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Original Articles

Novel carbazole sulfonamide microtubule-destabilizing agents exert potent antitumor activity against esophageal squamous cell carcinoma

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ABSTRACT

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. Microtubules are an attractive target for cancer therapy since they play a central role in various fundamental cell functions. We investigated the anti-ESCC activity and mechanisms of the small molecule tubulin ligands, SL-3-19 and SL-1-73, which are two carbazole sulfonamide derivatives, *in vitro* and *in vivo* for the first time. These drugs were previously screened from a small molecule library with over 450 compounds and optimized for high aqueous solubility [1,2]. Here, we reveal the promising activities of these compounds against esophageal cancer. Mechanistically, both SL-3-19 and SL-1-73 inhibited ESCC cell growth by inducing cell apoptosis and arresting the cell cycle at G2/M phase in a dose-dependent manner. These drugs effectively inhibited microtubule assembly, greatly disrupted microtubule maturation by down-regulating acetylated α-tubulin, and significantly disrupted the vascular structure by obstructing the formation of capillary-like tubes *in vitro*. Consistent with their *in vitro* activities, SL-3-19 and SL-1-73 inhibited the growth of ESCC xenografts and inhibited the microvessel density *in vivo*. In summary, 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 IC_{50} value and an observable antitumor activity *in vivo*. These results indicate that SL-3-19 may be a new therapeutic candidate for ESCC treatment.

1. Introduction

Esophageal cancer is the eighth most common type of malignancy and the sixth leading cause of cancer mortality worldwide [3,4], and it is the fourth leading cause of cancer death among men and women due to its high incidence and mortality rates in China [5]. The two pathologically classified subtypes are esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) [6]. Esophageal cancer has a serious impact on human health in many Eastern Asian countries and regions, particularly in northern China [4,5].

For advanced esophageal cancer treatment, esophagectomy or chemoradiotherapy are commonly used methods. Both methods play a vital role in esophageal cancer treatment, particularly chemoradiotherapy for unresectable patients [3,7]. Unfortunately, many patients eventually develop acquired resistance and high toxicity after a period of chemotherapy use [8,9] (e.g., treatment

Abbreviations: AC-α-tubulin, acetylated-α-tubulin; CA4, combretastatin A-4; DAPI, 6-diamidino-2-phenylindole; ESCC, esophageal squamous cell carcinoma; IC50, half maximal inhibitory concentration; MDAs, microtubule-destabilizing agents; MTAs, microtubule-targeted agents; Pgp, P-glycoprotein; SL-1-73, N-(2,6-dimethoxypyridine-3-yl)-6-hydroxy-9-methyl-3-carbazole-3-sulfonamide; SL-3-19, N-(2,6-dimethoxypyridine-3-yl)-7-hydroxy-9-methyl-3-carbazole-3-sulfonamide; VDAs, vascular-disrupting agents.

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with Taxol, cisplatin, and 5-fluorouracil). In the absence of effective drugs, novel small molecule drugs for esophageal cancer that are expected to improve therapeutic effects and overcome chemotherapy resistance are urgently needed.

Microtubules play a critical role in a variety of cellular functions, such as cell morphology maintenance, cell movement, intracellular transport, and cell division. In particular, microtubules participate in the formation of mitotic spindles. These essential roles make microtubules an attractive target for cancer treatment [10]. Therefore, microtubule-targeting agents (MTAs) have become a significant trend in the development of anticancer agents. Usually, MTAs directly target and break spindle microtubules by altering microtubule dynamics during the mitotic stage, which eventually leads to cell death via different signaling pathways [11]. Some MTAs also exert a unique vascular-disrupting effect, which is considered one of the best characteristics that clinical chemotherapy drugs can have [12]. Our previous study with N-(2,6-dimethoxypyridine-3-yl)-9-methylcarbazole-3-sulfonamide (Compound 3, also termed IG-105, see Fig. 1A) revealed potent activity against human leukemia and solid tumors [1]. IG-105 is a novel, small molecular weight, synthetic tubulin ligand that is not susceptible to the P-glycoprotein (P-gp) drug efflux pump. IG-105 binds at the colchicine site on tubulin (Fig. 1A). Next, we optimized IG-105 on the carbazole-ring to provide a series of new carbazole sulfonamide derivatives with the aim of significantly increasing the water-solubility and improving the oral bioavailability [2]. SL-3-19 and SL-1-73 were identified by the screening of a small molecule microtubule protein ligand library. These compounds showed a strong antiproliferative activity in various types of human cancer cells, such as breast, liver and pancreatic cancer cell lines. The present study was designed to evaluate the activity of SL-1-73 and SL-3-19 against ESCC *in vitro* and *in vivo*, and explore their molecular mechanisms with the aim of improving chemotherapy responses.

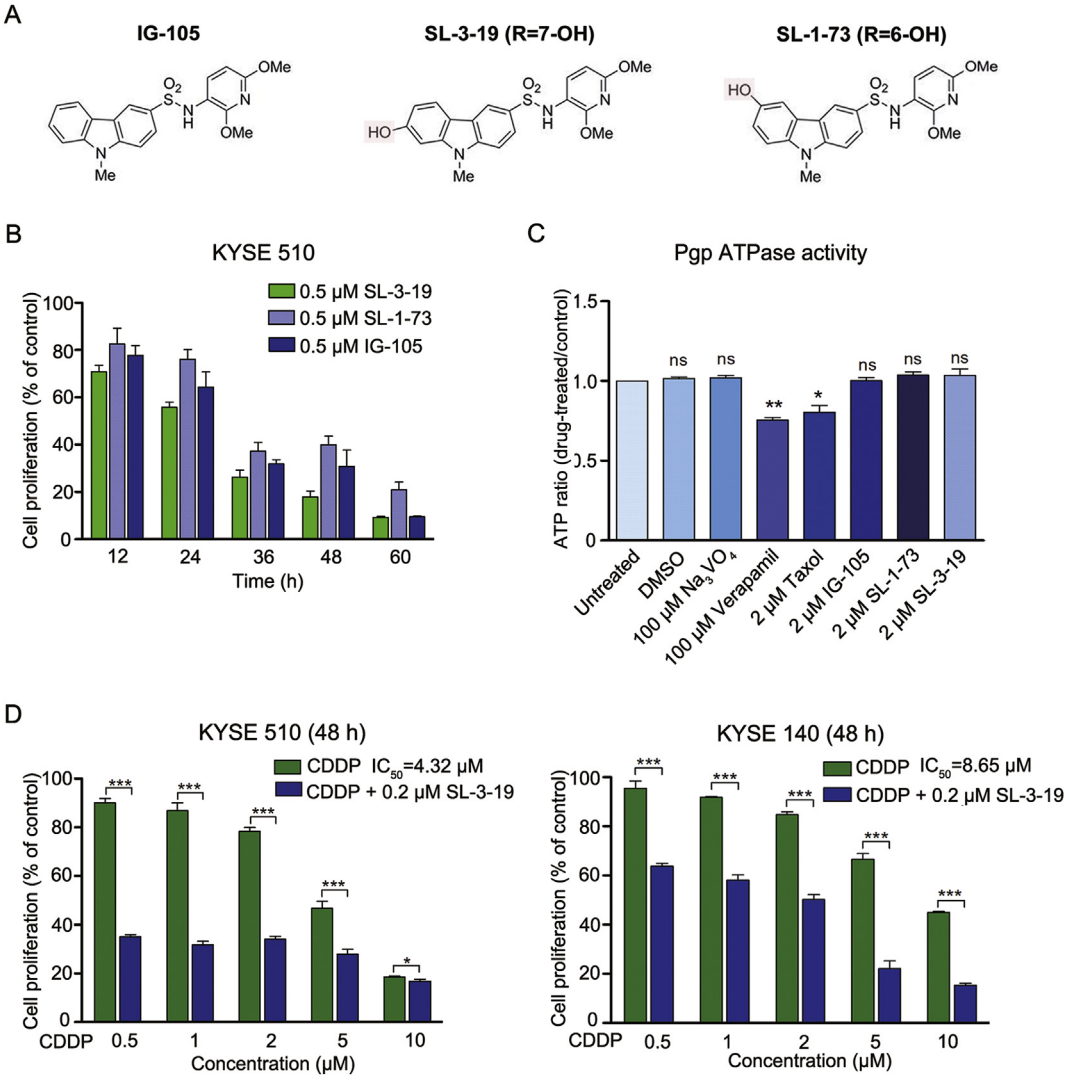

Fig. 1. SL-3-19 and SL-1-73 inhibited proliferation of human esophageal cancer cells. (A) Structures of IG-105, SL-3-19 and SL-1-73. (B) Human esophageal cancer cell viability was evaluated by CCK-8 assays. KYSE 510 cells were treated with SL-3-19, SL-1-73, and IG-105 at 0.5 μM for different periods (12, 24, 36, 48 and 60 h) prior to the calculation of cell proliferation. (C) Drug-stimulated activity of Pgp ATPase was detected by the Pgp-Glo assay system according to the user instructions. The ATP ratio (experimental/control) is presented which is inversely related to Pgp ATPase activity. Na_3VO_4 was used as a negative control (not a substrate of Pgp), verapamil and Taxol are positive controls (substrates of Pgp), and IG-105 served as a reference for comparison. 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 (CDDP) or CDDP and 0.2 μM 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.