Bioorganic & Medicinal Chemistry Letters 21 (2011) 2324–2326

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

The solvolysis of topotecan in alcohols and acetic anhydride

Jiajun Li [a], Guolin Wang [b], Mengjie Dong [b,*], Qian Zhang [a,*]

[a] Department of Medicinal Chemistry, School of Pharmacy, Fudan University, Shanghai 201203, China
[b] PET Center, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, China

ARTICLE INFO

Article history:
Received 7 October 2010
Revised 3 February 2011
Accepted 23 February 2011
Available online 26 February 2011

Keywords:
Topotecan
Derivatives of 10-hydroxycamptothecin
Anti-tumor activity
Solvolysis

ABSTRACT

Six derivatives of 10-hydroxycamptothecin were prepared via solvolysis of topotecan in corresponding alcohols and acetic anhydride. We attributed the specific reactivity of topotecan to the internal hydrogen-bonding between 10-hydroxy and the nitrogen atom in position 9. As a result the reaction underwent through an intermediate *ortho*-quionomethlide species to reach equilibrium. Bioactivity screening data showed all products could potentially inhibit the proliferation of several cancer cell lines in vitro and a bigger size group in 9-position would be favorable for the anti-tumor activities observably.

Camptothecin (CPT, **1**, Fig. 1), a pentacyclic alkaloid, was isolated by Wall et al. in 1966 from the Chinese tree *Camptotheca acuminata*[1] and was reported to possess potent anti-tumor activity. However, its clinical application has been hindered due to severe toxicity and extremely poor solubility.[2] Until the action mechanism of camptothecin, it can inhibit DNA topoisomerase I, was discovered in 1985, this compound was then revived, which brought a worldwide attention and led to the successful clinical application of several CPT derivatives including topotecan (**2**), irinotecan and 10-hydroxycamptothecin.[3,4] Now hundreds of CPT derivatives are undergoing investigation,[5a–c] in particular structure modifications in positions 7, 9 and 10,[6a–d] some of which even go into various stages of clinical trials.

In our research, a ^{11}C-labeled quaternary ammonium salt of topotecan, namely ^{11}C-9-(*N*,*N*,*N*-trimethylaminomethyl)-10-hydroxycamptothcin, was attempted to prepare as PET (Positron-Emission Tomography) molecule for tumor diagnosis. In the process of synthesizing the related reference substance, the methylation of topotecan was performed via iodomethane and methanol was selected as solvent due to it could dissolve the substrate very well. Following the reaction was monitored by TLC until it reached equilibrium, the main product was separated, purified and determined via ^{1}H NMR and MS as 10-hydroxy-9-(methoxymethyl)camptothcin (**3**)[7] unexpectedly, that is, instead of methylation the dimethylamino group of topotecan was substituted by a methoxyl group. The fact that the solvent participated in the reaction made us wonder if the quaternary ammonium salt of topotecan had been formed before

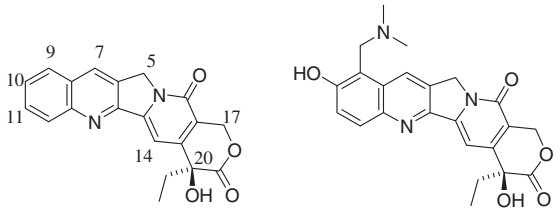


Figure 1. Structures of camptothecin and topotecan.

methanol attacked the carbon-atom in benzyl since the strong leaving-activity of trimethylamino group.

To testify the hypothesis, topotecan was refluxed in methanol without iodomethane. Out of expectation, the reaction still reached equilibrium after refluxing 20 h and afforded the same product in 31.7% as before (recovered substrate in 42.0%). From these results, we attributed the specific chemical behavior of topotecan to the internal hydrogen-bonding between 10-hydroxy group and the nitrogen atom in position 9, of which the unshared pair of electrons is locked by hydrogen that makes the methylation of nitrogen atom with iodomethane more difficult and enhances the leaving ability of dimethylamino group as dimethylamine entity. If that happens, topotecan will transfer into an intermediate *ortho*-quinonemethide species as shown in Figure 2. Herein, both topotecan (**2**) and 10-hydroxy-9-(methoxymethyl)camptothcin (**3**) could be considered as *ortho*-quinonemethide precursors (QMPs).[8] Dimethylamine or methanol can come off easily to afford *ortho*-quinonemethide and inversely they both can react with the

* Corresponding authors. Tel.: +86 21 51980119.
E-mail addresses: mengjiedong@163.com (M. Dong), zhangqian511@shmu.edu.cn (Q. Zhang).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.02.080

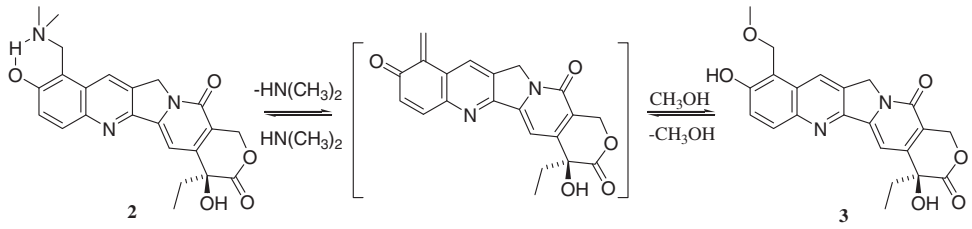
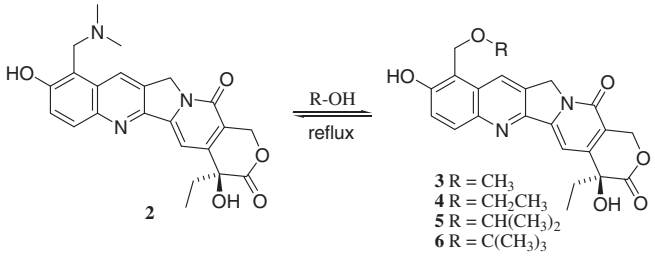
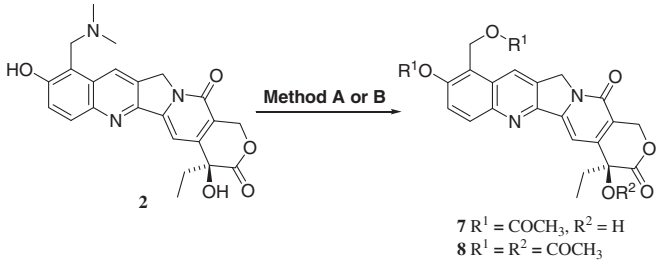


Figure 2. Mechanism of the solvolysis of topotecan in methanol.



Scheme 1. Solvolysis of topotecan in different alcohols.



Scheme 2. Solvolysis of topotecan in acetic anhydride. Reagents and conditions: Method A: $(CH_3CO)_2O$, 50 °C. Method B: $(CH_3CO)_2O$/DMAP, $CHCl_3$, reflux.

intermediate as nucleophiles to establish reaction equilibrium as observed.

Under similar protocol, the solvolysis of topotecan in ethyl alcohol, isopropyl alcohol and *tert*-butyl alcohol afforded **4**, **5** and **6**,[7] respectively, with the yields from 16.0% to 43.2% (Scheme 1). While cutting off the quantity of starting material recovered from the reaction, the translation rates would range from 54.6% to 69.8%.

Other than in alcohols, the solvolysis of topotecan in acetic anhydride, acetic acid, ethanethiol and water was also explored, respectively. After the solution of topotecan in acetic anhydride was stirred under 50 °C for 49 h, the reaction afforded compound **7** with a yield of 38.2%. While 1 N of DMAP was added into the reaction, another 10, 20-diacetylation product **8** was separated besides **7** (Scheme 2). However, the reactions did not afford the relevant products when acetic acid, ethanethiol and water were employed as solvents, possibly due to their stronger acidity and weaker nucleophilicity than alcohols.

All of these 9-alkoxymethyl and 9-acetoxymethyl derivatives were screened against three cancer cell lines (KB, HepG2 and C26) by WST-1 assay and using 10-hydroxycamptothecin (HCTP) as reference compound. The bioactivity data (shown in Table 1) demonstrated most of the compounds possessed potential antiproliferative activities and concentration dependence (Fig. 3). Especially, **7** and **6** showed more potent cytotoxic activity on KB and HepG2, respectively, than that of the reference compound. On C26 cell line test, 3 derivatives (**6**, **7** and **8**) exhibited stronger activity than 10-hydroxycamptothecin, in which **7** was even three-fold more active than the reference compound. Moreover, there was an evident trend among 9-alkoxymethyl derivatives from the test results that the bulkier the group in position 9 became, the higher cytotoxic activity the compound possessed except for compound **4** on HepG2. A possible structure–activity relationship between 9-position group and its bioactivity was suggested that a bigger size group in the 9-position would be observably favorable for

Table 1
Anti-tumor activity of solvolysis products **3**–**8** against KB, HepG2 and C26

Compounds	Cytotoxicity in vitro (IC_{50}, μM)		
	KB	HepG2	C26
10-Hydroxy CPT	0.829	0.981	0.496
3	25.367	92.071	100.776
4	19.727	3.909	29.106
5	7.246	13.480	3.877
6	1.376	0.802	0.273
7	0.794	1.004	0.157
8	1.151	1.306	0.424

KB, human oral squamous cell carcinoma; HepG2, human liver cancer; C26, mice colon carcinoma.

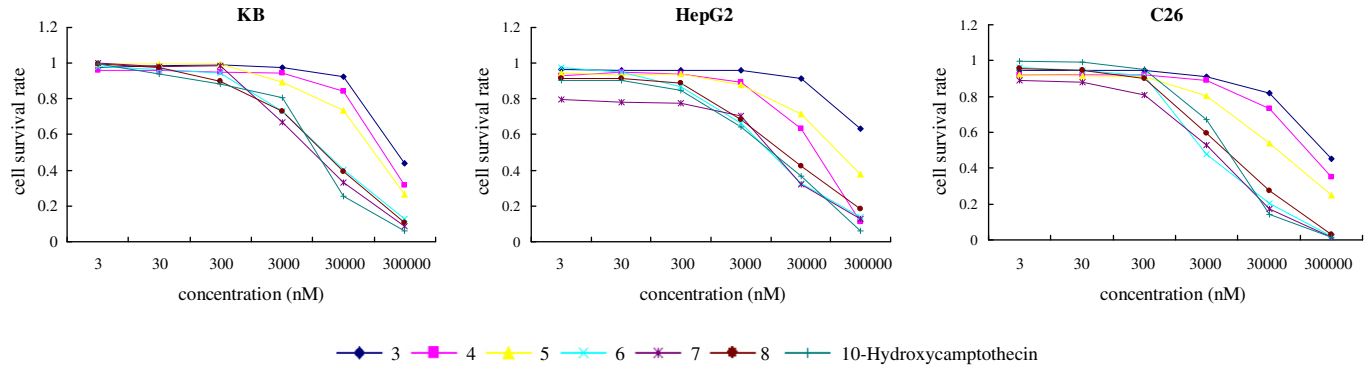


Figure 3. Concentration-versus-cell survival rate curves.

anti-tumor activities, which was consistent with the bioactivity screening results of some 9-substituted such as CHO, CN and CH=NOC$(CH_3)_3$ 10-hydroxycamptothecins reported by Dallavalle et al.[6b] As to 9-acetoxymethyl derivatives, the data of **7** was slightly smaller than that of **8**, indicting that 20-acetyl group was not compatible for its bioactivities.

In conclusion, 6 derivatives of 10-hydroxycamptothecin were obtained via solvolysis of topotecan in corresponding alcohols and acetic anhydride, and screened on several cancer cell lines as anti-tumor agents. The mechanism of this kind of reaction is attributed to the existence of internal hydrogen-bonding between 10-hydroxy and 9-nitrogen atom in topotecan. Consequently the reaction undergoes through an intermediate *ortho*-quionomethlide species to reach an equilibrium. It is worth mentioning that the lipo-solubility of all the 10-hydroxy 9-alkoxymethyl and 9-acetoxymethylcamptothecins is improved substantially compared with 10-hydroxycamptothecin or topotecan base.

Acknowledgements

This work was supported by National Drug Innovative Program (No. 2009ZX09301-011), National Natural Science Foundation of China (No. 30870730, to Dr. Mengjie Dong) and the Science and Technology Planning Project of Zhejiang province (No. 2009C33109, to Dr. Mengjie Dong).

References and notes

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.
2. Gottlieb, J. A.; Guarino, A. M.; Call, J. B.; Oliverio, V. T.; Block, J. B. *Cancer Chemother. Rep.* **1970**, *54*, 461.
3. Kingsbury, W. D.; Boehm, J. C.; Jakas, D. R.; Holden, K. G.; Hecht, S. M.; Gallagher, G.; Caranfa, M. J.; McCabe, F. L.; Faucette, L. F.; Johnson, R. K.; Hertzberg, R. P. *J. Med. Chem.* **1991**, *34*, 98.
4. Sawada, S.; Okajima, S.; Aiyama, R.; Nokata, K.-I.; Furuta, T.; Yokokura, T.; Sugino, E.; Yamaguchi, K.; Miyasaka, T. *Chem. Pharm. Bull.* **1991**, *39*, 1446.
5. (a) Thomas, C. J.; Rahier, N. J.; Hecht, S. M. *Bioorg. Med. Chem.* **2004**, *12*, 1585; (b) Basili, S.; Moro, S. *Expert Opin. Ther. Pat.* **2009**, *19*, 555; (c) Venditto, V. J.; Simanek, E. E. *Mol. Pharm.* **2010**, *7*, 307.
6. (a) Gao, H. Y.; Zhang, X. W.; Chen, Y.; Shen, H. W.; Sun, J.; Huang, M.; Ding, J.; Li, C.; Lu, W. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2003; (b) Dallavalle, S.; Rocchetta, D. G.; Musso, L.; Merlini, L.; Morini, G.; Penco, S.; Tinelli, S.; Beretta, G. L.; Zunino, F. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2781; (c) Li, Q. Y.; Zu, Y. G.; Shi, R. Z.; Yao, L. P.; Fu, Y. J.; Yang, Z. W.; Li, L. *Bioorg. Med. Chem.* **2006**, *14*, 7175; (d) Wang, S.; Li, Y. Y.; Liu, Y. H.; Lu, A. J.; You, Q. D. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4095.
7. *Compounds data*: *10-Hydroxy-9-methoxymethyl-(20S)-camptothecin* (***3***). Yield 31.7%, mp >300 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.88 (3H, t, J = 7.24 Hz, 18-CH_3), 1.87 (2H, m, 19-CH_2), 3.33 (3H, s, O-CH_3), 4.87 (2H, s, 9-CH_2), 5.26 (2H, s, 5-CH_2), 5.41 (2H, s, 17-CH_2), 6.49 (1H, s, 20-OH), 7.26 (1H, s, 14-CH), 7.53 (1H, d, J = 9.00 Hz, 11-CH), 8.02 (1H, d, J = 9.00 Hz, 12-CH), 8.65 (1H, s, 7-CH), 10.49 (1H, s, 10-OH); MS-ESI m/z 409.1 (MH^+); HRMS: calcd for $C_{22}H_{20}N_2O_6$ 409.1394, found: 409.1398.
9-Ethoxymethyl-10-hydroxy-(20S)-camptothecin (***4***). Yield 35.0%, mp >300 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.89 (3H, t, J = 7.24 Hz, 18-CH_3), 1.15 (3H, t, J = 7.04 Hz, O–CH_2–CH_3), 1.87 (2H, m, 19-CH_2), 3.57 (2H, q, J = 7.04 Hz, O–CH_2–CH_3), 4.89 (2H, s, 9-CH_2), 5.25 (2H, s, 5-CH_2), 5.41 (2H, s, 17-CH_2), 6.49 (1H, brs, 20-OH), 7.26 (1H, s, 14-CH), 7.51 (1H, d, J = 9.00 Hz, 11-CH), 8.00 (1H, d, J = 8.99 Hz, 12-CH), 8.65 (1H, s, 7-CH), 10.42 (1H, s, 10-OH); MS(ESI) m/z 423.1 (MH^+); HRMS: calcd for $C_{23}H_{22}N_2O_6$ 423.1551, found: 423.1556.
10-Hydroxy-9-isopropoxymethyl-(20S)-camptothecin (***5***). Yield 43.2%, mp >300 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.89 (3H, t, J = 7.24 Hz, 18-CH_3), 1.16 (3H, s, O–CH–CH_3), 1.17 (3H, s, O–CH–CH_3'), 1.87 (2H, m, 19-CH_2), 3.74 (1H, m, O–CH), 4.89 (2H, s, 9-CH_2), 5.26 (2H, s, 5-CH_2), 5.42 (2H, s, 17-CH_2), 6.51 (1H, s, 20-OH), 7.26 (1H, s, 14-CH), 7.51 (1H, d, J = 9.39 Hz, 11-CH), 7.97 (1H, d, J = 9.00 Hz, 12-CH), 8.63 (1H, s, 7-CH), 10.41 (1H, s, 10-OH); MS(ESI) m/z 437.2 (MH^+); HRMS: calcd for $C_{24}H_{24}N_2O_6$ 437.1707, found: 437.1699.
10-Hydroxy-9-(tert-butoxymethyl)-(20S)-camptothecin (***6***). Yield 16.0%, mp >300 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.89 (3H, t, J = 7.24 Hz, 18-CH_3), 1.28 (9H, s, O–C–$(CH_3)_3$), 1.84 (2H, m, 19-CH_2), 4.80 (2H, s, 9-CH_2), 5.25 (2H, s, 5-CH_2), 5.39 (2H, s, 17-CH_2), 6.49 (1H, s, 20-OH), 7.23 (1H, s, 14-CH), 7.47 (1H, d, J = 9.00 Hz, 11-CH), 7.96 (1H, d, J = 9.39 Hz, 12-CH), 8.58 (1H, s, 7-CH), 10.33 (1H, s, 10-OH); MS(ESI) m/z 451.1 (MH^+); HRMS: calcd for $C_{25}H_{26}N_2O_6$ 451.1864, found: 451.1856.
10-Acetoxy-9-acetoxymethyl-(20S)-camptothecin (***7***). Yield 38.2%, mp 236–239 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05 (3H, t, J = 7.33 Hz, 18-CH_3), 1.90 (2H, m, 19-CH_2), 2.07 (3H, s, 9-$COCH_3$), 2.46 (3H, s, 10-$COCH_3$), 3.82 (1H, s, 20-OH), 5.32 (1H, d, J = 16.49 Hz, 9-CH_2), 5.35 (2H, s, 5-CH_2), 5.59 (2H, s, 17-CH_2), 5.75 (1H, d, J = 16.49 Hz, 9-CH_2'), 7.58 (1H, d, J = 9.17 Hz, 11-CH), 7.67 (1H, s, 14-CH), 8.26 (1H, d, J = 9.47 Hz, 12-CH), 8.69 (1H, s, 7-CH); MS(ESI) m/z 479.2 (MH^+); HRMS: calcd for $C_{25}H_{22}N_2O_8$ 479.1449, found: 479.1461.
10, 20(S)-Diacetoxy-9-acetoxymethyl-(20S)-camptothecin (***8***). Yield 8.67%, mp 260–263 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (3H, t, J = 7.55 Hz, 18-CH_3), 2.07 (3H, s, 20-$COCH_3$), 2.15 (1H, q, J = 7.15 Hz, 19-CH_2), 2.23 (3H, s, 9-$COCH_3$), 2.29 (1H, q, J = 7.15 Hz, 19-CH_2'), 2.47 (3H, s, 10-$COCH_3$), 5.33 (2H, s, 5-CH_2), 5.41 (1H, d, J = 17.55 Hz, 9-CH_2), 5.60 (2H, s, 17-CH_2), 5.68 (1H, d, J = 17.06 Hz, 9-CH_2'), 7.21 (1H, s, 14-CH), 7.60 (1H, d, J = 9.27 Hz, 11-CH), 8.25 (1H, d, J = 8.77 Hz, 12-CH), 8.70 (1H, s, 7-CH); MS(ESI) m/z 521.2 (MH^+); HRMS: calcd for $C_{27}H_{24}N_2O_9$ 521.1555, found: 521.1567.
8. Weinert, E. E.; Dondi, R.; Colloredo-Melz, S.; Frankenfield, K. N.; Mitchell, C. H.; Freccero, M.; Rokita, S. E. *J. Am. Chem. Soc.* **2006**, *128*, 11940.