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Synthesis and biological evaluation of (±)-cryptotanshinone and its simplified analogues as potent CDC25 inhibitors

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Abstract—(±)-Cryptotanshinone and its simplified analogues were synthesized via SmI_2 promoted radical cyclization to construct the furan ring. Analogues **18** and **26** were identified as effective inhibitors of dual specificity protein phosphatase CDC25B which is a key enzyme for cell cycle progression, and they also inhibited growth in A-549 human lung cancer cell line.

1. Introduction

Quinones are biologically important compounds, especially because of their cytotoxic activity and pharmacological action. Many efficient antineoplastic drugs are quinones (anthracyline derivatives, mitoxantrone, actinomycin), quinonoid derivatives (quinolones, genistein, bactracyclin) or drugs that can easily be converted to quinones by in vivo oxidation (etoposide).[1] The antitumor activities of the quinones, including naphthoquinone, were revealed more than three decades ago when the National Cancer Institute published a report in which 1500 synthetic and natural quinones were screened for their anticancer activities.[2] Many 1,2-naphthoquinones were reported to be cytotoxic against a number of tumor cell lines.[3] Because of their effectiveness in drug-resistant cells, these agents appear to hold promise as effective chemotherapeutic agents.[3]

The dual specificity phosphatases (CDC25), play a pivotal role in the regulation of the cell cycle by activating cyclin-dependent kinases (CDK)[4] and participating in Raf-1-mediated cell signaling.[5] Three CDC25 homologues exist in human, CDC25A, CDC25B and CDC25C.[4,6–8] Over-expression of CDC25A and B was observed in a variety of cancers with a striking association with tumor aggressiveness and poor prognosis,[9–12] which making CDC25 an attractive drug target for cancer therapy. Over the last few years, some small molecule CDC25 inhibitors have been described in the literature.[13–28] Some quinones, including *ortho*-quinones, displayed inhibitory activity against CDC25.

The rhizome of *Salvia miltiorrhiza* Bunge, also known as 'Tanshen' or 'Danshen', is an ancient drug in Chinese traditional medicine,[29] which has been used widely to treat coronary heart disease, menstrual disorders, miscarriage, hypertension, and viral hepatitis.[30] There have been more than 50 *ortho*-quinone diterpenes, called tanshinones, isolated from Danshen. In our program of high-throughput screening for CDC25 protein phosphatases inhibitors, the tanshinones were chosen for evaluation. We found that (−)-cryptotanshinone (Fig. 1) was a moderate inhibitor of CDC25B phosphatase (IC_{50} 10.98 μM). Cryptotanshinone is a typical compound having *ortho*-quinone skeleton among these tanshinones. Cryptotanshinone has been reported to be an effective inhibitor of topoisomerase I[31] and exhibit significant cytotoxicity against a number of cultured human tumor cell lines.[32]

Although there have been many synthetic studies of the tanshinones,[33–39] these synthetic routes are not convenient for us to further modify these compounds because of the unavailable materials and harsh reactions. In order to further study the biological activities of the tanshinonnes, we have developed a facile strategy to prepare (±)-cryptotanshinone **11** via a SmI_2 promoted radical cyclization reaction.[40] Using this strategy, we synthesized new simplified analogues of

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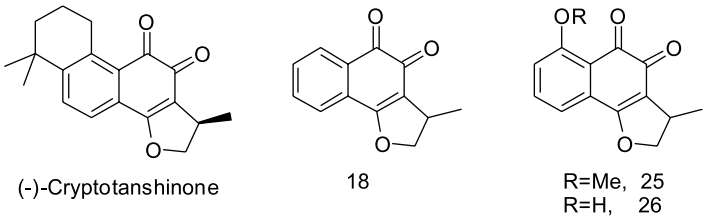


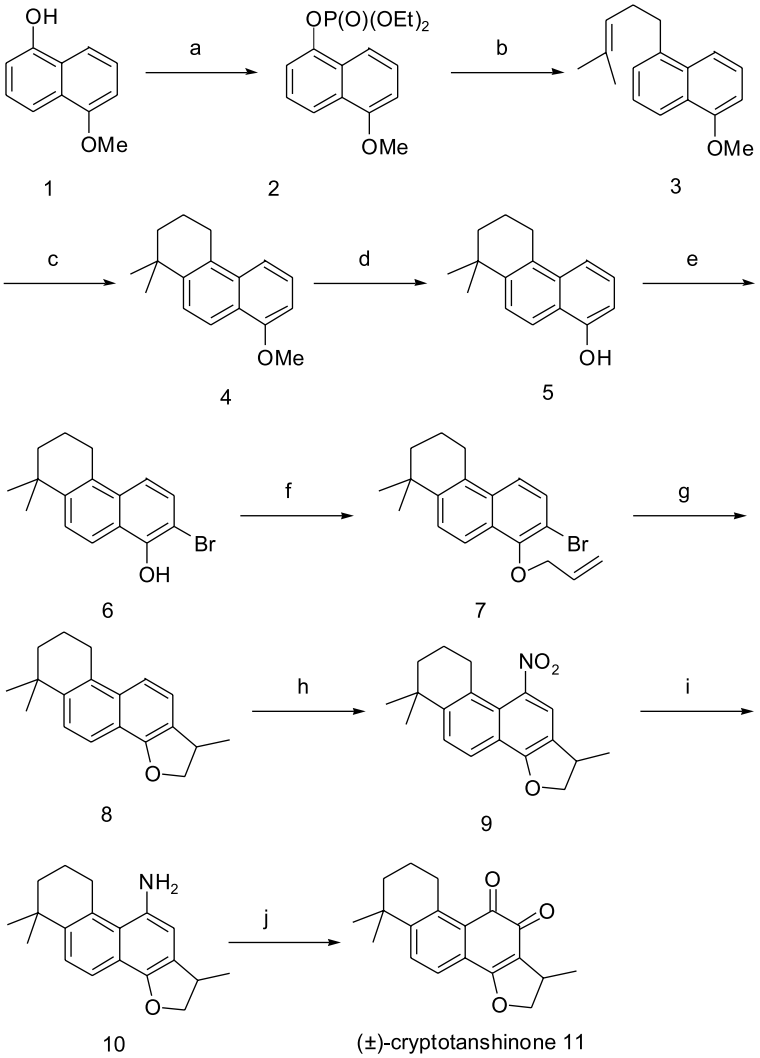
Figure 1.

(±)-cryptotanshinone **11** to investigate the structure–activity relationship as CDC25 inhibitors. The simplified analogues **18** and **26** demonstrated powerful inhibitory activity against protein phosphatase CDC25B and cytotoxic activity against A-549 tumor cell lines.

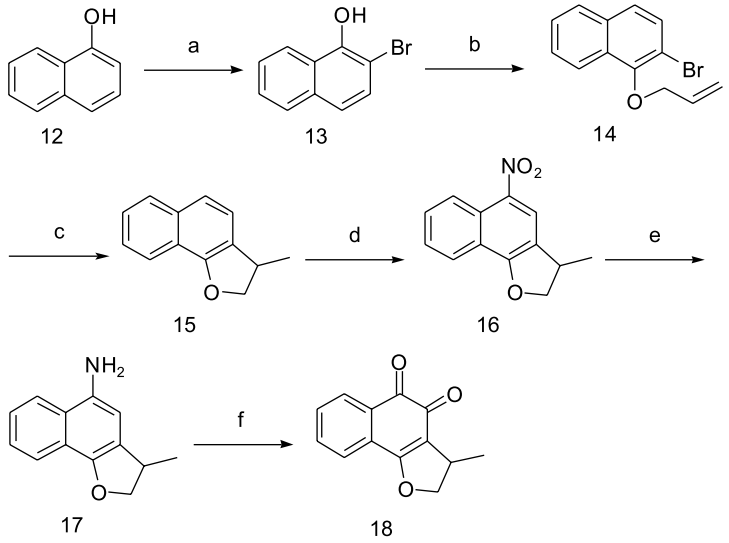
2. Results and discussion

Cryptotanshinone is tetracyclic compound, and its B and C rings are basic naphthalene structure. We use the accessible 5-methoxy-naphthalen-1-ol **1**[41] as starting material. Firstly, we constructed the A ring of cryptotanshinone. Compound **1** was treated with NaH and diethyl phosphorochloridate in dry THF to give aryl diethyl phosphates **2**. In the presence of $NiCl_2$(dppp), compound **3** was obtained via cross-coupling of **2** with Grignard reagent derived from 1-bromo-4-methylpent-3-ene.[42] Then, cyclization of **3** to **4** proceeded in excellent yield upon exposure $AlCl_3$ in CH_2Cl_2 at 0 °C for 30 min[39] to finish the building of A ring (Scheme 1).

Then, we built the naphthofuran (D ring) through the SmI_2-initiated radical cyclization reaction.[43] The treatment of **4** with BBr_3 at 0 °C provided the naphthol **5** in 95%.[44] Bromine diluted with carbon tetrachloride was added dropwise to **5** in carbon tetrachloride at the condition of



Scheme 1. Reagents and conditions: (a) $ClP(O)(OEt)_2$, NaH, THF; (b) $C_6H_{11}MgBr$, cat. $NiCl_2$ (dppp), Et_2O; (c) $AlCl_3$, CH_2Cl_2, 0 °C; (d) BBr_3, CH_2Cl_2, 0 °C; (e) Br_2, CCl_4; (f) allyl bromide, K_2CO_3, acetone; (g) SmI_2, HMPA, THF; (h) HNO_3, AcOH, 0 °C; (i) H_2, Pd/C, EtOH; (j) Fremy's salt, 0.06 M NaH_2PO_4.



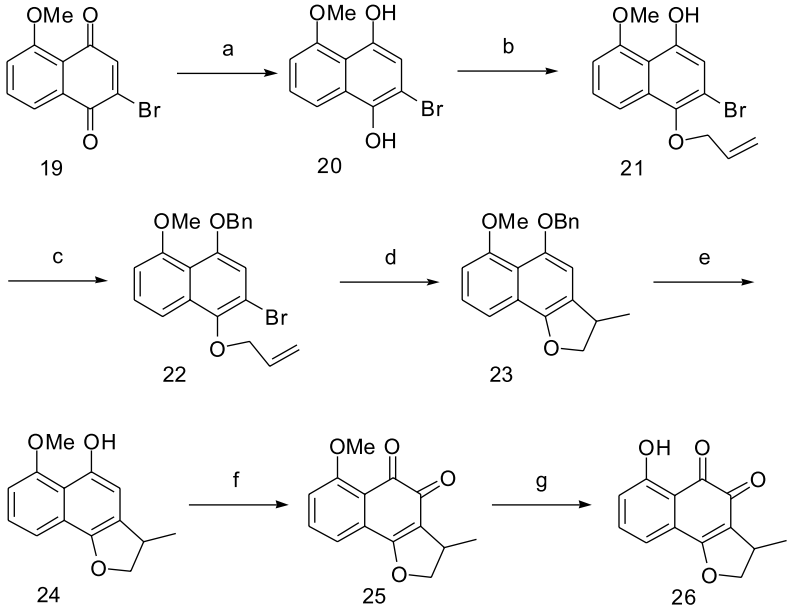
Scheme 2. Reagents and conditions: (a) Br_2, CCl_4, 24 h; (b) allyl bromide, K_2CO_3, acetone; (c) SmI_2, HMPA, THF; (d) HNO_3, AcOH; (e) H_2, Pd/C, EtOH; (f) Fremy's salt, 0.06 M NaH_2PO_4.

ice-water bath to afford **6**.[41] The desired product **6** was treated with allyl bromide and K_2CO_3 to yield allyl ether **7** as colorless oil.[45] Conversion of **7** into naphthofuran compound **8** via SmI_2-promoted intramolecular cyclization was executed in good yield.[43]

After constructing of A and D rings, we need to build *ortho*-quinone group to finish our synthesis of (±)-cryptotanshinone. By using mild aromatic nitration condition, **8** was smoothly converted into the nitrated derivative **9**, which was catalytically reduced to amine **10**.[46] Without further purification, compound **10** was oxidized directly with Fremy's salt to give (±)-cryptotanshinone **11** as an orange needles.[47]

Compound **18**, a simplified analogue of (±)-cryptotanshinone without the A ring, was prepared in six steps with a similar procedure, using 1-naphthol as the starting material (Scheme 2).

The analogue **26**, which possesses a hydroxy group instead of A ring was synthesized, as shown in Scheme 3. The intermediate **19** was prepared in four steps in high yield according to the literature method.[48] The reduction of **19** with $Na_2S_2O_4$ afforded the unstable naphthol **20**. Compound **20** was dissolved in acetone immediately, and treated with allyl bromide and potassium carbonate to give **21**. The protection of another hydroxy group of **21** with a benzyl group afforded **22** in 51% yield (from **19**). The dihydronaphthofuran was constructed using a similar procedure as described above. The catalytic hydrogenation of **23** and the following oxidation of **24** with $AgNO_3$ in absolute ethanol



Scheme 3. Reagents and conditions: (a) $Na_2S_2O_4$, Et_2O/H_2O; (b) allyl bromide, K_2CO_3, acetone; (c) BnBr, K_2CO_3, acetone; (d) SmI_2, HMPA, THF; (e) H_2, Pd/C, EtOAc; (f) $AgNO_3$, absolute EtOH; (g) $AlCl_3$, CH_2Cl_2.

Table 1. CDC25B inhibitory activities of cryptotanshinones and analogues

Compound	(−)-Cryptotanshinone	(±)-Cryptotanshinone **11**	**18**	**25**	**26**
IC_{50} (μM)	10.98±1.16	20.63±1.29	4.96±0.22	>81.89	3.21±0.76

Table 2. Cytotoxic activity against A-549 tumor cell line of cryptotanshinones and analogues

Compound	(−)-Cryptotanshinone	(±)-Cryptotanshinone **11**	**18**	**25**	**26**
IC_{50} (μM)	6.39	8.23	3.65	9.46	2.07

yielded the desired product **25** as orange solid. Compound **26** was prepared by the treatment of **25** with $AlCl_3$ in CH_2Cl_2.

Cryptotanshinones and its analogues were evaluated for their CDC25B inhibitory activity (Table 1). Natural (−)-cryptotanshinone, our synthetic (±)-cryptotanshinone (**11**) and its analogues **18** and **26** inhibited CDC25B in vitro with IC_{50} values from 3.21 to 20.63 μM. Interestingly, (−)-cryptotanshinone showed a CDC25B inhibitory activity 2-fold higher than its racemate (**11**), which proved that the stereochemistry of the C-3 in furan ring played an important role in the activity against CDC25B phosphatase. The absence of the A ring of cryptotanshinone could increase the activity. The simplified analogues **18** (IC_{50} 4.96 μM), which lack of the A ring, showed a higher CDC25B inhibitory activity than (±)-cryptotanshinone in four folds. So the naphthofuran *ortho*-quinone skeleton seems necessary for CDC25B inhibitory activity. The analogue **26**, which possesses a hydroxy group instead of A ring, was the most potent (IC_{50} 3.21 μM). However, protecting the hydroxy group with a methyl group (compound **25**), led to a complete loss of activity. In cytotoxic assay, (−)-cryptotanshinone, **11**, **18**, **25** and **26** were cytotoxic against A-549 tumor cells with IC_{50} values of 6.39, 8.23, 3.65, 9.46 and 2.07 μM (Table 2), respectively. The simplified analogues **18** and **26** also demonstrated more potent cytotoxic activity against tumor cells than cryptotanshinones.

3. Conclusion

In summary, we reported a novel synthetic method for (±)-cryptotanshinone and its new simplified analogs. The simplified analogues **18** and **26** were identified as micromolar inhibitors of CDC25B, and demonstrated powerful cytotoxic activity against A-549 tumor cell line. The A ring of cryptotanshinone was not necessary for its CDC25B inhibitory activity and cytotoxic activity. The stereochemistry of the C-3 in furan ring played an important role in the activity against CDC25B phosphatase. Thus these naphthofuran *ortho*-quinones would serve as attractive lead molecules of drug development efforts against the CDC25 phosphatase. The further investigation of the effect of the stereochemistry of the C-3 in the analogues on the biological activity is in process in our laboratory.

4. Experimental

4.1. General

Melting points were taken on Buchi510 apparatus and were uncorrected. ^1H and ^{13}C NMR spectra were recorded on either Gemini-300 or Bruker AM-400. Chemical shifts (δ ppm) were reported for signal center, and coupling constant J are reported in units of Hz. High-resolution mass spectra were recorded on Varian MAT-711, MAT-95 or HT-5989 mass spectrometer. Column chromatography was performed on 200–300 mesh silica gel. All reagents were used directly as obtained commercially, unless otherwise noted.

4.1.1. Phosphoric acid diethyl ester 5-methoxy-naphthalen-1-yl ester (2). To a solution of **1** (1.74 g, 10 mmol) in 30 mL of THF at 0 °C was added NaH (80% dispersion in mineral oil) (0.36 g, 12 mmol). After 30 min, diethyl phosphorochloridate (1.60 mL, 11 mmol) was added and stirred for additional 20 min. Water was added and the mixture was extracted with ethyl acetate. The organic extracts were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The crude residue was purified by column chromatography (petroleum/CH_2Cl_2/acetone=15:4:1) to afford **2** as a red-brown oil (2.48 g, 80%); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.32 (t, J=7.1 Hz, 6H), 3.96 (s, 3H), 4.22 (q, J=7.1 Hz, 4H), 6.82 (d, J=7.7 Hz, 1H), 7.33 (m, 2H), 7.49 (d, J=7.7 Hz, 1H), 7.72 (d, J=8.5 Hz, 1H), 8.07 (d, J=8.2 Hz, 1H).

4.1.2. 1-Methoxy-5-(4-methyl-pent-3-enyl)-naphthalene (3). To a mixture of $C_6H_{11}MgBr$ (4.8 mmol in 3.6 mL THF) and $NiCl_2$ (dppp) was added a solution of **2** (1.6 mmol, 0.5 g) in THF (3 mL), and the mixture was stirred under an atmosphere of nitrogen overnight. The mixture was poured into ice water and extracted with ethyl acetate. The organic extract was washed with 1 N HCl and brine, dried over $MgSO_4$, and concentrated in vacuo. The crude residue was purified by column chromatography (petroleum/ethyl acetate=20:1) to afford **3** as colorless oil (0.174 g, 45%); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.58 (s, 3H), 1.71 (s, 3H), 2.47 (m, 2H), 3.11 (t, J=7.9 Hz, 2H), 4.02 (s, 3H), 5.33 (m, 1H), 6.84 (d, J=7.5 Hz, 1H), 7.34–7.42 (m, 3H), 7.66 (d, J=8.6 Hz, 1H), 8.18 (d, J=8.2 Hz, 1H); EIMS (*m*/*z*): 240 (M^+), 171, 158, 143, 128, 115.

4.1.3. 8-Methoxy-1,1-dimethyl-1,2,3,4-tetrahydro-phenanthrene (4). A solution of 3 (2.0 g, 8.33 mmol) in 60 mL of CH_2Cl_2 was cooled to 0 °C. $AlCl_3$ (1.2 g, 9 mmol)

was added in one portion. The solution was stirred at 0 °C for 30 min and then poured into ice water. The aqueous phase was separated and extracted with diethyl ether, and the combined organic phase was washed with water, saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated in vacuo. Column chromatography (petroleum/ethyl acetate=20:1) furnished **4** as a yellow oil (1.9 g, 95%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.35 (s, 6H), 1.73 (m, 2H), 1.94 (m, 2H), 3.10 (t, J=6.5 Hz, 3H), 3.98 (s, 3H), 6.78 (d, J=7.6 Hz, 1H), 7.38 (m, 1H), 7.48 (d, J=8.2 Hz, 1H), 7.57 (d, J=8.2 Hz, 1H), 8.10 (d, J=9.0 Hz, 1H); EIMS (*m*/*z*): 240 (M^+), 225, 158, 143, 115; HRMS calcd for $C_{17}H_{20}O$ 240.1514, found 240.1519.

4.1.4. 8,8-Dimethyl-5,6,7,8-tetrahydro-phenanthren-1-ol (5). A solution of **4** (2.0 g, 8.33 mmol) in 40 mL of dry CH_2Cl_2 was cooled to 0 °C. BBr_3 (3 mL) was added dropwise. The solution was stirred at 0 °C for 2 h, then saturated $NaHCO_3$ solution was added slowly. The mixture was extracted with diethyl ether, and the combined organic phase was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Column chromatography (petroleum/ethyl acetate=8:1) furnished **5** (1.79 g, 95%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.35 (s, 6H), 1.72 (m, 2H), 1.94 (m, 2H), 3.08 (t, J=6.3 Hz, 2H), 6.76 (d, J=7.5 Hz, 1H), 7.29 (dd, J=8.5, 7.5 Hz, 1H), 7.49 (d, J=7.3 Hz, 1H), 7.55 (d, J=8.7 Hz, 1H), 7.98 (d, J=8.8 Hz, 1H); EIMS (*m*/*z*): 226 (M^+), 211, 115; HRMS calcd for $C_{16}H_{18}O$ 226.1358, found 226.1354.

4.1.5. 2-Bromo-8,8-dimethyl-5,6,7,8-tetrahydro-phenanthren-1-ol (6). Compound **5** (1.0 g, 4.42 mmol) was dissolved in CCl_4 and bromine (0.71 g, 4.43 mmol) was added dropwise at 0 °C. The mixture was stirred for 1 h, and then 3% $Na_2S_2O_3$ was added. After being stirred for additional 10 min, the mixture was extracted with CH_2Cl_2. The organic phase was washed sequentially with sodium thiosulfate, water and brine, dried over $MgSO_4$, and concentrated in vacuo. The crude residue was purified by column chromatography (petroleum/ethyl acetate=15:1) to afford **6** (1.21 g, 90%) as colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.34 (s, 6H), 1.72 (m, 2H), 1.93 (m, 2H), 3.05 (t, J=6.32 Hz, 2H), 5.87 (s, 1H), 7.41 (d, J=8.79 Hz, 1H), 7.45 (d, J=9.06 Hz, 1H), 7.50 (d, J=9.06 Hz, 1H), 8.04 (d, J=8.78 Hz, 1H); EIMS (*m*/*z*): 306, 304, 291, 289, 210; HRMS calcd for $C_{16}H_{17}OBr$ 304.0463, found 304.0467.

4.1.6. 8-Allyloxy-7-bromo-1,1-dimethyl-1,2,3,4-tetrahydro-phenanthrene (7). K_2CO_3 (1.98 g, 15.7 mmol) was added to a solution of **6** (1.2 g, 3.93 mmol) dissolved in 40 mL of acetone. Allyl bromide was added in one portion and the mixture was stirred at room temperature for 4 h. The mixture was filtered and concentrated in vacuo. The crude residue was purified by column chromatography (petroleum/ethyl acetate=20:1) to afford **7** as colorless oil (1.29 g, 95%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.36 (s, 6H), 1.72–1.75 (m, 2H), 1.92–1.97 (m, 2H), 3.09 (t, J=6.42 Hz, 2H), 4.61 (m, 2H), 5.36 (m, 1H), 5.55 (m, 1H), 6.25 (m, 1H), 7.54 (d, J=8.99 Hz, 1H), 7.57 (d, J=9.16 Hz, 1H), 7.64 (d, J=9.17 Hz, 1H), 7.97 (d, J=8.98 Hz, 1H); EIMS (*m*/*z*): 346, 344, 305, 303, 196; HRMS calcd. for $C_{19}H_{21}OBr$ 344.0776, found 344.0779).

4.1.7. 1,6,6-Trimethyl-1,2,6,7,8,9-hexahydro-phenanthro[1,2-*b*]furan (8). To a 0.1 M THF solution of SmI_2 (40 mL, 2 mmol) and HMPA (2.4 mL, 14 mmol) was added a solution of **7** (0.317 g, 0.92 mmol) in dry THF at 25 °C. The solution was stirred under an atmosphere of nitrogen for 3 h. The reaction was quenched with saturated NH_4Cl and extracted with ethyl acetate. The organic extracts were washed with H_2O, 3% $Na_2S_2O_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by column chromatography (petroleum/ethyl acetate=20:1) to afford **8** as a colorless oil (0.215 g, 88%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.38–1.42 (m, 9H), 1.74–1.78 (m, 2H), 1.93–1.99 (m, 2H), 3.11 (t, J=6.30 Hz, 2H), 3.73 (m, 1H), 4.30 (dd, J=7.00, 8.70 Hz, 1H), 4.89 (t, J=8.70, 9.00 Hz, 1H), 7.34 (d, J=8.70 Hz, 1H), 7.45 (d, J=8.65 Hz, 1H), 7.51 (d, J=8.62 Hz, 1H), 7.82 (d, J=8.70 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.5, 142.4, 132.9, 130.8, 127.8, 125.6, 124.6, 121.5, 119.2, 115.8, 79.1, 38.8, 37.3, 37.2, 34.2, 31.5, 31.4, 27.2, 19.9, 19.8, 19.6; EIMS (*m*/*z*): 266, 251, 169; HRMS calcd for $C_{19}H_{22}O$ 266.1671, found 266.1662.

4.1.8. 1,6,6-Trimethyl-10-nitro-1,2,6,7,8,9-hexahydro-phenanthro[1,2-*b*]furan (9). Compound **8** (0.212 g, 0.8 mmol) and AcOH (0.35 mL) were cooled to 0 °C and concentrated HNO_3 (0.04 mL) was added dropwise. After this addition, the mixture was placed in the refrigerator at 10 °C for 20 min and then diluted with H_2O. The resulting precipitate was extracted with ethyl acetate. The organic extracts were washed with H_2O and brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by column chromatography (petroleum/ethyl acetate=20:1) to afford **9** as yellow oil (0.216 g, 87%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.35 (s, 6H), 1.38 (d, J=6.87 Hz, 3H), 1.68–1.77 (m, 4H), 2.76 (t, J=6.05 Hz, 2H), 3.75 (m, 1H), 4.37 (dd, J=7.14, 8.93 Hz, 1H), 4.96 (dd, J=9.07, 9.20 Hz, 1H), 7.59 (d, J=8.93 Hz, 1H), 7.68 (s, 1H), 7.87 (d, J=8.93 Hz, 1H); EIMS (*m*/*z*): 311, 294, 251; HRMS calcd for $C_{19}H_{21}NO_3$ 311.1521, found 311.1524.

4.1.9. (±)-Cryptotanshinone 11. Compound **9** (0.2 g, 0.643 mmol) in EtOH was hydrogenated over 10% Pd/C (40 mg) for 4.5 h. The catalyst was filtered off and the solvent was removed in vacuo to give the amine **10**. The crude amine was dissolved in acetone (10 mL) and treated with Fremy's salt (550 mg, 2.06 mmol) in 55 mL of 0.06 M NaH_2PO_4. The mixture was stirred at room temperature for 30 min and extracted with CH_2Cl_2. The combined extracts were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by column chromatography (petroleum/ethyl acetate=6:1) to afford **11** as an orange solid (95 mg, 50%), mp 172–173 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.31 (s, 6H), 1.33 (d, J=6.87 Hz, 3H), 1.64–1.67 (m, 2H), 1.76–1.81 (m, 2H), 3.22 (t, J=6.42 Hz, 2H), 3.60 (m, J=6.79 Hz, 1H), 4.36 (dd, J=6.04, 9.33 Hz, 1H), 4.89 (dd, J=9.48, 9.48 Hz, 1H), 7.50 (d, J=8.10 Hz, 1H), 7.62 (d, J=8.10 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 184.2, 175.6, 170.7, 152.3, 143.6, 132.5, 128.3, 126.2, 122.4, 118.2, 81.4, 37.7, 34.8, 34.5, 31.8 (2C), 29.6, 19.0, 18.8; EIMS (*m*/*z*): 296, 253, 171; HRMS calcd for $C_{19}H_{20}O_3$ 296.1412, found 296.1404.

4.1.10. 2-Bromo-1-naphthalenol (13). According to the

procedure described above for **6**, compound **12** (4.14 g, 28.7 mmol) was converted to **13** (5.314 g, 83%) as white needles: mp: 46–47 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.00 (s, 1H), 7.32 (d, J=8.8 Hz, 1H), 7.47 (d, J=8.9 Hz, 1H), 7.49–7.54 (m, 2H), 7.78 (m, 1H), 8.24 (m, 1H); EIMS(*m*/*z*): 224 (M^+), 222 (M^+), 143, 144, 114, 115.

4.1.11. 1-Allyloxy-2-bromonaphthalene (14). According to the procedure described above for **7**, compound **13** (4.00 g, 17.9 mmol) was converted to **14** (4.615 g, 98%) as a pale yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.65 (d, J= 5.5 Hz, 2H), 5.35 (dd, J=10.2, 1.2 Hz, 1H), 5.54 (dd, J= 17.1, 1.4 Hz, 1H), 6.25 (m, 1H), 7.48–7.62 (m, 4H), 7.84 (dd, J=6.8, 2.3 Hz, 1H), 8.15 (dd, J=8.2, 1.8 Hz, 1H); EIMS (*m*/*z*): 264 (M^+), 262 (M^+), 223, 221, 195, 193, 183, 181, 114.

4.1.12. 2,3-Dihydro-3-methylnaphtho[1,2-*b*]furan (15). According to the procedure described above for **8**, compound **14** (0.526 g, 2.00 mmol) was converted to **15** (0.360 g, 98%) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (d, J=6.8 Hz, 3H), 3.75 (m, 1H), 4.32 (dd, J=7.4, 8.6 Hz, 1H), 4.92 (dd, J=9.1, 8.8 Hz, 1H), 7.36 (d, J=8.1 Hz, 1H), 7.41–7.52 (m, 3H),7.84 (dd, J=6.8, 2.7 Hz, 1H), 8.01 (dd, J=7.8, 2.4 Hz, 1H); EIMS (*m*/*z*): 184 (M+), 169, 141, 115.

4.1.13. 2,3-Dihydro-3-methy-5-nitronaphtho[1,2-*b*]furan (16). According to the procedure described above for **9**, compound **15** (0.360 g, 1.957 mmol) was converted to **16** (0.251 g, 56%) as an orange solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.45 (d, J=6.9 Hz, 3H), 3.80 (m, 1H), 4.45 (dd, J=7.0, 9.1 Hz, 1H), 5.04 (dd, J=9.1, 9.1 Hz), 7.56 (dd, J= 8.0, 8.2 Hz, 1H), 7.70 (dd, J=8.3, 8.8 Hz, 1H), 8.06 (d, J= 8.4 Hz, 1H), 8.36 (s, 1H), 8.83 (d, J=9.0 Hz, 1H); EIMS (*m*/*z*): 229 (M^+), 214, 199, 168, 139.

4.1.14. 2,3-Dihydro-3-methylnaphtho[1,2-*b*]furan-4,5-dione (18). According to the procedure described above for (±)-cryptotanshinone **11**, compound **16** (0.20 g, 0.87 mmol) was converted to **18** (102 mg, 55%) as a red solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.38 (d, J=6.7 Hz, 3H), 3.64 (m, 1H), 4.42 (dd, J=9.31, 9.47 Hz, 1H), 4.93 (dd, J=9.62, 9.63 Hz, 1H), 7.55–7.66 (m, 3H), 8.08 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.3, 175.4, 169.8, 134.6, 131.9, 130.8, 129.4, 127.6, 124.3, 120, 81.5, 34.4, 18.9; EIMS (*m*/*z*): 214 (M^+), 186, 171; HRMS calcd for $C_{13}H_{10}O_3$ 214.06, found 214.0617.

4.1.15. 1-Allyloxy-4-benzoxy-5-methoxynaohthalene (22). A suspension of **19** (1.03 g, 3.86 mmol) in 50 mL diethyl ether was stirred with a freshly prepared solution of $Na_2S_2O_4$ (5.00 g, 28.7 mmol) in 50 mL water. After the mixture was stirred for 30 min, the organic layer was separated, washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo to give **20** as a tan solid. Without purification, **20** was dissolved in 20 mL of acetone and K_2CO_3 (2.92 g, 38.6 mmol) was added. After the suspension was stirred for several minutes, allyl bromide (0.934 g, 7.72 mmol) was added dropwise. The endpoint of the reaction was detected by TLC. Then the mixture was concentrated in vacuo to remove the solvent and remaining allyl bromide. The residue was dissolved in 20 mL of acetone, and benzyl bromide (1.915 g, 7.72 mmol) was added. The mixture was stirred at room temperature for 12 h, filtered with celite, and concentrated in vacuo. Purification by column chromatography (petroleum/ethyl acetate=30:1) afforded **22** (0.785 g, 51% from **19**) as colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.94 (s, 3H), 4.56 (m, 2H), 5.16 (s, 2H), 5.34 (m, 1H), 5.50 (m, 1H), 6.24 (m, 1H), 6.90–7.70 (m, 9H); EIMS (*m*/*z*): 400, 398, 307, 227; HRMS calcd for $C_{21}H_{19}BrO_3$ 398.0518, found 398.0538.

4.1.16. 5-Benzoxy-6-methoxy-3-methylnaphtho[1,2-*b*]furan (23). According to the procedure described above for **8**, compound **22** (0.64 g, 1.60 mmol) was converted to **23** (0.44 g, 85%) as a colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.36 (d, J=6.87 Hz, 3H), 3.71 (m, 1H), 3.82 (s, 3H), 4.25 (dd, J=7.83, 8.53 Hz, 1H), 4.85 (dd, J=8.53, 8.72 Hz, 1H), 5.22 (s, 2H), 6.83–7.57 (m, 9H); EIMS (*m*/*z*): 320, 229, 149; HRMS calcd for $C_{21}H_{20}O_3$ 320.1412, found 320.1419.

4.1.17. 2,3-Dihydro-6-methoxy-3-methylnaphtho[1,2-*b*]furan-4,5-dione (25). Compound **23** (0.312 g, 1.357 mmol) was dissolved in 20 mL of ethyl acetate, and hydrogenated over 10% Pd/C (50 mg) overnight. The catalyst was filtered off, and the solvent was removed in vacuo to give **24**. The crude product **24** was dissolved in 40 mL of absolute ethanol, and powdered $AgNO_3$ (1.454 g, 8.507 mmol) was added. After the mixture had been gently refluxed for 15 min, water was added to quench the reaction, followed by an extraction with dichloromethane. The extracts were washed with water and brine, dried over $MgSO_4$ concentrated in vacuo. The crude product was purified by chromatography (petroleum/ethyl acetate=3:1) to afford **25** (0.116 g, 50%) as an orange red solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.37 (d, J=6.89 Hz, 3H), 3.61 (m, 1H), 3.98 (s, 3H), 4.36 (dd, J=6.21, 9.23 Hz, 1H), 4.89 (dd, J=9.57, 9.57 Hz, 1H), 7.17 (d, J=8.56 Hz, 1H), 7.27 (d, J=7.39 Hz, 1H), 7.59 (dd, J=7.39, 8.56 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 18.5, 34.5, 56.1, 81.1, 116.5, 117.0, 117.5, 119.0, 129.1, 135.8, 161.5, 169.3, 175.2, 180.1; EIMS (*m*/*z*): 246, 244, 216, 201, 115; HRMS calcd. for $C_{14}H_{12}O_4$ 244.0736, found 244.0733.

4.1.18. 2,3-Dihydro-6-hydroxy-3-methylnaphtho[1,2-*b*]furan-4,5-dione (26). To the solution of **25** (80 mg, 0.328 mmol) in 10 mL of dry CH_2Cl_2, was added powdered $AlCl_3$ (0.660 g, 4.96 mmol) at 0 °C. The mixture was stirred at 0 °C for 30 min, then at room temperature for 6 h, poured into ice water, and concentrated hydrochloric acid was added. The resulting mixture was extracted with ethyl acetate. The extracts were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the crude product by chromatography (petroleum/ethyl acetate=1:1) afforded **26** (74.5 mg, 98%) as a red solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40 (d, J=6.8 Hz, 3H), 3.60 (m, 1H), 4.38 (dd, J=6.04, 9.34 Hz, 1H), 4.90 (dd, J=9.62, 9.61 Hz, 1H), 7.12 (d, J=8.65 Hz, 1H), 7.18 (d, J= 7.32 Hz, 1H), 7.54 (dd, J=8.65, 7.28 Hz, 1H), 11.94 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 18.8, 34.8, 81.7, 113.4, 117.6, 120.1, 123.3, 127.4, 137.8, 164.5, 169.4, 175.0, 185.5; EIMS (*m*/*z*): 230, 187, 159; HRMS calcd for $C_{13}H_{10}O_4$ 230.0579, found 230.0570.

4.2. CDC25B inhibition assay

CDC25B phosphatase catalytic domain was expressed with the Glutathionine *S*-transferase (GST) and purified by the GSTrap affinity chromatograph. GST-CDC25B active enzyme was stored in 50 mM Tris–HCl Ph 8.0, 50 mM NaCl, 10 mM Glutathionine, 2 mM DTT and 2 mM EDTA at −80 °C. The typical inhibition assay was carried out in 100 μL system containing 50 mM Tris–HCl PH8.0, 50 mM NaCl, 2 mM DTT, 2 mM EDTA, 1% glycerol, 10 μM OMEP, 2% DMSO and 70∼100 nM GST-CDC25B. The reaction was monitored by Victor (Perkin–Elmer; excitation filter 485 nm and emission filter 530 nm) at the room temperature.

4.3. Cytotoxicity assay

Cytotoxicity assay was performed on human lung cancer (A-549) cell line. Cells (6000–10,000) in 100 μL culture medium per well were seeded into 96-well microtest plates (Falcon, CA). Cells were treated in triplicate with gradient concentration of test drugs and incubated at 37 °C for 72 h. The growth inhibitory effect on A-549 cell line was measured by the Sulforhodamine B (SRB; Sigma, St. Louis, MO) assay. The drug concentration required for 50% growth inhibition (IC_{50}) of tumor cells was determined from the dose-response curve.

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References and notes

1. Gantchev, T. G.; Hunting, D. J. *Biochem. Biophys. Res. Commun.* **1997**, *237*, 24–27.
2. Driscoll, J. S.; Hazard, G. F. Jr.; Wood, H. B. Jr.; Goldin, A. *Cancer. Chemother. Rep.* **1974**, *4*, 1–362.
3. Dolan, M. E.; Frydman, B.; Thompson, C. B.; Diamond, A. M.; Garbiras, B. J.; Safa, A. R.; Beck, W. T.; Marton, L. J. *Anti-Cancer Drugs* **1998**, *9*, 437–448.
4. Nilsson, I.; Hoffmann, I. *Prog. Cell Cycle Res.* **2000**, *4*, 107–114.
5. Xia, K.; Lee, R. S.; Narsimhan, R. P.; Mukhopadhyay, N. K.; Neel, B. C.; Roberts, T. M. *Mol. Cell. Biol.* **1999**, *19*, 4819–4824.
6. Galaktionov, K.; Beach, D. *Cell* **1991**, *67*, 1181–1194.
7. Sadhu, K.; Reed, S. I.; Richardson, H.; Russell, P. *Prog. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 5139–5143.
8. Nagata, A.; Igarashi, M.; Jinno, S.; Suto, K.; Okayama, H. *New Biol.* **1991**, *3*, 959–968.
9. Sasaki, H.; Yukiue, H.; Kobayashi, Y.; Tanahashi, M.; Moriyama, S.; Nakashima, Y.; Fukai, I.; Kiriyama, M.; Yamakawa, Y.; Fujii, Y. *Cancer Lett.* **2001**, *173*, 187–192.
10. Cangi, M. G.; Gukor, B.; Soung, P.; Signoretti, S.; Moreira, G. Jr.; Ranashinge, M.; Cady, B.; Pagano, M.; Loda, M. *J. Clin. Investig.* **2000**, *106*, 753–761.
11. Takemasa, I.; Yamamoto, H.; Sekimoto, M.; Ohue, M.; Noura, S.; Miyake, Y.; Matsumoto, T.; Aihara, T.; Tomita, N.; Tamaki, Y.; Sakita, I.; Kikkawa, N.; Matsuura, N.; Shiozaki, H.; Monden, M. *Cancer Res.* **2000**, *60*, 3043–3050.
12. Hernandez, S.; Bessa, X.; Bea, S.; Hernmandez, L.; Nadal, A.; Mallofre, C.; Muntane, J.; Castells, A.; Fernandez, P. L.; Cardesa, A.; Campo, E. *Lab. Investig.* **2001**, *81*, 465–473.
13. Wu, F. Y. H.; Sun, T. P. *Eur. J. Cancer* **1999**, *35*, 1388–1393.
14. Tamura, K.; Rice, R. L.; Wipf, P.; Lazo, J. S. *Oncogene* **1999**, *18*, 6989–6996.
15. Tamura, K.; Southwick, E. C.; Kerns, J.; Rosi, K.; Carr, B. I.; Wilcox, C.; Lazo, J. S. *Cancer Res.* **2000**, *60*, 1317–1325.
16. Takahashi, M.; Dodo, K.; Sugimoto, Y.; Aoyagi, Y.; Yamada, Y.; Hashimoto, Y.; Shirai, R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2571–2574.
17. Peng, H.; Zalkow, L. H.; Abraham, R. T.; Powis, G. *J. Med. Chem.* **1998**, *41*, 4677–4680.
18. Gunasekera, S. P.; McCarthy, P. J.; Kelly-Broger, M.; Lobkovsky, E.; Clardy, J. *J. Am. Chem. Soc.* **1996**, *118*, 8759–8760.
19. Blanchard, J. L.; Epstein, D. M.; Boisclair, M. D.; Rudolph, J.; Pal, K. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2537–2538.
20. Cebula, R. E.; Blanchard, J. L.; Boisclair, M. D.; Mansuri, M.; Pal, K.; Bockovich, N. J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2015–2020.
21. Juan, C. C.; Wu, F. Y. *Biochem. Biophys. Res. Comm.* **1993**, *190*, 907–913.
22. Rice, R. L.; Rusnak, J. M.; Yokokawa, F.; Messner, D. J.; Boynton, A. L.; Wipf, P.; Lazo, J. S. *Biochemistry* **1997**, *36*, 15965–15974.
23. Bergnes, G.; Gilliam, C. L.; Boisclair, M. D.; Blanchard, J. L.; Blake, K. V.; Epstein, D. M.; Pal, K. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2849–2854.
24. Peng, H.; Xie, W.; Otterness, D. M.; Cogswell, J. P.; McConnel, R. T.; Carter, H. L.; Powis, G.; Abraham, R. T.; Zalkow, L. H. *J. Med. Chem.* **2001**, *44*, 834–848.
25. Lazo, J. S.; Aslan, D. C.; Pestell, K. E.; Cooley, K.; Ducruet, A. P.; Joo, B.; Vogt, A.; Wipf, P. *J. Med. Chem.* **2001**, *44*, 4042–4049.
26. Lazo, J. S.; Nemoto, K.; Pestell, K. E.; Cooley, K.; Southwick, E. C.; Mitchell, D. A.; Furey, W.; Gussio, R.; Zaharevitz, D. W.; Joo, B.; Wipf, P. *Mol. Pharmacol.* **2002**, *61*, 720–728.
27. Pu, L.; Amoscato, A. A.; Bier, M. E.; Lazo, J. S. *J. Biol. Chem.* **2002**, *277*, 46877–46885.
28. Wipf, P.; Joo, B.; Nguyen, T.; Lazo, J. S. *Org. Biomol. Chem.* **2004**, *2*, 2173–2174.
29. Duke, J. A.; Avensu, E. S. In *Medicinal Plants of China*, *Vol. 2*; Reference: Algonac, MI, 1985; p 381.
30. Chang, H. M., But, P., Eds.; Pharmacology and Applications of Chinese Materia Medica; World Science: Singapore, 1986; Vol. 1, pp 255–268.
31. Lee, D. S.; Hong, S. D. *J. Microbiol. Biotechnol.* **1998**, *1*, 89–91.
32. Ryu, S. Y.; Lee, C. O.; Choi, A. U. *Planta Med.* **1997**, *63*, 339–342.
33. Baillie, A. C.; Thomson, R. H. *J. Chem. Soc. C* **1968**, 48–52.
34. Kakisawa, H.; Tateishi, M.; Kusumi, T. *Tetrahedron Lett.* **1968**, *34*, 3783–3786.
35. Kakisawa, H.; Inouye, Y. *J. Chem. Soc., Chem. Commun.* **1968**, 1327–1328.
36. Inouye, Y.; Kakisawa, H. *Bull. Chem. Soc. Jpn* **1969**, *42*, 3318–3323.
37. Lee, J.; Snyder, J. K. *J. Am. Chem. Soc.* **1989**, *111*, 1522–1524.

38. Lee, J.; Snyder, J. K. *J. Org. Chem.* **1990**, *55*, 4995–5008.
39. Danheiser, R. L.; Casebier, D. S.; Firooznia, F. *J. Org. Chem.* **1995**, *60*, 8341–8350.
40. Jiang, Y. Y.; Li, Q.; Lu, W.; Cai, J. C. *Tetrahedron Lett.* **2003**, *44*, 2073–2075.
41. Hannan, R. L.; Barber, R. B.; Rapoport, H. *J. Org. Chem.* **1979**, *44*, 2153–2158.
42. Hayashi, T.; Katsuro, Y.; Okamoto, Y.; Kumada, M. *Tetrahedron Lett.* **1981**, *22*, 4449–4452.
43. Inanaga, J.; Ujikawa, O.; Yamaguchi, M. *Tetrahedron Lett.* **1991**, *32*, 1737–1740.
44. Demuynck, M.; Clercq, M. *J. Org. Chem.* **1979**, *44*, 4863–4866.
45. Martin, S. F.; Garricon, P. J. *J. Org. Chem.* **1992**, *47*, 1513–1518.
46. Alves, G. B. C.; Lopes, R. S. C.; Lopes, C. C. *Synthesis* **1999**, 1875–1877.
47. Best, T. M.; Wege, D. *Aust. J. Chem.* **1986**, *39*, 647–666.
48. Jung, M. E.; Hagenah, J. A. *J. Org. Chem.* **1987**, *52*, 1889–1902.