H5}$ show differences between compounds **1** and **6**–**9** (Table 2). It thus appears that the position of the oxygen atoms of ring E in camptothecin is significantly different from the methylenedioxy compounds. These predictions concerning the geometry of camptothecin are in accord with those made previously by Fan et al.[14]

Electronic attributes

The carbonyl oxygen atom of ring E is the site for the most negative potential in all the compounds, which

Table 2. Selected AM1 geometric parameters of the 20(*S*)-camptothecin analogues

Compound	1	6	7	8	9
Torsion angles					
$C_{16}C_{17}O_{18}C_{19}$	32.2	33.8	33.7	33.8	35.5
$C_{17}O_{18}C_{19}O_{CO}$	−175.6	177.3	177.3	177.3	175.5
$C_{17}O_{18}C_{19}C_{20}$	3.1	−2.9	−2.8	−2.9	−4.9
$O_{18}C_{19}C_{20}O_{OH}$	−158.7	−147.6	−147.6	−147.6	−147.1
$O_{CO}C_{19}C_{20}O_{OH}$	19.8	32.1	32.1	32.1	32.3
$O_{18}C_{19}C_{20}C_{C2H5}$	84.8	92.2	92.2	92.2	92.1
Bond angles					
$C_{16}C_{17}O_{18}$	113.0	113.4	113.4	113.4	114.5
$O_{18}C_{19}C_{20}$	121.4	122.1	122.1	122.1	123.2
$O_{18}C_{19}O_{CO}$	113.6	113.9	113.9	113.9	113.4
$C_{19}C_{20}O_{OH}$	110.2	109.0	109.0	109.0	109.7
$C_{19}C_{20}C_{ringD}$	108.4	109.9	109.9	109.9	109.8
$O_{OH}C_{20}C_{C2H5}$	106.6	110.6	110.6	110.6	111.3
Bond distance					
$C_{19}O_{CO}$	1.23	1.23	1.23	1.23	1.24
$C_{20}O_{OH}$	1.42	1.42	1.42	1.42	1.42
$H_{OH}\cdots O_{CO}$	2.49	2.38	2.38	2.38	2.39

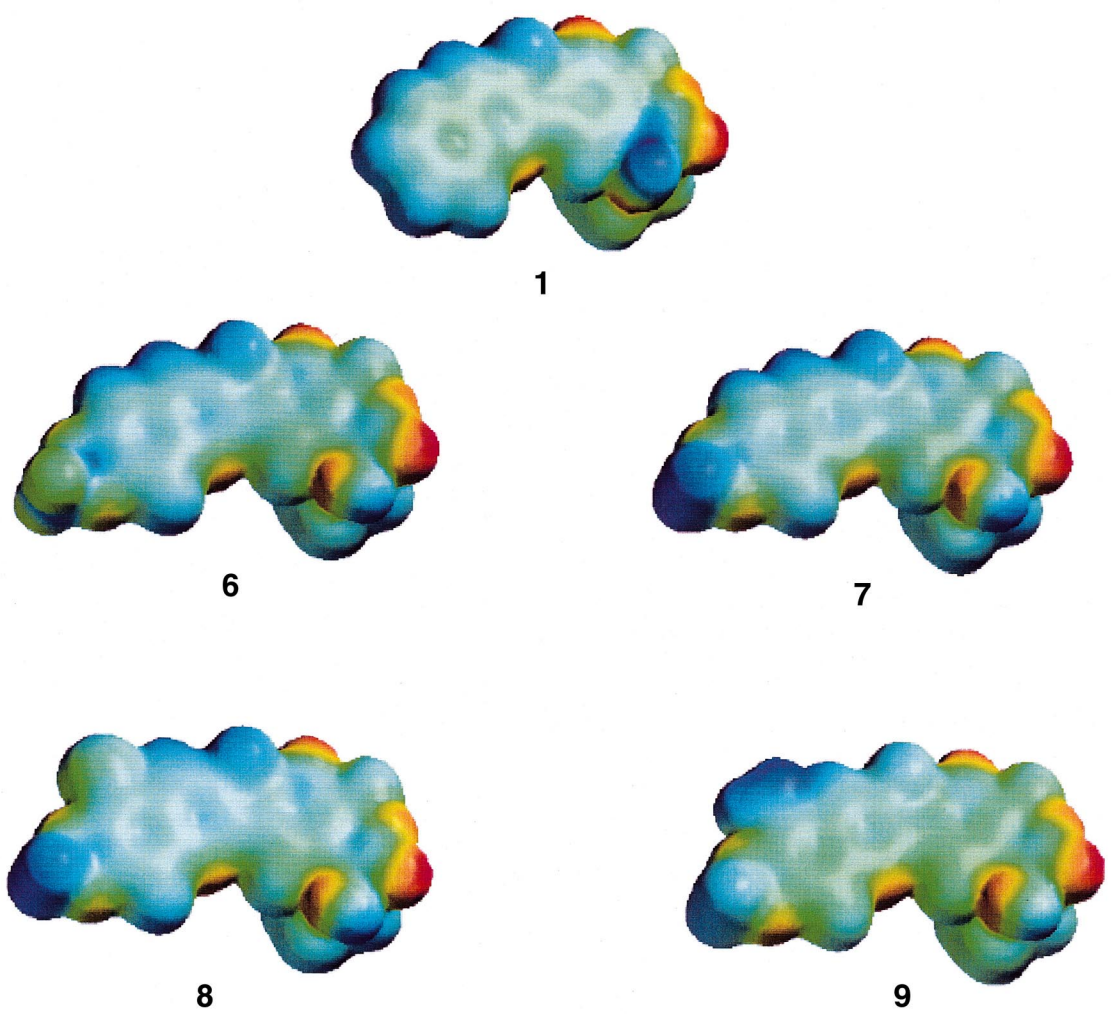


Figure 1. Molecular electrostatic potentials (MEPs) plotted onto a surface of constant electron density (0.002e/au^3) showing the most positive potential (deepest blue color), the most negative potential (deepest red color) and the intermediate potential regions (intermediate shades) of the compounds.

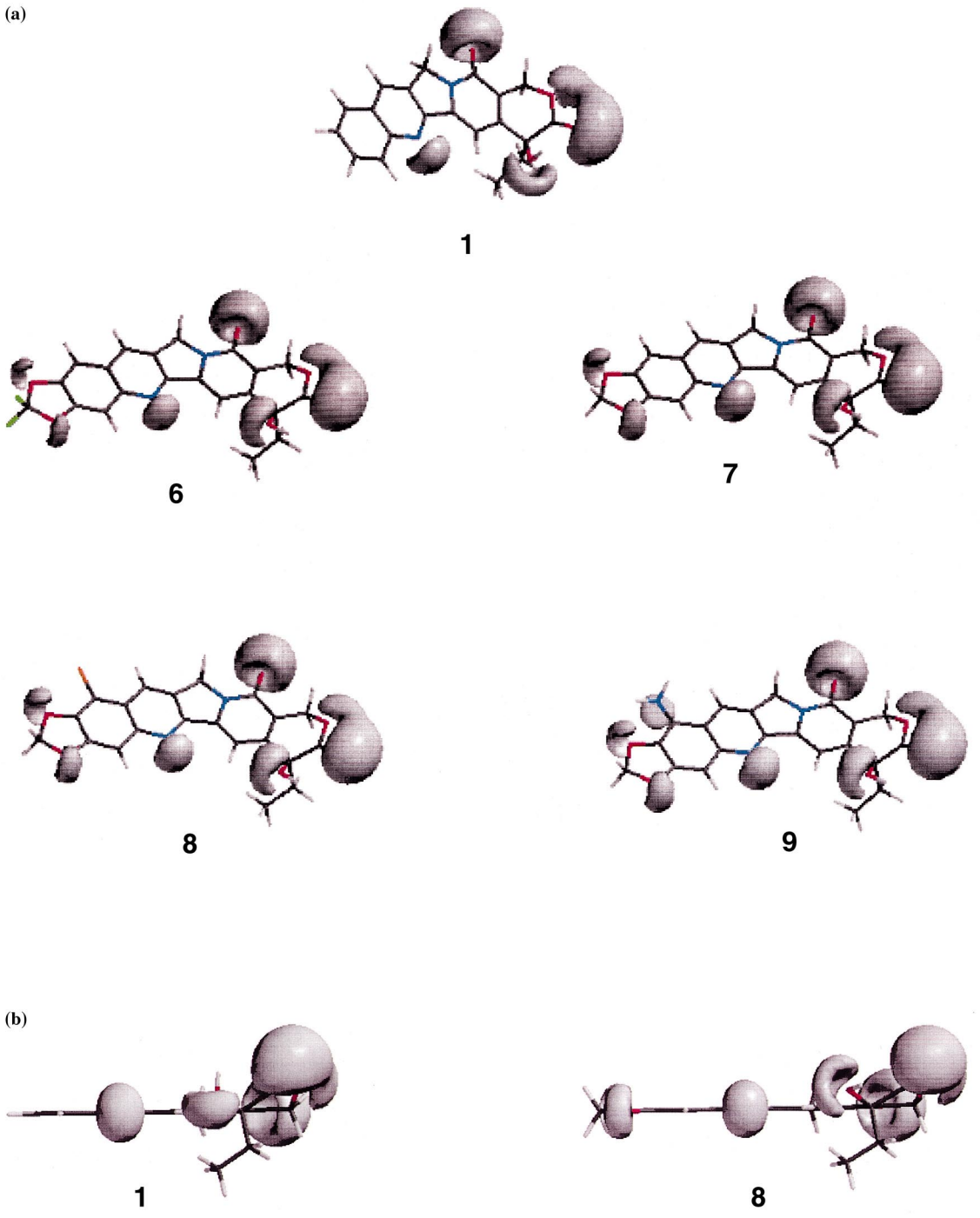


Figure 2. Three-dimensional isopotential surface of the molecules. The solid contours represent a constant potential of −30.0 kcal/mol showing the position and orientation of the lone pair electrons at approximately 1.4 Å away from the van der Waals surface of the molecule. Figure 2A depicts a view from above each of the five molecules, while Figure 2B observes compounds **1** and **8** in the ring plane, approximately 90° from the view in Figure 2A. The methylenedioxy group is at the far left for compound **8** in Figure 2B, and the observer is looking directly at C20 (with its attached hydroxyl and ethyl groups) on the right side of the molecule.

indicates that this atom is intrinsically the most nucleophilic site in the molecule. However, the site for positive potential is scattered among the different hydrogen atoms in the compounds except in **1**, where it is found to be more localized by the hydroxyl proton of ring E (Fig. 1). Unlike camptothecin, the intrinsic electrophilicity of 10,11-methylenedioxy analogues does not seem to have directional characteristics. The large delocalization of positive potential regions as evident from the scattered positive potentials of the molecules (indicated by the deep blue colors in Fig. 1) implies larger electron deficiency in molecules **6**–**9**. Camptothecin and its analogues interact with DNA directly and this interaction stabilizes the active lactone form of these molecules.[5] A model was proposed recently for the interaction between camptothecin, DNA, and topoisomerase I, where the lowest energy ternary complex placed camptothecin in a 'pseudo-intercalation' mode with DNA.[14] The large

Table 3. Selected values of AM1-derived electronic properties of the 20(*S*)-camptothecin analogues

Property	1	6	7	8	9
Dipole Moment (Debye)	7.95	4.52	7.10	6.54	7.89
Orbital Energy (eV)					
HOMO	−9.116	−9.230	−8.990	−9.073	−8.590
LUMO	−1.429	−1.702	−1.439	−1.552	−1.370
Gap	7.68	7.53	7.55	7.52	7.22
MEP Values (kcal/mol)					
Most negative	−69.6	−66.5	−69.5	−69.0	−69.9
Most positive	36.7	35.3	32.0	32.6	47.5
Proton Affinity[a] (kcal/mol)	180.6	179	181.5	180.7	181.9
Aqueous Stabilization Energy (kcal/mol)	24.6	23.2	28.9	27.6	38.1

[a]Proton affinity was calculated by protonating the C20-hydroxyl oxygen atom.

delocalization of positive potential in the methylenedioxy camptothecins may have a contributing effect toward association of these compounds with DNA, as it is well known that positively charged aromatic systems such as ethidium bromide and propidium iodide have a high affinity for DNA. Although these effects in the methylenedioxy camptothecin analogues would be much weaker than those described for ethidium and propidium, the large distribution of positive potential in molecules **6**–**9** is likely to lead to 'recognition effects' with the amino sites in DNA. Such recognition effects would draw the ligand closer to its target,[15] in this case DNA, for further specific interactions. Although no quantitative correlation of positive potential with antileishmanial activity could be established, the delocalization of positive potential regions in the methylenedioxy camptothecin analogues may be a contributing factor to their increased activity by enhancing their affinity for DNA.

The three-dimensional electrostatic potential profiles of the compounds beyond the van der Waals surface (approximately 1.4 Å away) have also been determined (Fig. 2). A view from above the camptothecin ring plane is given for each of the compounds in Figure 2A, which clearly shows the electrostatic potential profiles for all of the sites in the molecules. A common large negative potential region extends from the hydroxyl oxygen atom to the ether oxygen atom of ring E for all the compounds. Despite the similarity of the negative potential profiles, a careful examination of these maps beyond the van der Waals surface (approximately 1.4 Å away) indicates that the lone pair electrons of the hydroxyl oxygen atom in camptothecin are close to the aromatic ring plane. Figure 2B views camptothecin and compound **8**, a representative of the methylenedioxy class, in the plane of the ring system. This view clearly demonstrates the different orientations of the hydroxyl oxygen lone pairs in the E ring between camptothecin and its methylenedioxy analogues. The lone pair electrons of the hydroxyl oxygen atom in ring E of camptothecin may thus be delocalized in the pentacyclic ring. In the methylenedioxy analogues, on the other hand, this lone pair is located approximately perpendicular to the ring plane. The outward localized orientation of lone pair electrons in the methylenedioxy analogues may enable them to be better hydrogen bond donors than camptothecin. Although positive potential regions may influence the interaction of the molecules with DNA, the negative potential region could play an important role in drug-topoisomerase I interactions. In the model advanced by Fan et al., the C20 hydroxyl and C21 carbonyl present in the E ring of camptothecin were implicated in hydrogen bonding with Asn722 near the active site of topoisomerase I.[14] If this model is correct, the orientation of these residues would play a critical role in the stabilization of the camptothecin/DNA/topoisomerase I ternary complex. The localized orientation of the hydroxyl oxygen lone pair electrons in the methylenedioxy camptothecin analogues could result in more efficient hydrogen bonding with topoisomerase I and could also contribute to the increased activity of these analogues.

The HOMO and LUMO orbital energies (Table 3) of the molecules are consistently negative and the energy gap is more or less similar indicating little variation in the nature of reactivity of the compounds. In order to assess the role of the C20-hydroxyl oxygen atom in the mechanism of action of the compounds we have also calculated the proton affinity of this atom for each of the analogues. The calculated proton affinities are similar, varying between 0 and 3.0 kcal/mol (Table 3). Intrinsic nucleophilicity of the C20-hydroxyl oxygen atom thus does not seem to have any significant effect on the biological activity of the agents, although the activity appears to be related to the orientation of the lone pair electrons of this oxygen atom.

Conclusion

Although the methylenedioxy-substituted camptothecins are highly effective antileishmanials in vitro, the concentrations required for activity are similar to those which inhibit the growth of murine L1210 leukemia cells[7] and produce cleavage complexes with purified mammalian topoisomerase I in vitro.[13] The pattern of toxicity of the compounds toward parasites is also similar to that against mammalian cells. Thus the camptothecin analogues are not ideal antileishmanial drug candidates. However, our investigation revealed two key differences between camptothecin and 10,11-methylenedioxy camptothecin derivatives: a large delocalization of positive potential regions in the methylenedioxy analogues compared to camptothecin and differences in the geometry of the C20 substituents. These findings are consistent with the model proposed by Fan et al. concerning the camptothecin/DNA/topoisomerase I ternary complex[14] and provide new insight

into the potent biological activity of the methylenedioxy camptothecin derivatives. These insights will hopefully provide direction for the synthesis of more potent camptothecin analogues.

Experimental

Chemistry

Camptothecin and amphotericin B were obtained from Sigma. Pentostam was a generous gift of Burroughs-Wellcome. Camptothecin analogues **7**,[12] **8**,[13] and **9**[13] have been reported previously. The corresponding compounds synthesized for this study possessed spectral data consistent with that reported in the literature. The synthesis of **6** and several of its intermediates have not been described previously and are given below.

3,4-(Difluoromethylenedioxy)-6-nitrobenzaldehyde (3). To a solution of trifluoromethanesulfonic acid (8 mL) in anhydrous CH_2Cl_2 (100 mL) was added 90% nitric acid (2.5 mL). A white solid separated from the solution. The suspension was cooled to −60 °C and 3,4-(difluouromethylenedioxy)benzaldehyde[16] (**2**, 1.7 g, 9.1 mmol) was added, dropwise. The mixture was stirred at −60 °C for 2 h, at 0 °C for 1.5 h, then stored at 4 °C overnight. The cool mixture was poured over ice (50 g) and extracted with CH_2Cl_2 (2×200 mL). The extracts were washed with water (2×200 mL), dried with $MgSO_4$, then concentrated in vacuo to an oil. The oil was purified by chromatography on a silica gel column using hexane:CH_2Cl_2:EtOAc (10:1:1) as the eluant. Fractions containing pure product were combined and concentrated to give 1.52 g (73%) of **3**, after crystallization from hexane/ether; mp 35–36 °C. ^{1}H NMR (DMSO-d_6) δ 7.91 (s, 1H), 8.37 (s, 1H), 10.13 (s, 1H); IR (KBr) 3127, 3074, 2920, 1702, 1622, 1537, 1489, 1425, 1343, 1273, 1253, 1216, 1179, 1129, 1069, 893, 856, 829, 802, 753, 710, 669, 603 cm^{-1}. Anal. $C_8H_3F_2NO_5$: C, H, N.

6-Amino-3,4-(difluoromethylenedioxy)benzaldehyde (4). To a solution of ferrous sulfate (8.0 g, 28.8 mmol) in boiling H_2O (40 mL) was added, dropwise, a solution of **3** (0.7 g, 3 mmol) in EtOAc (10 mL) then ammonium hydroxide (10 mL), in small portions until the solution remained alkaline. The mixture was refluxed for 5 min, cooled, diluted with EtOAc (50 mL), then filtered. The aqueous portion was washed with brine (2×10 mL), dried with $MgSO_4$, then concentrated to an oil. The oil was purified by silica gel chromatography using hexane:CH_2Cl_2:EtOAc (10:1:1) as the eluant to yield 200 mg (33.2%) of **4**, mp 80–81 °C. ^{1}H NMR (DMSO-d_6) δ 6.76 (s, 1H), 7.53 (s, 2H), 7.60 (s, 1H), 9.74 (s, 1H). Anal. $C_8H_5F_2NO_3$: C, H, N.

(*S*)-7-Ethyl-7-hydroxy-10*H*-2,2-difluoro-1,3-dioxolo(4,5-g)-pyrano(3′,4′,:6,7)indolizino(1,2-*b*)quinoline-8,11(7*H*,13*H*)-dione (6). A stirred mixture of **4** (200 mg, 1 mmol), (*S*)-4-ethyl-7,8-dihydro-4-hydroxy-1*H*-pyrano(3,4-*f*)indolizine-3,6,10-(4*H*)-trione[4] (**5**, 265 mg, 1 mmol), toluene (1.5 mL) and glacial acetic acid (0.5 mL) was heated until solution was obtained (~60 °C). *p*-Toluenesulfonic acid (50 mg) was added, and the mixture was heated under reflux for 17 h, cooled, concentrated, then diluted with ethanol. The precipitate was collected and recrystallized (EtOH); yield 120 mg, mp 269–270 °C. Additional product (40 mg) was obtained from the mother liquor by chromatography on silica gel using EtOAc:hexanes (10:1) as the eluant; total yield 160 mg (37.4%). ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H, $J_1 = 7.2$ Hz, $J_2 = 7.4$ Hz), 1.83 (m, 2H), 5.25 (s, 2H), 5.40 (s, 2H), 6.51 (s, 1H), 7.29 (s, 1H), 8.09 (s, 1H), 8.11 (s, 1H), 8.67 (s, 1H). IR (KBr) 3420, 3097, 3067, 2976, 2940, 2883, 1749, 1661, 1618, 1601, 1559, 1506, 1465, 1387, 1240, 1156, 1109, 1035, 1002, 928, 912, 859, 808, 708, 667, 583; $[\alpha]_D^{20}$ −17.5° (*c* 0.104, DMSO). Anal. $C_{21}H_{14}F_2N_2O_6$: C, H, N.

Parasites

Leishmania donovani parasites (WHO designation: MHOM/SD/62/1S-CL2$_D$) were a kind gift of Dr. Dennis Dwyer of the National Institutes of Health. These parasites were maintained as axenic amastigote-like forms by serial passage at 37 °C in a humid atmosphere containing 5% CO_2 using a slightly modified version of the medium described by Joshi et al.[17]

Antileishmanial drug susceptibility assay

Drugs other than Pentostam were dissolved in DMSO to give stock solutions (2.5–10 mM) which were stored in the dark at 4 °C prior to antileishmanial testing. Pentostam was supplied in sterile saline at a concentration of 100 mg Sb mL^{-1}, and further dilutions were made from this solution. The *L. donovani* axenic amastigote drug susceptibility assay was performed as described previously.[18]

Molecular modeling

A thorough conformational search using the Monte Carlo techniques as implemented in SPARTAN (*SPARTAN* version 4.1, Wavefunction, Inc., 18401 Von Karman Ave., #370, Irvine, CA 92715) was adopted to the structures of each of the five compounds to determine the minimum energy and the most abundant conformer. The geometry of this conformer was completely optimized using the AM1 semiempirical quantum chemical methods[19] and the stereoelectronic properties are calculated on the optimized geometry in the neutral form of each of the molecules as available in the SPARTAN package. The log P values were calculated on the optimized geometry of the molecules using the Villar scale as implemented in SPARTAN.

Molecular electrostatic potential (MEP) maps for all molecules were generated at −30.0 kcal/mol (approximately 1.4 Å from the van der Waals surface) of the molecules. The electrostatic potentials were sampled over the entire accessible surface of a molecule (corresponding roughly to a van der Waals contact surface) which provides a measure of charge distribution from the point of view of an approaching reagent. The regions of positive electrostatic potential indicate excess positive charge, (i.e., repulsion for the positively-charged test probe) while regions of negative potential indicate areas

of excess negative charge (i.e., attraction of the positively charged test probe). This encoding is done by the use of color; colors toward the deepest blue representing the most positive potential and colors toward the deepest red representing the most negative potential.

Acknowledgements

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References

1. Wall, M.; Wani, M.; Cook, C.; McPhail, A.; Sim, G. *J. Am. Chem. Soc.* **1966**, *88*, 3888.
2. Pommier, Y. *Biochimie* **1998**, *80*, 255.
3. Wani, M.; Nicholas, A.; Manikumar, G.; Wall, M. *J. Med. Chem.* **1987**, *30*, 1774.
4. Wani, M.; Nicholas, A.; Wall, M. *J. Org. Chem.* **1987**, *30*, 2317.
5. Yang, D.; Strode, J.; Spielmann, H.; Wang, A.; Burke, T. *J. Am. Chem. Soc.* **1998**, *120*, 2979.
6. *Camptothecins: New Anticancer Agents*; Potmesil, M., Pinedo, H., Eds.; CRC: Boca Raton, 1995.
7. Bodley, A.; Wani, M.; Wall, M.; Shapiro, T. *Biochem. Pharmacol.* **1995**, *50*, 937.
8. Bodley, A.; Cumming, J.; Shapiro, T. *Biochem. Pharmacol.* **1998**, *55*, 709.
9. Berman, J. D. *Clin. Infect. Dis.* **1997**, *24*, 684.
10. Chakraborty, A.; Majumder, H. *Biochem. Biophys. Res. Comm.* **1988**, *152*, 605.
11. Lucumi, A.; Robledo, S.; Gama, V.; Saravia, N. *Antimicrob. Agents Chemother.* **1998**, *42*, 1990.
12. Wani, M.; Nicholas, A.; Wall, M. *J. Med. Chem.* **1986**, *29*, 2358.
13. Wall, M.; Wani, M.; Nicholas, A.; Manikumar, G.; Tele, C.; Moore, L.; Truesdale, A.; Leitner, P.; Besterman, J. *J. Med. Chem.* **1993**, *36*, 2689.
14. Fan, Y.; Weinstein, J.; Kohn, K.; Shi, L.; Pommier, Y. *J. Med. Chem.* **1998**, *41*, 2216.
15. Murray, J.; Zilles, B.; Jayasuriya, K.; Politzer, P. *J. Am. Chem. Soc.* **1986**, *108*, 915.
16. Stogryn, E. *J. Org. Chem.* **1972**, *37*, 673.
17. Joshi, M.; Dwyer, D. M.; Nakhasi, H. L. *Mol. Biochem. Parasitol.* **1993**, *58*, 345.
18. Werbovetz, K.; Brendle, J.; Sackett, D. *Mol. Biochem. Parasitol.* **1999**, *98*, 53.
19. Dewar, M.; Zoebisch, E.; Horsely, E.; Stewart, J. *J. Am. Chem. Soc.* **1985**, *107*, 302.