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Synthesis and antitumor activity of 10-arylcamptothecin derivatives

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ABSTRACT

A series of 10-arylcamptothecin derivatives was designed and synthesized. The key step of the synthesis was achieved by employing Suzuki cross-coupling chemistry. All of the new derivatives were tested for cytotoxicity against three human tumor cell lines, BEL-7402, A549, and HL-60; most of the derivatives exhibited potent cytotoxicity. The stability study showed that compound **30** was more stable than its lead compound 10-hydroxycamptothecin under the physiological condition. Mechanistic study demonstrated that compound **30** and its hydrochloride **31** had a pharmacological profile similar with camptothecin.

Camptothecin (CPT, **1**), an alkaloid isolated by Wall and Wani from *Camptotheca acuminate*, showed potent inhibitory activity against a broad spectrum of tumors.[1,2] Clinical use of camptothecin in cancer therapy, however, was limited by its poor water solubility and its severe adverse effects including neutropenia, thrombocytopenia, hemorrhagic cystitis, and G.I. symptoms with significant diarrhea. Since the discovery of its antitumor mode of action which was closely associated with inhibition of topoisomerase I (Topo I), an essential enzyme that catalyzes the relaxation of supercoiled DNA during DNA replication,[3] intensive efforts have been devoted to identify novel camptothecin analogues.[4–8] As a result, three drugs, topotecan (**2**),[4] irinotecan (**4**),[5] and belotecan (**5**)[9,10] (Fig. 1), have been approved in treatment of human cancers.

Structure–activity relationship (SAR) studies revealed that modification at northwestern positions of camptothecin may result in increase in its potency. The substitution at the positions 7–10 and fusion of an additional ring with the ring A/B have led to the discovery of a series of potent compounds which are in clinical studies, such as gimatecan (**7**),[6] silatecan (**8**),[7] lurtotecan (**9**),[8] exatecan (**10**)[11,12] (Fig. 1). These successful examples imply that those

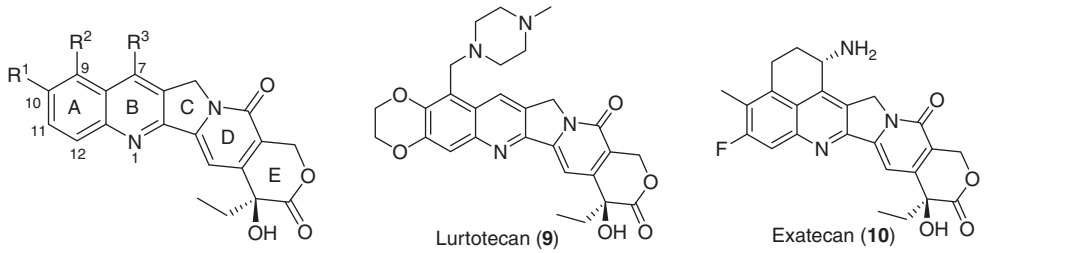

Figure 1. Camptothecin and its derivatives in clinic use and in clinic trial.

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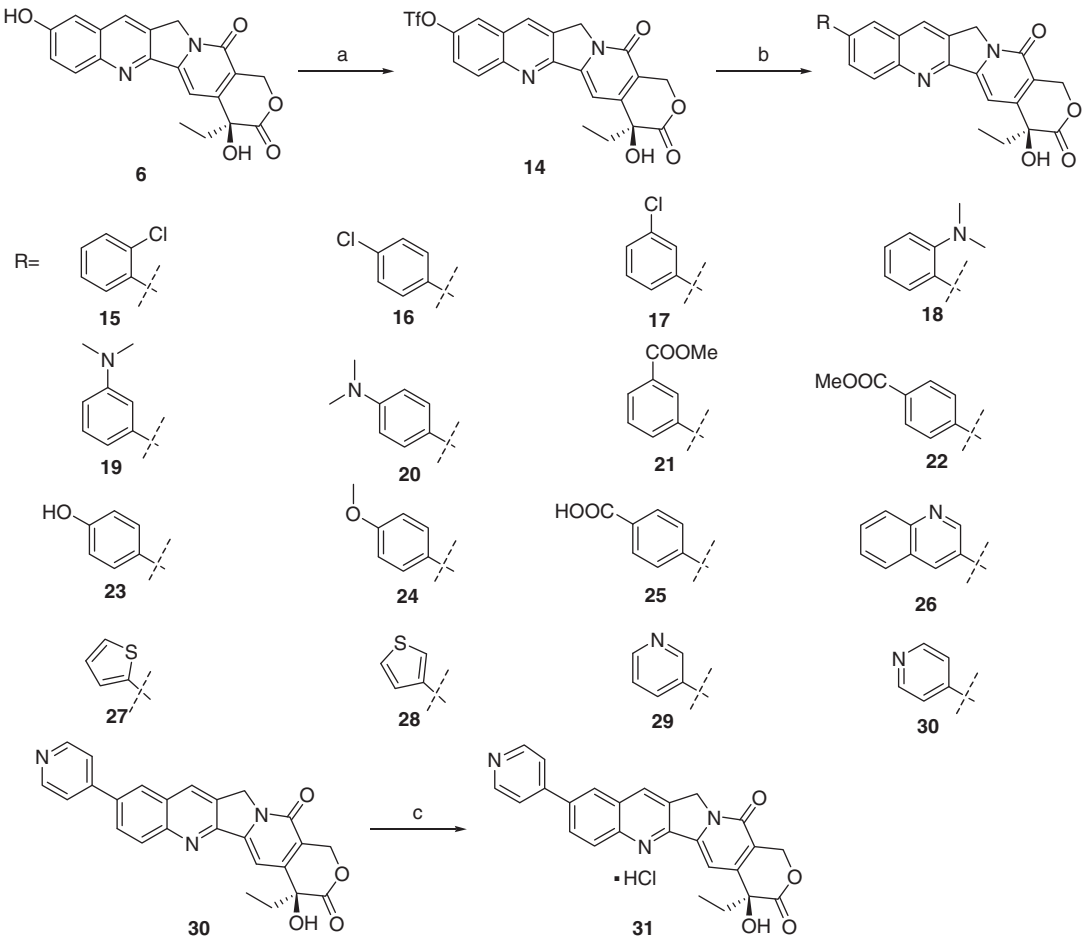

Scheme 1. The synthesis of the new derivatives. Reagents and conditions: (a) *N*,*N*-bis(trifluoromethylsulfonyl)aniline, TEA, DMF, rt; (b) $Pd(PPh_3)_4$, potassium carbonate, $RB(OH)_2$, 1,4-dioxane, 80 °C; (c) HCl in EtOAc.

positions would tolerate a large group; therefore there remains wide possibility for structural modification. On this basis we designed a class of camptothecin derivatives with an aryl substituent at the position 10. Herein we reported the synthesis of a series of 10-arylcamptothecin and their preliminary biological results.

10-Hydroxycamptothecin (**6**), a commercial available natural product, was served as the starting material; the 10-hydroxyl group was transformed into various aryl groups using a two-step sequence (Scheme 1). Compound **6** was first converted into triflate **14** in high yield (96.7%) using the literature method.[13] Then **14** was arylated using Suzuki cross-coupling chemistry: treatment of the triflate with $Pd(PPh_3)_4$, potassium carbonate, and an appropriate aryl boronic acid in 1,4-dioxane afforded corresponding 10-arylcamptothecin (35.4–77.8% yields) (compounds **15**–**30**).[14,15] Finally, compound **30** was treated with a hydrogen chloride solution in ethyl acetate to give the water soluble quaternary salt **31**.

The new derivatives were then subjected to testing in three cancer cell lines: leukemia HL60, liver cancer BEL-7402, and lung cancer A549, using the marketed drug topotecan as a reference drug. The data show that most of the new 10-arylcamptothecins exhibited potent cytotoxicity as shown in Table 1. The IC_{50} of the new derivatives is in a broad range from 9 nM to 36.5 μM, indicating that both aryl variants and substitution pattern might greatly influence cytotoxicity of the new CPT derivatives.

The primary structure–activity relationships were generalized from the in vitro cytotoxicity data (Table 1). The derivatives with *m*-substituted phenyl (**17**, **19**, and **21**) had a comparable activity to topotecan; whereas *o*-/*p*-substituted phenyl derivatives exhibited lower activity than their *m*-substituted isomers (**15** and **16** vs. **17**, **18** and **20** vs. **19**, **22** vs. **21**). When a bulky bicyclic aryl group quinolyl was introduced, a sharp drop in potency was

Table 1
The cytotoxicity of the new derivatives against human tumor cell lines HL60, A549, and BEL-7402[a]

Compounds	In vitro cytotoxicity (IC_{50}, μmol L^{-1})		
	HL60	A549	BEL-7402
TPT	0.052	0.071	0.47
15	0.59	1.12	2.46
16	0.30	0.32	3.12
17	0.10	0.067	0.93
18	0.16	0.23	1.53
19	0.013	0.040	0.18
20	2.88	2.68	36.48
21	0.049	0.023	5.12
22	0.28	0.51	0.65
23	0.020	0.14	1.05
24	0.78	0.50	5.89
25	1.45	11.59	13.81
26	1.44	5.95	6.59
27	0.048	0.010	0.08
28	0.037	0.012	0.23
29	0.032	0.068	0.38
30	0.009	0.067	0.42
31	0.021	0.062	0.46

[a] The in vitro cytotoxicity of the CPT derivatives against three cell lines, HL60(human leukemia), A549 (human lung cancer) and BEL-7402 (human liver cancer) was measured by the MTT assay and SRB assay after 3 days of incubation and expressed as the half maximal inhibitory concentration (IC_{50}, μmol L^{-1}).

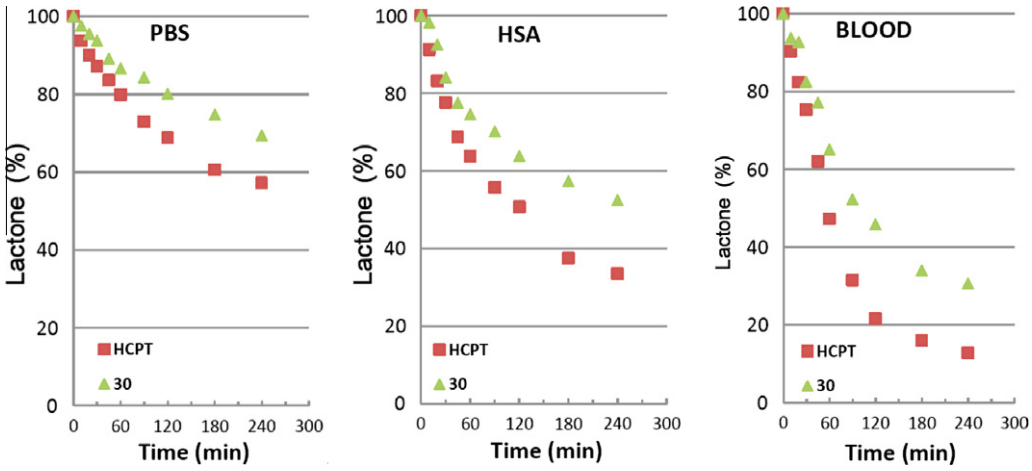

Figure 2. The stability of compound **30** in PBS (left panel), PBS with HSA (middle panel), and blood (right panel), HCPT (10-hydroxycamptothecin) as control. Stability profiles were determined on HPLC with a fluorescence detector. Drug concentrations of 50 μmol L^{-1} were used, and drug samples were incubated at pH 7.4 and 37 °C. Each data point represents the average of three determinations with an uncertainty of 10% or less.

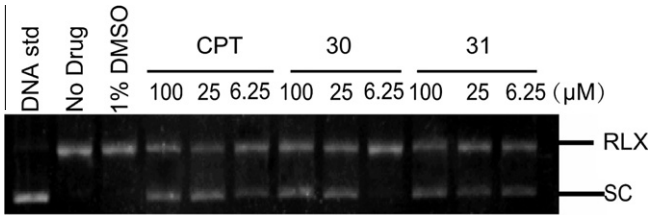

Figure 3. Topo I-mediated supercoiled DNA pBR322 relaxation assay. pBR322 DNA was incubated with topoisomerase I (1 unit) in the presence or absence of indicated drugs at 37 °C for 30 min and terminated by the addition of 10% sodium dodecyl sulfate. The mixtures then were analyzed on a 1% agarose gel. CPT (camptothecin) was used as reference drug. The position of supercoiled DNA (SC) and relaxed DNA (RLX) were indicated.

observed (compound **26**), indicating that 10-monocyclic aryl is more favorable than 10-bicyclic aryl for maintaining the potency of 10-aryl derivatives. Four 10-monoheteroaryl derivatives (**27**, **28**, **29**, and **30**) exhibited potent cytotoxicity at sub-nanomolar range. Moreover, 10-(2-thienyl)camptothecin (**27**) showed a selective cytotoxicity against Bel-7402, a liver cancer cell line, with IC_{50} of 9 nM, suggesting that compound **27** might have a potential in treatment of the notorious liver cancers. However, the position of heteroatom had little impact on cytotoxicity (**27** vs. **28**, **29** vs. **30**).

Water solubility plays a very important role in improving the potency of camptothecin derivatives. The hydrochloride salt **31**, the water soluble version of **30** was prepared to increase solubility, its solubility was improved greatly (10 mg in 1 mL of water for **31** vs. less than 1 mg in 1 mL of water for **30**).

It is well-known that only the lactone form of camptothecin derivatives is active in vivo,[16–19] the higher ratio of the lactone form in blood, the better activity they might possess. The stability of **30** and its lead compound 10-hydroxycamptothecin were tested in three systems: phosphate buffered saline (PBS) at pH 7.4 (left panel), PBS with human serum albumin (HSA) (middle panel), and human blood (right panel) (Fig. 2). The result showed that compound **30** displayed consistently more stable than 10-hydroxycamptothecin in all three systems. The result indicated that the introduction of an aryl group at the position 10 of camptothecin would enhance the stability of its lactone structure.

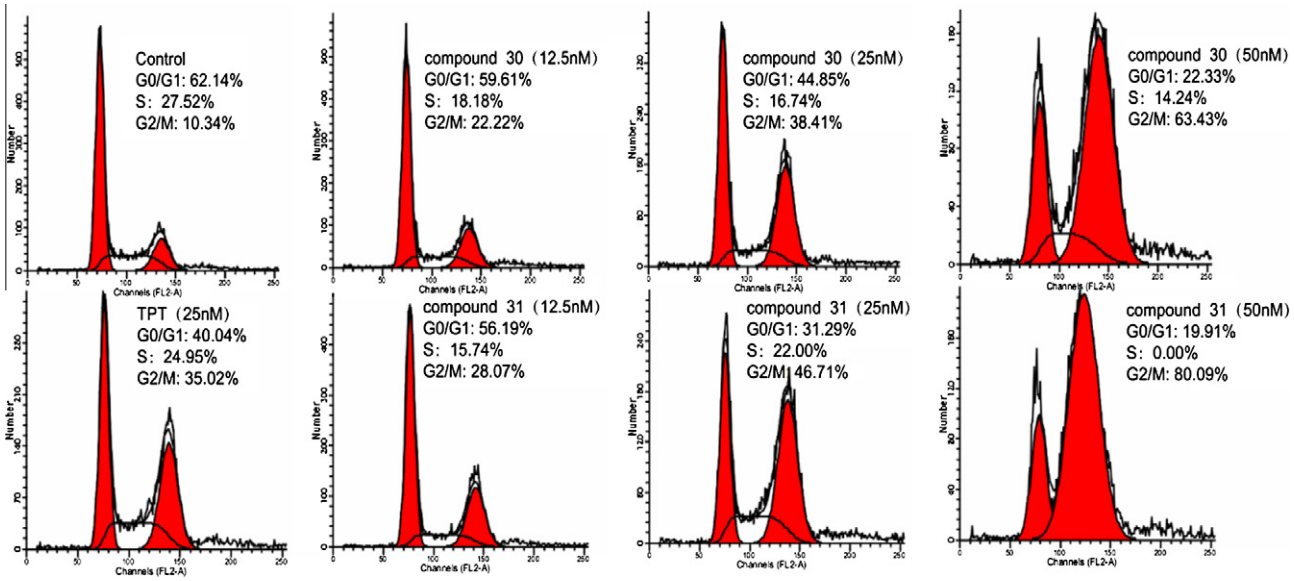

Figure 4. Cell cycle alterations in response to compounds treatment. A549 cells were treated with TPT (topotecan), compounds **30** and **31** for 24 h. Cell cycle profiles were analyzed by flow cytometry, and the percentage of the cells in G0/G1, S, and G2 /M phases were calculated by the ModFit program A.

Two separate assays were carried out to explore the mode of action of the new derivatives. Compounds **30** and **31** were preferentially tested for Topo I inhibitory activity using camptothecin as a reference drug. As shown in Figure 3, both two compounds exhibited strong Topo I inhibitory activity with an almost equal potency as camptothecin. A typical characteristic of camptothecin is its ability to induce cell cycle arrest at G2/M phase; we therefore assessed perturbation of the cell cycle using flow cytometry. We found that treatment of compounds **30** and **31,** at concentration from 12.5 nM to 50 nM, caused a marked increase in the proportion of G2/M phase cells from 10% to 63% and 80%, respectively (Fig. 4). Moreover, compounds **30** and **31** showed better inhibitory effect than topotecan at the concentration of 25 nM.

In summary, seventeen 10-arylcamptothecins were designed and synthesized. Preliminary in vitro biological evaluation demonstrated that some of the derivatives showed very potent cytotoxicity with IC_{50} up to 9 nM. In particular, 10-(4-pyridyl)camptothecin (**30**) and its water soluble hydrochloride **31** displayed comparable potency to the clinically used drug topotecan in cytotoxic settings. The stability study revealed that an additional aryl group at the position 10 of camptothecin would increase the ratio of lactone form which is beneficial to the efficacy of camptothecin derivatives. Mechanistic studies showed that compound **30** had a similar pharmacological profile with typical camptothecin derivatives both in Topo I inhibitory assay and in cell circle arrest assay. These results together suggested that compound **30** was a promising lead compound for antitumor drugs. Further biological evaluation of **30** is currently underway in our laboratory.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.005.

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14. *General procedure for synthesis of 10-aryl substituted camptothecins* (**15**–**30**): the appropriate organoboron compound (0.24 mmol), powdered K_2CO_3 (40 mg, 0.29 mmol), and 14 (100 mg, 0.20 mmol) were added to a sealed tube that had been purged with argon. A solution of $Pd(PPh_3)_4$ (10 mg) in degassed dioxane (1 mL) was added, and the reaction mixture was stirred under argon at 80 °C for 5 h. The reaction mixture was cooled and filtered through a short celite pad. The filtrate was concentrated under reduced pressure to remove the solvents. The crude product was purified by flash chromatography (silica gel: 1.5–2.5% MeOH in CH_2Cl_2) to give compounds **15**–**30** (35.4–77.8%).
15. *The analytical data of* **30** and **31**. Compound **30**: mp 287–288 °C.^{1}H NMR (300 MHz $CDCl_3$) δ 1.06 (3H, t, J = 7.5 Hz), 1.85-1.95 (2H, m), 5.34 (2H, s), 5.32 (1H, d, J = 14.4 Hz), 5.68 (1H, d, J = 14.4 Hz), 7.67 (2H, d, J = 6.0 Hz), 7.71 (1H, s), 8.10 (1H, d, J = 7.8 Hz), 8.19 (1H, s), 8.36 (1H, d, J = 7.8 Hz), 8.43 (1H, s), 8.78 (2H, d, J = 6.0 Hz).^{13}C NMR (100 MHz DMSO-d_6) 175.5, 156.7, 153.2, 150.5(2), 149.9, 148.0, 145.7, 145.2, 135.7, 132.1, 130.4, 129.9, 128.8, 128.0, 126.8(2), 121.5, 119.3, 97.0, 72.4, 65.3, 50.3, 30.3, 7.8. MS (EI): m/z 425. HRMS (EI) calcd for $C_{25}H_{19}N_3O_4$ 425.1376; found 425.1383. Compound **30** has >98% chemical purity as measured by HPLC.**31**: mp >300 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.89 (3H, t, J = 7.5 Hz), 1.86–1.90 (2H, m), 5.35 (2H, S), 5.45 (2H, S), 8.01 (2H, d, J = 6.0 Hz), 8.23 (1H, s), 8.34 (1H, d, J = 7.8 Hz), 8. 45 (1H, s), 8.50 (1H, d, J = 7.8 Hz), 8.65 (1H, s), 8.95 (2H, d, J = 6.0 Hz). MS (EI): m/z 425. Anal. Calcd for $C_{25}H_{19}N_3O_4$·HCl: C, 65.01; H, 4.36; Cl, 7.68; N, 9.10. Found: C, 64.89; H, 4.47; Cl, 7.79; N, 8.98.
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