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How can tricyclic sophoridinic derivatives be used as autophagy inhibitors for cancer treatments?

“The tricyclic sophoridinic derivatives constitute a novel class of autophagy inhibitors, which induce autophagy-dependent apoptosis in cancer cells and indicate the potential value of antitumor drug candidates.”

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The tricyclic sophoridinic analog, *N*-3,4,5-trimethoxybenzyl-12*N*-*p*-chlorobenzyl sophoridinamine (**6b**), blocks autophagic flux in tumor cells [1], severely suppresses lysosomal acidification and thereby inhibits tumor cell survival. The 12*N*-*p*-chlorobenzyl sophoridinic methyl ester **IMB-6G** induced lysosomal membrane permeabilization (LMP) caused lysosomal release of cathepsin B (CTSB)/ cathepsin D (CTSD) and eventually triggered mitochondrial apoptosis [2]. The tricyclic sophoridinic derivatives constitute a novel class of autophagy inhibitors, which induce autophagy-dependent apoptosis in cancer cells and indicate the potential value of antitumor drug candidates.

Fufang Kushen injection, a traditional Chinese medicine, was approved by the China FDA in 1995 for treatment of non-small-cell lung cancer, liver cancer and gastric cancer [3–5]. It has been widely used for the treatment of diverse cancer patients in combination with other anticancer drugs, such as vinorelbine, cisplatin and taxol. Sophoridine as a quinolizidine alkaloid, one of the key active ingredients in *Fufang Kushen injection*, was also approved by the China FDA in 2005 to treat cancer patients with malignant trophoblastic tumors. However, the moderate antitumor activity of sophoridine limited its

use as a drug for clinical applications, therefore, a lot of attention has been drawn for further development of its analogs to discover more effective drug candidates.

Taking sophoridine as the lead compound, we have constructed a compound library for tricyclic sophoridinic derivatives, and summarized the structure–activity relationship for antiproliferative activity of this kind of compounds. We have successfully identified that the 12-*N*-*p*-chlorobenzyl sophoridinic derivatives with a tricyclic core were more favorable than sophoridine with a four-ring scaffold [1,2,6–8]. Moreover, the tricyclic sophoridinic derivatives own multiple drug-like properties such as structural flexibility, high solubility and good pharmacokinetic and safety profiles, and constitute a novel family of potent antiproliferative agents. The representative compounds **6b** and **IMB-6G** demonstrated a potent antitumor activity against a variety of human carcinoma cell lines. Especially, both of them displayed a promising potency against both wild type and drug-resistant carcinoma cell lines.

Autophagy is a highly conserved process where cells get rid of unwanted cellular components including damaged organelles, lipid vesicles or protein aggregates through lysosomal degradation [9]. It is a dynamic process

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in which useless cellular components get sequestered in double-membranous vesicles called autophagosomes. Autophagosomes fuse with lysosomes to generate autolysosomes, in which the autophagic cargo is degraded by acidic hydrolases and released into the cytosol [10].

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Our previous studies demonstrated that tricyclic sophoridin derivatives showed a potent antitumor activity via inhibiting the DNA topoisomerase I activity, inducing cell-cycle arrest at the G0/G1 phase, and cause apoptotic cell death in tumor cells, similar to that of parent sophoridine [4–6]. However, little is known about the ability of this kind of compounds to affect autophagy in tumor cells. Recently, we identified **6b** and **IMB-6G**, as a novel autophagy inhibitor with chemical structure distinct from other known ones. Both of them inhibit the growth of A549, HepG2, MiaPaCa-2 and HupT-3 cancer cells and induce caspase-mediated apoptosis. Meanwhile, treatment with **6b** and **IMB-6G** leads to substantial accumulation of both LC3-II and p62/SQSTM1 in human cancer cells, which indicated the blockade of autophagy by **6b** and **IMB-6G**. LC3 turnover assay and mCherry-EGFP-LC3 confocal microscopy assay also confirmed that the autophagic flux was indeed blocked by **6b** and **IMB-6G**, these results thus support the conclusion that the inhibition of autophagy has a central role in tricyclic sophoridin derivatives-mediated apoptotic cell death.

On the basis of the upregulation of autophagy in cancer cells treated with chemotherapeutic agents, autophagy inhibition has become a promising strategy for cancer treatment [11]. In autophagy, the lysosome is the only way to degrade cargos, so complete inhibition of the lysosome always causes serious impairment of autophagic flux. Low pH (pH < 4.5) is critical for the proteolytic activity in the lysosome [12]. Chloroquine (CQ) is a weak base that inhibits lysosomal acidification, which prevents the fusion of autophagosomes with lysosomes and subsequent autophagic degradation. Currently, CQ and its derivative hydroxychloroquine are widely used as late-stage autophagy inhibitors and have been evaluated in several Phase I and II clinical trials in combination with chemotherapy drugs in a variety of tumor types [13,14]. However, the safety concerns, including ocular toxicity and irreversible retinopathy limit their clinical use, and thereby it is highly desirable for new inhibitors of autophagy [15].

Sophoridinamine **6b** is a basic nitrogen-containing compound, mainly resulting from the secondary amine (NH) at the side chain, which makes the solution of it alkaline. It thus can be protonated and accumulates in lysosomes, resulting in the deacidification of their lumens. Similar to CQ, our finding demonstrate that **6b** is a potent lysosomal-alkalizing agent that exhibits anti-tumor activity in various human tumor cell lines. Compound **6b** actually blocks rather than activates autophagic flux in tumor cells, severely suppresses lysosomal acidification, thus preventing the activation of lysosomal enzymes and thereby their ability to degrade the cargo of the autolysosome, which in turn leads to a blockade of autophagic flux and suppress tumor cell survival [8].

In addition, sophoridin ester **IMB-6G** promotes autophagosome accumulation from the early stage of treatment but blocks autophagic flux in the degradation stage, mainly through attenuation of lysosomal cathepsin activity in pancreatic cancer cells [16]. Compound **IMB-6G** caused LMP in pancreatic cancer cells after treatment for 12 h. Induction of LMP has been abundantly linked to cell death [17]. This suggests that LMP may have occurred after autophagic flux inhibition in **IMB-6G**-treated pancreatic cancer cells. Furthermore, knocking down Atg5 alleviated **IMB-6G**-induced LMP and the abrogation of cathepsin activity by CA-074 Me and E-64 attenuated cell death in the presence of **IMB-6G**, implying that increased LMP is involved in the death-promoting effects of **IMB-6G**. On the other hand, the increased levels of CTSB/CTSD in cytosolic extracts confirmed the **IMB-6G**-induced LMP in pancreatic cancer cells. Elevated levels of cytosolic CTSB/CTSD were detected after treatment with 5 μ M **IMB-6G** for 12 h in the cells. Inhibition of autophagosome formation with siRNA against Atg5 attenuated **IMB-6G**-induced lysosomal release of CTSB/CTSD. CTSB/CTSD is an important mediator of apoptosis after LMP [18]. Upon LMP, the released CTSD induces conformational change of Bax and its translocation to the mitochondria, subsequently promoting apoptosis [19]. Previously, we demonstrated that **IMB-6G** induced mitochondrial apoptosis through releasing cytochrome c into the cytosol and Bax translocation into mitochondrial [20]. Our data indicated that **IMB-6G**-induced LMP caused lysosomal release of CTSB/CTSD and eventually triggered mitochondrial apoptosis.

Altogether, our findings demonstrate that sophoridinamine **6b** blocks autophagic flux in tumor cells, severely suppresses lysosomal acidification, and thereby inhibiting tumor cell survival, and sophoridin ester **IMB-6G**-induced LMP caused lysosomal release of CTSB/CTSD and eventually triggered mitochondrial apoptosis. The tricyclic sophoridin derivatives constitute a novel class of autophagy inhibitors, which induce autophagy-dependent

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Financial & competing interests disclosure

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