Novel carbazole sulfonamide microtubule-destabilizing agents exert potent antitumor activity against ESCC

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Conclusion
- SL-3-19 and SL-1-73 are novel microtubule-destabilizing agents that have a potential antitumor effect on ESCC both in vitro and in vivo, and SL-3-19 had a higher activity than SL-1-73, with a low IC50 value and an observable antitumor activity in vivo.
- These results indicate that SL-3-19 may be a new therapeutic candidate for ESCC treatment.

Introduction
Esophageal squamous cell carcinoma (ESCC) is one of the most common cancers worldwide due to its chemoresistance and poor prognosis. Currently, there is a lack of effective small molecule drugs for the treatment of ESCC. These results indicate that SL-3-19 may be a new therapeutic candidate for ESCC treatment.

Novel carbazole sulfonamide microtubule-destabilizing agents that have a potential antitumor effect on ESCC both in vitro and in vivo, and SL-3-19 had a higher activity than SL-1-73, with a low IC50 value and an observable antitumor activity in vivo.

These results indicate that SL-3-19 may be a new therapeutic candidate for ESCC treatment.

Fig. 1. SL-3-19 and SL-1-73 inhibited proliferation of human esophageal cancer cells. (A) Structures of IG-105, SL-3-19 and SL-1-73. (B) Cell viability was evaluated by CCK-8 assays. (C) Drug-stimulated activity of Pgp ATPase was detected by the Pgp-Glo assay system according to the user instructions. Data are shown as the mean ± SD from three independent replicates. (D) The cells viability of KYSE 510 (left) and KYSE 140 (right) treated with cisplatin (CDDDP) or CDPP and 0.2 mM SL-1-19 were evaluated using the CCK-8 assay. Data are shown as the mean ± S.D. *P < 0.05; **P < 0.01; ***P < 0.001, ns, not significant.

Fig. 2. SL-3-19 and SL-1-73 disrupted the microtubule network in cells and in cell-free assays. (A) Representative images of intracellular microtubule assembly in KYSE 510 cells stained for AC-a-tubulin (green) and nuclei (blue) after incubation with or without SL-3-19 or IG-105 (20, 50, or 200 nM) for 6 h. Scale bar: 30 mm. (B) Western blots were used to measure expression levels of AC-a-tubulin in KYSE 510 cells treated with DMSO (0 nM), SL-3-19, or IG-105 (20, 50, 100, or 100 nM) for 6 h. Both SL-3-19 (C) and SL-1-73 (D) inhibited tubulin polymerization according to cell-free tubulin polymerization assays.

Fig. 3. SL-3-19 induced the G2/M cell cycle arrest and upregulated cyclin B1. (A-B) Flow cytometric cell cycle analysis and quantitative analyses were conducted using KYSE 510 cells treated with the indicated concentrations [Conc: (nM)] of drugs for 24 h. (C) Western blots showed the expression of cyclin B1 after treatment. (D) ImageJ was employed to quantify the relative expression of cyclin B1.

Fig. 4. SL-3-19 induced apoptosis in human esophageal cancer cells. (A) For apoptosis analyses, KYSE 510 cells were treated with DMSO (control), SL-3-19 (1 mM), or IG-105 (1 mM) for 24 or 48 h. The cells were collected, fixed with 4% paraformaldehyde, stained with annexin V and PI. Data are shown as the mean ± SEM. (B) KYSE 510 cells were treated with DMSO (control), SL-3-19 (0.5 mM), or IG-105 (0.5 mM) for 24 or 48 h and then stained with a DAPI solution (blue). The percentage of apoptotic cells was measured by counting condensed and fragmented nuclei. Data are shown as the mean ± SEM. Magnification: 40. (C) KYSE 510 cells were treated with SL-3-19 (0.5 or 1 mM) or IG-105 (1 mM) and harvested at different time points. Expression levels of apoptotic markers were determined by WB. Data are shown as mean ± SD; *P < 0.05.

Fig. 5. SL-3-19 and SL-1-73 treatment inhibited tumor growth in an ESCC xenograft model in vivo. (A) Representative photos showing the appearance of tumors. Control (Ctrl), saline; SL-3-19 (L indicates Low, 25 mg/kg; H indicates High, 50 mg/kg), and IG-105 (10 mg/kg).

**P < 0.01; ***P < 0.001, ns, not significant.