J Incl Phenom Macrocycl Chem (2011) 69:85–89
DOI 10.1007/s10847-010-9817-1

ORIGINAL ARTICLE

Preparation and characterization of inclusion complexes of topotecan with sulfonatocalixarene

Guo-Song Wang · Heng-Yi Zhang · Fei Ding · Yu Liu

Received: 20 April 2010 / Accepted: 2 June 2010 / Published online: 25 June 2010

Abstract Inclusion complex of sulfonatocalix[4]arene (SC4A) with topotecan (TPT) was prepared, and its inclusion complexation behaviors, such as stoichiometry, complex stability constants, and inclusion mode, were investigated by means of UV/Vis spectroscopy, DSC, and 2D NMR. The obtained results show that the quinoline ring and the dimethylaminomethyl group of TPT can be efficiently encapsulated in SC4A, and the complex is more soluble than free TPT.

Keywords Sulfonatocalixarenes · Topotecan · Supramolecular chemistry · Inclusion complexation

Introduction

Topotecan (TPT, Fig. 1), a derivative of camptothecin, is a chemotherapy agent that is a topoisomerase I inhibitor [1–3], and is used clinically in the treatment of ovarian [4, 5], small cell lung [6, 7], and more recently, cervical cancer [8, 9]. However, its low solubility makes people have to prepare TPT hydrochloride for improving its solubility.

Calixarenes, which contain a repeating phenolic unit formed into a macrocycle via methylene bridges [10, 11], are noted for their ability to form host–guest complexes by trapping organic compounds, small ions, and gases in their toruslike cavities. The water-soluble sulfonatocalixarenes have been widely used in pharmaceutical fields [12–17] due to their innocuous nature [18–21] and good water solubility (up to 0.1 M) [22]. Recently, we explored the potential application of *para*-sulfonatocalixarenes to treat viologen poisoning [23]. These calixarenes provide not only a hydrophobic environment (benzene rings), but also hydrophilic heads (SO_3^-), so they can encapsulate some drug molecules into their cavity, leading to the increase of the solubility and the stability [12, 13], and the improvement of the bioavailability [14] for the drug.

In this study, the binding properties of sulfonatocalix[4]arene (SC4A) with TPT were investigated. Firstly, the interaction between SC4A and TPT was studied by using UV spectrophotometry. And then, inclusion complex of SC4A with TPT was prepared, and confirmed by NMR and DSC analysis.

Materials and methods

General methods

TPT was purchased from *Knowshine (Shanghai) Pharmachemicals Inc*. *Para*-sulfonatocalix[4]arene were synthesized and purified according to literature procedures, and verified by ^{1}H NMR [24–26]. All of the chemicals and solvents were of analytical reagent grade and were used as received.

NMR experiments were performed on a Varian Mercury VX300 spectrometer (300 MHz) at 298 K in a deuterium oxide solution. 2D Rotating Frame Overhauser Effect Spectroscopy (ROESY) was performed in D_2O (300 MHz) with a mixing time of 400 ms. DSC analysis was performed with a NETZSCH DSC 204 instrument from 25 to 400 °C with a heating rate of 10 K/min.

G.-S. Wang · H.-Y. Zhang · F. Ding · Y. Liu (✉)
Department of Chemistry, State Key Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, People's Republic of China
e-mail: yuliu@nankai.edu.cn

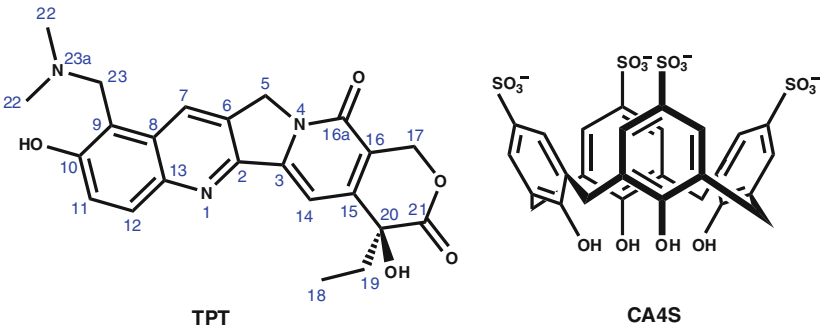

Fig. 1 Chemical structures of TPT and SC4A. Protons of TPT are shown for NMR purposes

Prediction of the interaction between TPT with SC4A by UV spectrophotometry

A fixed amount of TPT was added into a series of 10-mL comparison tubes. A different amount of SC4A was gently increased to be added into the tubes, respectively. These solutions were diluted to graduation with MeOH–H_2O, shook up and settled about 30 min. Then by using the same concentration of SC4A in MeOH–H_2O as blanks, the difference-UV absorption spectra of these solutions were determined, and the absorbance was measured at 230 nm.

For the titration of host into a solution of guest, the relationship between the change in absorbance of the guest and the host concentration was given by equation $(A - A_0)/[H] = K(A_\infty - A_0) - K(A - A_0)$, where A_0 is the absorbance of the guest in the absence of host, $[H]$ is the concentration of host at each titration point, A_∞ is the absorbance when all the guest molecules are complexed with host (i.e., guest with large excess of host), A is the observed absorbance at each titration point, and K is the binding constant (M^{-1}) [27].

Preparation of SC4A–TPT complex

To obtain SC4A–TPT complex, TPT (0.01 mM) and SC4A (0.01 mM) were completely dissolved in a mixed solution of methanol and water (v:v = 1:9) and stirred for about 48 h at room temperature. The mixed solution was evaporated to remove methanol and water, and dried in vacuum to give SC4A–TPT complex.

Characteristics of SC4A–TPT complex

^1H NMR and 2D ROESY were carried out on Varian Mercury VX300 spectrometer with 5 mm sample tube. The deuterated water was typically used in such studies as solvent in these experiments. No internal reference was used to avoid possible interference with the complexation between TPT and SC4A, therefore the solvent signal (D_2O, 4.694 ppm) was used as an internal reference.

TG/DTG analysis was carried out for pure TPT, pure SC4A, physical mixtures of TPT with SC4A at a 1:1 M ratio, and complex of TPT with SC4A. The TG/DTG patterns were recorded with a heating rate of 10 K/min from 25 to 400 °C with a heating rate of 10 K/min equipped with a thermal analysis data station.

Results and discussion

UV spectrum and binding constant

A series of solutions containing same amounts of TPT and different amounts of SC4A were determined by UV Spectrophotometry. The maximal absorption intensity of TPT increased markedly upon the addition of SC4A, which indicates that there exists the interaction with TPT and SC4A, and the formation of the complex between TPT with SC4A.

To determine the stoichiometry of the complexation, the Job plots were constructed from the UV titration data. The formation of the 1:1 complex was clearly confirmed (Fig. 2). The K_S value of the complex was calculated by

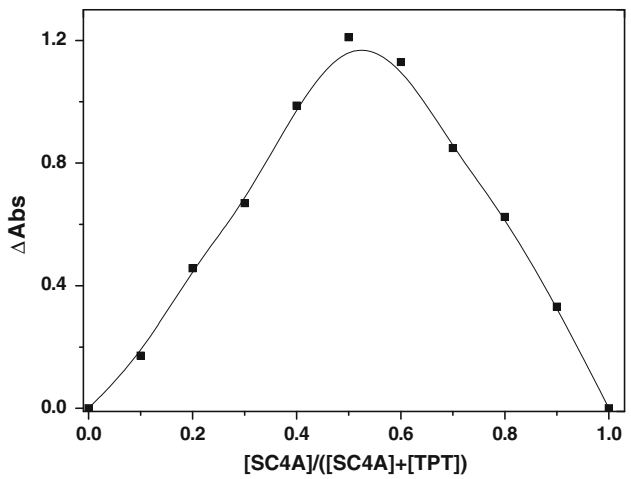

Fig. 2 Job plot for binding of TPT with SC4A, with the total concentration maintained at 0.5 mM and the changes of absorbance at 232 nm were measured

using the Scatchard method [27]. It is $5.6 \times 10^3\ M^{-1}$, indicating that the complex of TPT binding SC4A is remarkably stable.

Characteristics of the SC4A–TPT complex

^{1}H NMR experiments of TPT, SC4A, and the SC4A–TPT complex were performed to clarify the interaction between TPT and SC4A. On the one hand, chemical shift values of aromatic ring proton of SC4A increase from 7.33 to 7.63 ppm, and on the other hand, those in TPT also markedly change due to the formation of the complex between SC4A and TPT (Table 1). As can be seen from Table 1, the chemical shift values of the protons in quinoline ring (H7, H11, and H12), N–CH_3 (H22) and N–CH_2 (H23) change markedly upon complexation, but H17, H18, and H19 in hydroxyl lactone ring, as well as H5 and H14 do small. These observations suggest that the stronger interaction exists between the quinoline ring as well as the dimethylaminomethyl group of TPT and the SC4A aromatic rings, which indicates that the SC4A cavity includes mainly the quinoline ring and dimethylaminomethyl part of TPT.

To further confirm the binding mode of SC4A with TPT, and 2D ROESY experiment was performed for the SC4A–TPT complex (Fig. 3). There are three clear cross peaks (circled a, b, and c) between protons of quinoline ring in TPT and those of the aromatic ring in SC4A (ArH). The peak a represents the correlation involving H7 in quinoline ring of TPT, peaks b and c do those involving H12, and H11, respectively. The results prove that the SC4A cavity includes the quinoline ring of TPT. Moreover, the cross peak d suggests the interaction between protons of the aromatic ring in SC4A and H23 in TPT, and the cross peaks e and f come from those with H22. Based on above observations, we can deduce reasonably the binding mode of SC4A with TPT in Fig. 4. In this mode, the cavity of SC4A includes mainly the quinoline ring and the dimethylaminomethyl of TPT mainly through hrdrogen interaction, π–π interaction, and electrostatic interaction, etc.

The TG/DTG curves of the physical mixture exhibit thermal profiles associated to SC4A and TPT (not shown). The thermal behaviors of SC4A, TPT, and SC4A–TPT inclusion complex are entirely different (Fig. 5a, b). The TG curve of SC4A–TPT (Fig. 5a) presents a weight loss (8–9%) in the 30–120 °C range attributed to the release of water molecules. In the second loss of the 240–300 °C range, it is corresponding to partial TPT decomposition

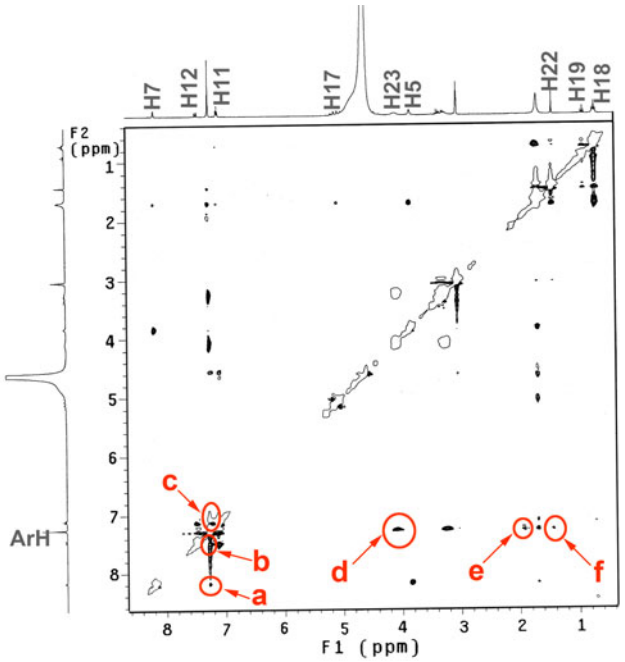

Fig. 3 2D ROESY spectrum of SC4A–TPT complex in D_2O. Annotated crosspeaks indicate intermolecular interactions between TPT and SC4A

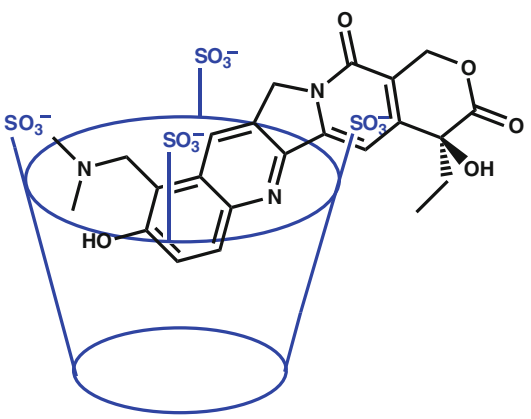

Fig. 4 Possible binding mode of SC4A–TPT complex according to 2D ROESY spectrum

Table 1 ^{1}H Chemical shifts (δ_H) (ppm) for TPT and complex

Proton	5	7	12	11	14	17	18	19	22	23
Free TPT	4.33	8.69	8.00	7.59	7.46	5.30	1.02	1.75	2.60/2.60	4.61
Complex	4.18	8.50	7.78	7.41	7.42	5.47	1.10	1.22	1.82/3.40	4.39
$\Delta\delta$	−0.15	−0.19	−0.22	−0.18	0.04	0.17	0.08	−0.53	−0.78/0.80	0.22

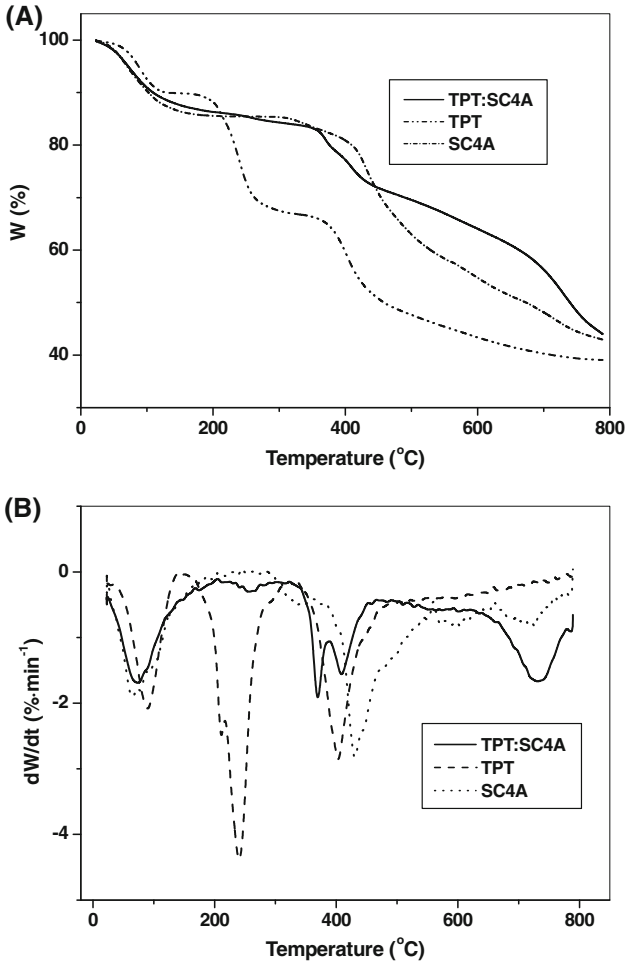

Fig. 5 TG-DTG thermograms for SC4A–TPT complex. **a** TG curves of SC4A, TPT, and SC4A–TPT inclusion complex, **b** DTG curves of SC4A, TPT, and SC4A–TPT inclusion complex

with the percentage weight loss (13–15%). Further decomposition occurs from 320 to 440 °C, as evidenced by the DTG curve (Fig. 5b). The complex begins to decompose at 320 °C with a strong exothermal peak at 360 °C in the DTG curve. During the range of 320–440 °C, the TG curve also shows a two-step weight loss corresponding to the decomposition of TPT.

Aqueous solubility

The water solubility of the SC4A–TPT complex was assessed by the preparation of its saturated solution [28]. Excess amount of complex was put into 5 mL of water and then was stirred for 1 h. After removing the insoluble substance by filtration, the filtrate was evaporated under reduced pressure to dryness and the residue was dosed by weighing method. Comparing with the water solubility of TPT (1.2 mg/mL), that of the SC4A–TPT complex is dramatically increased to approximately 6.4 mg/mL (enhancing about fivefold). In the control experiment, a clear solution was obtained after dissolving SC4A–TPT (16 mg) complex, which was equivalent to 6.4 mg of TPT in 1 mL of water at room temperature. This subsequently confirms the reliability of the water solubility of the SC4A–TPT complex.

Conclusion

In conclusion, the binding behaviors of SC4A with TPT were investigated. The formation of inclusion complex was confirmed by DSC and ^{1}H NMR. Aqueous solubility study shows that SC4A could enhance the water-solubilities of TPT. The complex should be regarded as an important choice in the design of novel formulation of TPT for medicine.

Acknowledgments We thank 973 Program (2006CB932900) and NNSFC (20932004 and 20721062) for financial support.

References

1. Voloschin, A.D., Betensky, R., Wen, P.Y., Hochberg, F., Batchelor, T.: Topotecan as salvage therapy for relapsed or refractory primary central nervous system lymphoma. J. Neurooncol. **86**, 211–215 (2008)
2. Calabrese, C.R., Almassy, R., Barton, S., Batey, M.A., Calvert, A.H., Canan-Koch, S., Durkacz, B.W., Hostomsky, Z., Kumpf, R.A., Kyle, S., Li, J., Maegley, K., Newell, D.R., Notarianni, E., Stratford, I.J., Skalitzky, D., Thomas, H.D., Wang, L.-Z., Webber, S.E., Williams, K.J., Curtin, N.J.: Anticancer chemosensitization and radiosensitization by the novel poly (ADP-ribose) polymerase-1 inhibitor AG14361. J. Natl. Cancer Inst. **96**, 56–67 (2004)
3. Macdonald, J.S., Jacobson, J.L., Ketchel, S.J., Weiss, G., Taylor, S., Mills, G., Kuebler, J.P., Rivkin, S., Conrad, M.: A phase II trial of topotecan in esophageal carcinoma: a Southwest Oncology Group study (SWOG 9339). Invest. New Drugs **18**, 199–202 (2000)
4. Sehouli, J., Oskay-Oezcelik, G.: Current role and future aspects of topotecan in relapsed ovarian cancer. Curr. Med. Res. Opin. **25**, 639–651 (2009)
5. McGuire, W.P., Blessing, J.A., Bookman, M.A., Lentz, S.S., Dunton, C.J.: Topotecan has substantial antitumor activity as first-line salvage therapy in platinum-sensitive epithelial ovarian carcinoma: a gynecologic oncology group study. J. Clin. Oncol. **18**, 1062–1067 (2000)
6. Beldner, M.A., Sherman, C.A., Green, M.R., Garrett-Mayer, E., Chaudhary, U., Meyer, M.L., Kraft, A.S., Montero, A.J.: Phase I dose escalation study of vinorelbine and topotecan combination chemotherapy in patients with recurrent lung cancer. BMC Cancer **7**, 231 (2007)
7. Nicum, S.J., O'Brien, M.E.R.: Topotecan for the treatment of small-cell lung cancer. Expert Rev. Anticancer Ther. **7**, 795–801 (2007)
8. Idris, T., Winter, R., Lang, U., Petru, E.: Topotecan-induced long-term remission of an advanced endometrial cancer after treatment with cisplatinum, an anthracycline and paclitaxel. Anticancer Res. **29**, 1761–1762 (2009)

9. Tao, X., Hu, W., Ramirez, P.T., Kavanagh, J.J.: Chemotherapy for recurrent and metastatic cervical cancer. Gynecol. Oncol. **110**, S67–S71 (2008)
10. Gutsche, C.D.: Calixarenes. The Royal Society of Chemistry, Cambridge (1989)
11. Asfari, Z., Böhmer, V., Harrowfield, J., Vicens, J.: Calixarenes. Kluwer Academic Publishers, Dordrecht (2001)
12. Yang, W., Otto, D.P., Liebenberg, W., de-Villiers, M.M.: Effect of para-sulfonato-calix(n)arenes on the solubility, chemical stability, and bioavailability of a water insoluble drug nifedipine. Curr. Drug Discov. Technol. **5**, 129–139 (2008)
13. Yang, W., De Villiers, M.M.: Effect of 4-sulfonato-calix[n]arenes and cyclodextrins on the solubilization of niclosamide, a poorly water soluble anthelmintic. AAPS J. **7**, E241–E248 (2005)
14. Yang, W., De Villiers, M.M.: Aqueous solubilization of furosemide by supramolecular complexation with 4-sulphonic calix[n]arenes. J. Pharm. Pharmacol. **56**, 703–708 (2004)
15. Yang, W., De Villiers, M.M.: The solubilization of the poorly water soluble drug nifedipine by water soluble 4-sulphonic calix[n]arenes. Eur. J. Pharm. Biopharm. **58**, 629–636 (2004)
16. Fernandes, S.A., Cabec, L.F., Marsaioli, A.J., de Paula, E.: Investigation of tetracaine complexation with beta-cyclodextrins and p-sulphonic acid calix[6]arenes by nOe and PGSE NMR. J. Incl. Phenom. Macrocycl. Chem. **57**, 395–401 (2007)
17. Millership, J.S.: A preliminary investigation of the solution complexation of 4-sulphonic calix[n]arenes with testosterone. J. Incl. Phenom. Macrocycl. Chem. **39**, 327–331 (2001)
18. Coleman, A.W., Jebors, S., Cecillon, S., Perret, P., Garin, D., Marti-Battle, D., Moulin, M.: Toxicity and biodistribution of para-sulfonato-calix[4]arene in mice. New J. Chem. **32**, 780–782 (2008)
19. Perret, F., Lazar, A.N., Coleman, A.W.: Biochemistry of the para-sulfonato-calix[n]arenes. Chem. Commun. 2425–2438 (2006)
20. Paclet, M.-H., Rousseau, C.F., Campion, Y., Morel, F., Coleman, A.W.: An absence of non-specific immune response towards para-sulphonato-calix[n]arenes. J. Incl. Phenom. Macrocycl. Chem. **55**, 353–357 (2006)
21. Silva, E.D., Shahgaldian, P., Coleman, A.W.: Haemolytic properties of some water-soluble para-sulphonato-calix-[n]-arenes. Int. J. Pharm. **273**, 57–62 (2004)
22. Shinkai, S., Araki, K., Tsubaki, T., Arimura, T., Manabe, O.: New syntheses of calixarene-p-sulphonates and p-nitrocalixarenes. J. Chem. Soc. Perkin Trans. **1**, 2297–2299 (1987)
23. Wang, K., Guo, D.-S., Zhang, H.-Q., Li, D., Zheng, X.-L., Liu, Y.: Highly effective binding of viologens by p-sulfonatocalixarenes for the treatment of viologen poisoning. J. Med. Chem. **52**, 6402–6412 (2009)
24. Da Silva, E., Coleman, A.W.: Synthesis and complexation properties towards amino acids of mono-substituted p-sulphonato-calix-[n]-arenes. Tetrahedron **59**, 7357–7364 (2003)
25. Liu, Y., Ma, Y.-H., Chen, Y., Guo, D.-S., Li, Q.: Molecular recognition thermodynamics of pyridine derivatives by sulfonatocalixarenes at different pH values. J. Org. Chem. **71**, 6468–6473 (2006)
26. Arena, G., Contino, A., Lombardo, G.G., Sciotto, D.: Water-soluble calix[6]arenes, characterization of 5,11,17,23,29,35-hexasulphonate-37,38,39,40,41,42-hexahydroxycalix[6]arene and thermodynamic study of proton complex formation. Thermochim. Acta **264**, 1–11 (1995)
27. Connors, K.A.: Binding Constants, the Measurements of Molecular Complex Stability. Wiley, New York (1987)
28. Montassier, P., Duchêne, D., Poelman, M.-C.: Inclusion complexes of tretinoin with cyclodextrins. Int. J. Pharm. **153**, 199–209 (1997)