Synthesis and Antitumor Activity of Staurosporine Derivatives

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Abstract
Twenty-four derivatives of staurosporine were synthesized by modification at the 3′-N, 3- and 7-positions. Of these compounds, 16 were synthesized for the first time and their structures were determined by NMR spectroscopy, ECD, and HRESIMS. Their inhibitory activities against seven tumor cell lines, MV4-11 (leukemia), MCF-7 (breast carcinoma), HCT-116 (colon cancer), TE-1 (esophageal carcinoma), PATU8988 T (pancreatic cancer), HOS (osteosarcoma) and GBC-SD (gallbladder cancer), and human normal liver cell L-02 were evaluated using a Cell Counting Kit-8. The IC50 values for 7-oxo-3-chloro-3′-N-benzoylstaurosporin (4) on MV4-11 and PATU8988 T cells were 0.078 and 0.666 μmol/L, and the selection indexes were 1254 and 147, respectively. The IC50 values of 7-oxo-3-chloro-3′-N-benzoylstaurosporin (5) and (7R)-7-hydroxy-3-bromo-3′-N-acetylstaurosporin (24) on MCF-7 cells were 0.029 and 0.021 μmol/L, and the selection indexes were 102 and 221, respectively. The above compounds have the potential to be developed further into antitumor drugs due to the advantages of high efficiency and low toxicity.

Keywords
alkaloids, staurosporine, halo-derivatives, antitumor activity, high efficiency and low toxicity

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Introduction
Staurosporine, with indolecarbazole skeleton, was first isolated from cultures of Streptomyces staurosorus in 19771 (Figure 1). This alkaloid is a broad-spectrum competitive protein kinase inhibitor and can strongly bind to about 90% of human ATP kinase.2,3 Due to its poor selectivity, staurosporine cannot be used as a candidate drug for further research. UCN-01, a new staurosporine derivative, was isolated from Streptomyces N-126 for the first time by Takahashi et al (Figure 1).4 The selective inhibitory activity of UCN-01 on kinases was significantly enhanced. However, in the clinical trials for monotherapy or combination therapy, UCN-01 showed poor clinical efficacy and serious adverse reactions.5–11 Midostaurin (PKC-412) (Figure 1), a semi-synthetic derivative of staurosporine, is a FMS-like tyrosine kinase 3 (Flt-3) inhibitor.12 In 2017, it was approved for the treatment of acute myeloid leukemia (AML) target to Flt-3. However, PKC-412 still has adverse reactions in clinical application, which caused 21% of the patients to stop treatment.13 A study of UCN-01 and PKC-412 found that structural modification at the 3′-N and C-7 positions could improve the druggability of staurosporine. In addition, halogen plays an important role in improving the effect of drugs.14 In the early research, we synthesized derivatives of staurosporine and their halogenated products.15–17 It was found that a halogen at the 3-position of staurosporine could increase the selectivity and activity against some tumor cells.

Based on the above studies, 24 derivatives (1-24) were synthesized by structural modification at the 3′-N, 3- and 7-positions of staurosporine, which was isolated from the fermentation of marine Streptomyces sp. OUCMDZ-3118.18 Then the Cell Counting Kit-8 (CCK-8) method was used to determine the inhibitory activity of 24 compounds on the proliferation of 7 tumor cells (human acute myeloid leukemia cell line MV4-11, human breast cancer cell line MCF-7, human colon cancer cell line HCT-116, human osteosarcoma cell line HOS, human pancreatic carcinoma cell line PATU8988 T, human esophageal carcinoma cell line TE-1, and human gallbladder carcinoma cell line GBC-SD) using a Cell Counting Kit-8 method. The inhibitory activity of the above compounds was determined by the Cell Counting Kit-8 method and the IC50 values were calculated. The results showed that the IC50 values of compounds (4) and (5) were 0.078 and 0.029 μmol/L, respectively. The selection indexes were 1254 and 102, respectively. The above compounds have the potential to be developed further into antitumor drugs due to the advantages of high efficiency and low toxicity.
cancer cell line MCF-7, human colon cancer cell line HCT-116, human esophageal cancer cell line TE-1, human pancreatic cancer cell line PATU8988 T, human osteosarcoma cell line HOS and human gallbladder cancer cell line GBC-SD, as well as human normal liver cell L-02, in order to obtain the target compounds with high inhibitory activity and low toxicity.

Results and Discussion

Design and Synthesis of Staurosporine Derivatives

The synthesis route is outlined in Figure 2. Compounds 1 (PKC-412) and 13 were obtained by the reaction of staurosporine with an acylation reagent. 3-Chloro derivatives 2 and 14, and 3-bromo derivatives 3 and 15 were synthesized by the electrophilic reaction of compounds 1 and 13 with N-chlorosuccinimide (NCS) and N-bromosuccinimide (NBS), respectively. Then the 7-position oxidation products 4-6 and 16-18 were obtained by oxidation of 1-3 and 13-15 with potassium tert-butoxide as base. Compounds 7-12 and 19-24 were synthesized by oxidation of compounds 1-3 and 13-15 with sodium hydroxide solution as base under an argon atmosphere.

The absolute configuration of the hydroxyl group at C-7 was determined by comparison of the relative strength of the Cotton effects in ECD spectra. The ECD patterns of compounds 8, 10, 12, 20, 22, 24, and UCN-01 were similar, which showed a strong positive Cotton effect around 300 nm. The ECD trends of compounds 7, 9, 11, 19, 21, 23, and UCN-02 were similar, which

Figure 1. Structures of staurosporine, UCN-01, and PKC-412.

Figure 2. Synthetic route of target compounds 1-24.
showed a weak positive effect around 300 nm (Figure 3). Therefore, the C-7 configuration of compounds 7, 9, 11, 19, 21, and 23 was determined to be 7S, and that of compounds 8, 10, 12, 20, 22, and 24 to be 7R. The ECD spectra showed that the configuration of the 7-hydroxyl group had a great influence on the ECD of such compounds, which could be applicable to determine the configuration of the 7-hydroxyl of staurosporine derivatives.

**Antitumor Activity**

Compounds 1-24 were evaluated for their inhibitory activities against seven tumor cells (MV4-11, MCF-7, HCT-116, TE-1, PATU8988 T, HO8, GBC-SD), as well as the human normal liver cell (L-02). In order to find highly active molecules, primary screening was carried out with a concentration of 1 μM, and the IC50 values were further tested with an inhibition rate greater than 50% at this concentration. The results showed that compounds 1-8, and 10-24 exhibited strong inhibitory activity against the human acute myelogenous leukemia MV4-11 cell line. Among them, the activities of compounds 13-15 and 18 (IC50 values from 0.011 to 0.015 μM) were stronger than that of PKC-412 (IC50 0.020 μM), but these compounds also showed high toxicity against the human L-02 cell line. Notably, compound 4 exhibited a strong selective inhibitory activity against the MV4-11 cell line (IC50 0.078 μmol/L), with a selection index (SI, the ratio of IC50 value of normal cell to tumor cell) of 1254, which was 3 times that of PKC-412 (SI 405). For human breast cancer MCF-7 cells, compounds 5, 12, and 24 exhibited selective inhibitory activity (IC50 values of 0.029, 0.183, and 0.021 μM) with SI values of 102, 76, and 221, respectively. The selectivity was significantly higher than that of the positive control UCN-01 (IC50 1.636 μM, SI 0.6) and adriamycin (IC50 0.084 μM, SI 0.5). Compound 4 had strong selective inhibitory activity in human pancreatic cancer PATU8988 T cell line, with an IC50 of 0.666 μM and SI of 147, which were better than those of adriamycin (IC50 1.118 μM, SI 0.04). Compound 18 had a strong selective inhibitory activity on human colon cancer HCT-116 cells, with an IC50 of 0.032 μM and a SI of 19, which were better than those of adriamycin (IC50 of 1.001 μM and SI of 0.04) (Table 1).

**Materials and Methods**

**Instruments and Chemicals**

Staurosporine was isolated from the fermentation broth of the marine *Streptomyces OUCMDZ-3118*.18 NMR spectra were recorded on an Avance III-600 MHz spectrometer with tetramethylsilane (TMS) as the internal standard, optical rotations using an AUTOPOLI polarimeter (Rudolph Research Analytical), ECD spectra with a JASCO J-715 spectropolarimeter, IR spectra on a Nicolet Nexus 470 spectrophotometer (Thermo Nicolet Corporation) as KBr disks, and electrospray ionization mass spectra (ESIMS) and HREIMS measurements on a Waters 2695 LCQ-MS liquid chromatography-mass spectrometer and Thermo Fisher Q Exactive Focus ion hydrazine mass spectrometer, respectively. Semipreparative high-performance liquid chromatography (HPLC) separations were performed using a Hitachi Primaide Organizer HPLC system with a YMC-Pack ODS-A column (S-5 μm, 12 mm, 250 × 10 mm). Some compounds were purified using a SepaBean machine equipped with SepaFlash columns (Santai Technologies Inc). Thin-layer chromatography (TLC) and column chromatography (CC) were performed on plates precoated with silica gel GF 254 (10-40 μm), and over silica gel (200-300 mesh, Qingdao Marine Chemical Factory), respectively.

**Synthesis of compounds 1-24.** UCN-01 and UCN-02 as shown in [Figure 2. Synthesis of compound 1.](#) Under an argon atmosphere, staurosporine (46.6 mg, 0.1 mmol) was added to a 10 mL 2-port reaction flask, dissolved in 3 mL dichloromethane, then triethylamine (0.1 mL) and benzoyl chloride (41 mg, 0.3 mmol) were added. After being stirred at room temperature for 3 h, the reaction mixture was quenched with water (10 mL), extracted with dichloromethane (10 mL), and the combined organic phase was dried over Na2SO4. After removal of the solvent in vacuo, the residue was purified by a SepaBean...
machine eluting with 25% to 75% MeOH/H2O (containing 1% trifluoroacetic acid) to afford compound 1 (39.9 mg) in 70% yield.

Synthesis of compound 13. Under an argon atmosphere, staur-osporine (46.6 mg, 0.1 mmol) was added to a 10 mL 2-port reaction flask, dissolved in 3 mL dichloromethane, and then triethylamine (0.1 mL) and acetyl chloride (23 mg, 0.3 mmol) was added. After stirring at room temperature for 3 h, the reaction mixture was quenched with water (10 mL), extracted with dichloromethane (10 mL), and the combined organic phase was dried over Na2SO4. After removal of the solvent in vacuo, the residue was purified by a SepaBean machine eluting with 25% to 75% MeOH/H2O (containing 1% trifluoroacetic acid) to afford compound 13 (39.1 mg) in 77% yield.

Synthesis of compounds 2 and 14. Under an argon atmosphere, compound 1 (570 mg, 1 mmol) was added to a 25 mL 2-port reaction flask, dissolved in 20 mL dichloromethane-methanol (1:1), and then NCS (180 mg, 1.3 mmol) was added. After stirring at room temperature for 6 h, the reaction mixture was quenched with water (10 mL), extracted with dichloromethane (20 mL), and the combined organic phase was dried over Na2SO4. After removal of the solvent in vacuo, the residue was purified by column chromatography eluting with light petroleum-ethyl acetate (2:1) to obtain compound 2 (308 mg) in 51% yield. Compound 14 was prepared from compound 13 by the same method.

Synthesis of compounds 3 and 15. Under an argon atmosphere, compound 1 (570 mg, 1 mmol) was added to a 25 mL 2-port reaction flask, dissolved in 20 mL dichloromethane-methanol (1:1), then NBS (192 mg, 1.05 mmol) was added. After stirring at −20 °C for 0.5 h, the reaction mixture was quenched with water (10 mL), extracted with dichloromethane (20 mL), and the combined organic phase was dried over Na2SO4. After removal of the solvent in vacuo, the residue was purified by column chromatography, eluting with light petroleum-ethyl acetate (2:1) to obtain compound 3 (369 mg) in 57% yield. Compound 15 was prepared from compound 13 by the same method.

Synthesis of compounds 4-6 and 16-18. Compound 1 (46 mg, 0.08 mmol) was dissolved in 2 mL dimethyl sulfoxide in a 25 mL 2-port reaction flask, then potassium tert-butoxide (1 M in tert-butanol solution, 0.4 mL, 0.4 mmol) was added. After stirring at room temperature for 6 h, the reaction mixture was quenched with water (10 mL), extracted with ethyl acetate (20 mL), and the combined organic phase was dried over Na2SO4. After removal of the solvent in vacuo, the residue was purified by a SepaBean machine eluting with 25% to 75% MeOH/H2O (containing 1% trifluoroacetic acid) to afford compound 4 (32 mg) in 68% yield. Compounds 5, 6, 16-18 were prepared by the same method using compounds 2, 3, and 13-15 as raw materials, respectively.

Synthesis of compounds 7-12, 19-24, UCN-01, and UCN-02. Under an argon atmosphere, compound 1 (123 mg, 0.22 mmol) was
added to a 25 mL 2-port reaction flask, dissolved in 10 mL dimethyl sulfoxide, and then sodium hydroxide solution (1.6 mL, 0.63 M) was added. After stirring at room temperature for 9 h, the reaction mixture was quenched with water (10 mL), extracted with ethyl acetate (20 mL), and the combined organic phase dried over Na2SO4. After removal of the solvent in vacuo, the residue was purified by a SepaBean machine eluting with 25% to 75% MeOH/H2O (containing 1% trifluoroacetic acid) to afford compound 7 (32 mg) in 25% yield and the crude compound 8. Pure compound 8 (29 mg) was obtained in 23% yield by semipreparative HPLC (75% MeOH/H2O). Compounds 9-12, 19-24, UCN-01, and UCN-02 were prepared by the same method with compounds 2, 3, 13-15, and staurosporine as raw materials, respectively.

Biological Activity
Cell proliferation was measured by the CCK-8 method. A cell suspension of 100 μL was dispensed (5 × 10⁴ cells/well) in 96-well plates. The plates were preincubated for 24 h, with UCN-01 and adriamycin as positive drugs, followed by treatments with either DMSO (control) or various concentrations of compounds 1-24 at different concentrations (from 1 μM to 0.001 μM) for MV4-11, MCF-7, HCT-116, TE-1, PATU8988 T, HOS, GBC-SD, and L-02 cell lines. The 96-well plates were maintained at 37 °C and cultured in a 5% CO2 incubator for 72 h. After aspiration of the old medium, the 10-fold diluted CCK-8 (100 μL) solution was added to each well of the plate, which was then incubated for 3 h. The absorbance was measured at 450 nm using an absorbance microplate reader (Thermo Scientific MULTISKAN MK3). The optical density values of each well represented the survival/proliferation of cells. The half-maximal inhibitory concentration (IC50) is defined as the concentration causing 50% inhibition.

Conclusions
Twenty-four compounds were synthesized, of which 16 were new halogenated staurosporine derivatives; the absolute configuration of the hydroxyl group at C-7 was confirmed by ECD spectra. The inhibitory activity of 3’-N acetylated derivatives on the proliferation of tumor cells was generally better than that of the corresponding benzoylated derivatives, but the inhibition of the human normal L-02 cell line also showed an increasing trend. After the 7-position carbonyl modification of PKC-412 (1), the selective inhibitory activity of compound 4 on MV4-11 and PATU8988 T cell lines was significantly enhanced. After 7-hydroxylation of compounds 1 and 13, the activities on tumor and normal cell lines were decreased, but their 3-bromo derivatives 12 and 24 showed high selective inhibitory activity on the MCF-7 cell line. Compounds 4 and 24 should be further studied to obtain anti-tumor candidate drugs with high efficiency and low toxicity.

Authors’ Note
Gang Li and Dan Wu contributed equally to this work.

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Supplemental Material
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