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HS-173, a novel phosphatidylinositol 3-kinase (PI3K) inhibitor, has anti-tumor activity through promoting apoptosis and inhibiting angiogenesis

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ABSTRACT

We synthesized a novel imidazopyridine analogue, a PI3Kα inhibitor HS-173 and investigated anti-cancer capacity in human cancer cells. HS-173 inhibited the PI3K signaling pathway, and showed anti-proliferative effects on cancer cells. Also, HS-173 induced cell cycle arrest at the G_2/M phase and apoptosis. In addition, HS-173 decreased the expression HIF-1α and VEGF which play an important role in angiogenesis. This effect was confirmed by the suppression of tube formation and migration assay *in vitro*. Furthermore, HS-173 diminished blood vessel formation in the Matrigel plug assay in mice. Therefore, HS-173 is considered as a novel drug candidate to treat cancer patients.

1. Introduction

Phosphatidylinositol 3-kinase (PI3K) was first identified over 20 years ago as a lipid kinase associated with viral oncoproteins [1–5]. PI3K signaling pathway regulates various cellular processes such as growth, cell cycle progression, apoptosis, migration, metabolism, and cytoskeleton rearrangement [6].

There are three classes of PI3K isoforms grouped according to structure and function. Among the different PI3K subtypes, PI3K class IA plays a key role in the biology of human cancer. The *PIK3CA* gene encoding p110α is frequently mutated and overexpressed in a large portion of human cancers [7]. Many researchers have identified somatic *PIK3CA* mutations in a wide range tumors including ones in the breast (40%), liver (35%), gastric system (6.5% and 25%), ovaries (6.6%), and lung (4%) [8–10]. Furthermore, these mutations have been observed in gliomas (5 and 27%), medulloblastomas (5%), and acute leukemia (1%). In cases of cancer, the PI3K pathway is activated by several different mechanisms including somatic mutation, amplification, and overexpression. In addition, PI3K signaling may perform integral functions in noncancerous cells in the tumor microenvironment. Abnormal regulation of these cellular processes in human cancers has encouraged researchers to develop therapies targeting individual enzymes involved in this signaling cascade [11–14].

Several PI3K pathway inhibitors specific for PI3K, Akt, and mTOR are in the early stages of development for clinical use [15–18]. Previously, we have reported that N-(5-(3-(5-methyl-1,2,4-oxadiazol-3-yl)imidazo[1,2-a]pyridin-6-yl)pyridin-3-yl)benzenesulfonamide (HS-104) has anti-tumor effects on breast cancer by inhibiting the PI3K pathway [19,20]. With a goal of developing a new structural class of potent PI3K inhibitors, we designed and synthesized a new series of imidazo[1,2-a]pyridine derivatives, HS-173 as a PI3Kα inhibitor using a fragment-growing strategy. In the present study, we determined whether HS-173 has anti-cancer effects on cancer cell lines and the molecular mechanism underlying these processes. Our results show that HS-173 promotes apoptosis while preventing proliferation and angiogenesis by inhibiting the PI3K pathway in human liver and breast cancer cells.

2. Materials and methods

2.1. Preparation of HS-173

Ethyl 6-(5-(phenylsulfonamido)pyridin-3-yl)imidazo[1,2-a]pyridine-3-carboxylate (HS-173) is a new PI3Kα inhibitor. This imidazopyridine derivative was synthesized in our previous study [20]. For all *in vitro* studies, HS-173 was dissolved in dimethylsulfoxide (DMSO) at a concentration of 10 mM before use.

2.2. Cell lines

Human breast cancer (SkBr3, T47D, and MCF-7) and human liver cancer (HepG2, Huh7, and Hep3B) cells were purchased from the Korean Cell Line Bank (KCLB, Seoul, Republic of Korea). T47D, MCF7, Huh7, and Hep3B cells were cultured in Roswell Park Memorial Institute 1640 (RPMI-1640) media. SkBr3 and HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Human umbilical

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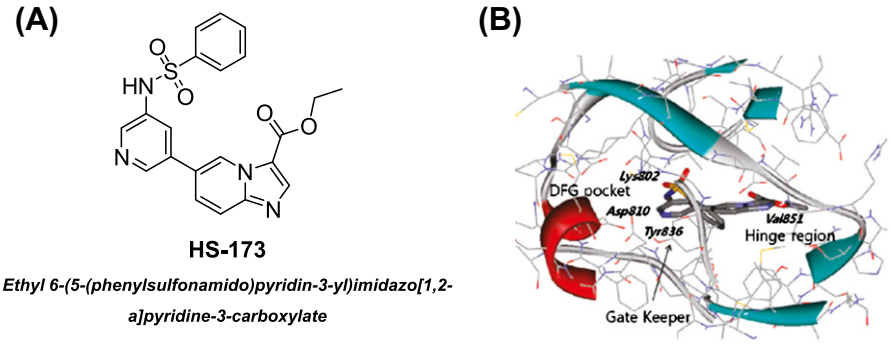

Fig. 1. Chemical structure of HS-173 and its docking mode. (A) Ethyl 6-(5-(phenylsulfonamido)pyridin-3-yl)imidazo[1,2-a]pyridine-3-carboxylate. (B) The putative binding mode of HS-173 in the ATP-binding site of PI3Kα.

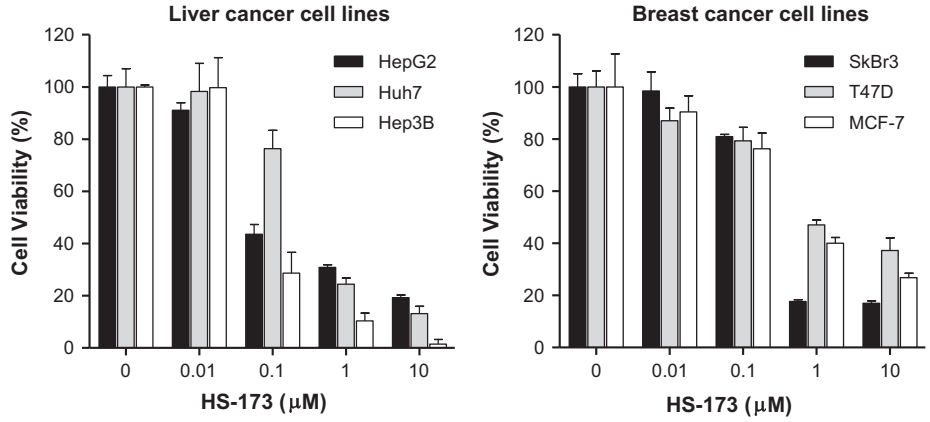

Fig. 2. HS-173 specifically binds to PI3Kα and inhibits cell proliferation. Cytotoxic effects of HS-173 on liver (HepG2, Hep3B, and Huh-7) and breast (SkBr3, T47D, and MCF-7) cancer cells. Inhibitory effect of HS-173 on liver and breast cancer cell proliferation was assessed with an MTT assay. Results are expressed as the percent cell proliferation relative to the control. Data are expressed as the mean ± SD from triplicate wells.

vein endothelial cells (HUVECs) were grown in a gelatin-coated 75-cm^2 flask with M199 medium containing 20 ng/mL basic fibroblast growth factor (bFGF), 100 U/mL heparin, and 20% FBS. Cell cultures were maintained at 37 °C in a CO_2 incubator with a controlled humidified atmosphere composed of 95% air and 5% CO_2.

2.3. Cell viability assay

Cell viability was assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, cells were plated at a density of 3–5 × 10^3 cells/well in 96-well plates for 24 h. The medium was then removed, and cells were treated with either DMSO as a control or various concentrations (0.1–10 μM) of HS-173. After the cells were incubated for 48 h, 100 μL MTT solutions (2 mg/mL) was added to each well and the plate was incubated for another 4 h at 37 °C. The formed formazan crystals were dissolved in DMSO (200 μL/well) with constant shaking for 5 min. Absorbance of the solution was then measured with a microplate reader at 540 nm. This assay was conducted in triplicate.

2.4. Western blotting

Total cellular proteins were extracted with lysis buffer containing 1% Triton X-100, 1% Nonidet P-40, and the following protease and phosphatase inhibitors: aprotinin (10 mg/mL), leupeptin (10 mg/mL; ICN Biomedicals, Asse-Relegem, Belgium), phenylmethylsulfonyl fluoride (1.72 mM), NaF (100 mM), $NaVO_3$ (500 mM), and $Na_4P_2O_7$ (500 mg/mL; Sigma–Aldrich). The proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose membranes. The blots were immunostained with the appropriate primary antibodies followed by secondary antibodies conjugated to horseradish peroxidase. Antibody binding was detected with an enhanced chemiluminescence reagent (Amersham Biosciences). Antibodies against p-mTOR (Ser^{2448}), mTOR, p-Akt (Ser^{473}), p-Akt (Thr^{308}), Akt, p-p70S6K1 (Thr^{389}), p70S6K1, p-GSK3β (Ser^{9}), GSK3β, PARP-1, cleaved caspase-9, Bcl-2, HIF-1α, VEGF, and α-tubulin were purchased from Cell Signaling Technology.

Table 1
Inhibitory concentration (IC_{50}) of HS-173 in different human cancer cells.

Cell lines	IC_{50} (μm)
HepG2	0.045
Huh7	0.2
Hep3B	0.05
SkBr3	0.13
T47D	0.4
MCF-7	0.28

Data represent the mean values from three independent experiments.

2.5. Immunofluorescence microscopy

Hep3B cells were plated on 18-mm cover glasses in RPMI-1640 medium and incubated for 24 h so that approximately 70% confluence was reached. The cells were then incubated in the presence or absence of 1 μM HS-173, washed twice with PBS, and fixed in an acetone: methanol solution (1:1) for 10 min at −20 °C. Cells were blocked in 1.5% horse serum in PBS for 30 min at room temperature, and then incubated overnight at 4 °C with primary antibody in a humidified chamber. After washing twice with PBS, the cells were incubated with mouse fluorescein-labeled secondary antibody (1:100, Dianova, Germany) for 20 min at 37 °C. The cells were also stained with 4,6-diamidino-2-phenylindole (DAPI) to visualize the nuclei. The slides were then washed twice with PBS and covered with DABCO (Sigma–Aldrich) before being view with a confocal laser scanning microscope (Olympus, Tokyo, Japan).

2.6. Cell cycle analysis

Hep3B and SkBr3 cells were plated in 100-mm culture dishes. The next day, the cells were treated with 1 μM HS-173. Floating and adherent cells were collected and fixed overnight in cold 70% ethanol at 4 °C. After washing with PBS, the cells