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Synthesis, antiproliferative activity and autophagic flux inhibition of new arylsparteine derivatives

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ABSTRACT

New series of arylsparteine derivatives were synthesized and evaluated for their cytotoxic activity against four human cancer cell lines (cervical epithelial carcinoma cells Hela, breast cancer cells MCF-7, lung cancer cells A549, and glioma cells U87 MG) and one normal fibroblast cell line. Structure-activity relationship revealed that introduction of 4-quinolinyl moiety to sparteine afforded a hybrid compound **10** with considerable antiproliferative activity against all tested cancer cell lines. Compound **10**, the most active agent in this study possessed IC_{50} values of 5.97 ± 1.1 and 9.52 ± 0.3 μM against A549 and Hela cancer cell lines, respectively. Inhibition of autophagic flux proved to be the underlying mechanism for the antiproliferative activity of **10** which was further validated by decreased levels of ATP in cancer cells treated with **10**. In addition, co-treatment of **10** and rapamycin restored cell viability which comes in good agreement with the proposed autophagic flux inhibition for **10**.

1. Introduction

Natural products and their derivatives are valuable leads for the development of cancer therapeutics (Khazir et al., 2014). Quinolizidine alkaloids have demonstrated promising activity against non-small cell lung carcinoma, liver cancer and gastric cancer (Bao et al., 2014; Guo et al., 2015; Xu et al., 2011; Yang et al., 2013; Zhao et al., 2014). In this context, sophoridine (**1**, Fig. 1), a quinolizidine natural product, emerged as a promising chemical entity for drug development owing to its structural flexibility and favorable pharmacokinetic profile (Wei et al., 2006). In addition, **1** exhibits considerable cytotoxic activity which is attributed to the induction of apoptosis and the cell cycle arrest at the S-phase (Xu et al., 2017). Matrine (**2**, Fig. 1), extracted from *Sophora flavescens Ait*, was approved by Chinese FDA as an antiproliferative drug for non-small cell lung cancer and liver cancer (Sun et al., 2012). Consequently, tremendous research efforts have been directed towards development of sophoridine and matrine derivatives as potential antiproliferative agents (Bi et al., 2014, 2016; Wang et al., 2012; Zhao et al., 2015). Sparteine (**3**, Fig. 1), a quinolizidine alkaloid, is known to be a principal constituent of *Lupinus mutabilis* (Hatzold et al., 1983). The biological activities of sparteine as an antiarrhythmic (Pugsley et al., 1995) and hypoglycemic agents (Garcia-Lopez et al., 2004) have been widely studied. However, development of sparteine derivatives with potential antiproliferative activity remains unexplored.

Autophagy is a cellular degradation pathway for the clearance of undesired cellular components *via* lysosomal degradation. Autophagic pathway sustains metabolic pathways required for survival of different cancer types. (Anding and Baehrecke, 2015; Galluzzi et al., 2014). Quinoline-based compounds have displayed the ability to accumulate in acidic lysosomes of cancer cells resulting in disruption of autophagy (Amaravadi et al., 2007; Carew et al., 2007; Golden et al., 2015). Chloroquine (CQ), the prototypical antimalarial drug, received considerable attention as a potential antiproliferative agent because of its ability to block autophagic flux. However, severe side effects of CQ including ocular toxicity developed a pressing need for identification of new inhibitors of autophagy (Wang et al., 2017). Recently, sophoridine derivatives emerged as novel autophagy inhibitors owing to their lysosomotropic property similarly to CQ (Bi et al., 2017). Taking all these findings into consideration, new arylsparteine derivatives were synthesized and evaluated for their antiproliferative activity against four human cancer cell lines (cervical epithelial carcinoma cells Hela, breast cancer cells MCF-7, lung cancer cells A549, and glioma cells U87 MG) and one normal fibroblast cell line. Moreover, the ability of the new arylsparteine derivatives to block autophagic flux is investigated.

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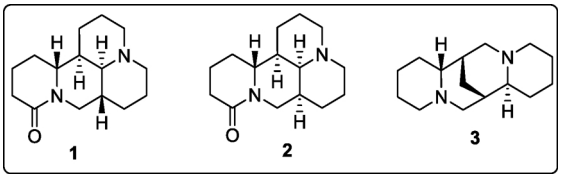


Fig. 1. Chemical structures of **1-3**.

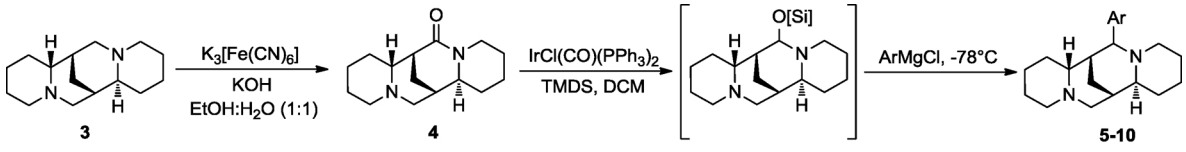
2. Results and discussion

A general approach for the synthesis of the target compounds is outlined in Scheme 1. 17-Oxosparteine **4** was obtained through oxidation of the parent sparteine **3** through a modification of previously described procedure (Golebiewski and Spenser, 1985). In addition, a two-stage iridium catalyzed reductive coupling of **4** and Grignard reagents furnished arylsparteine derivatives **5–10** in serviceable yields. All target compounds were characterized by HRMS, ^{1}H NMR, and ^{13}C NMR. The cytotoxic activity of **5–10** against four human cancer cell lines (cervical epithelial carcinoma cells Hela, breast cancer cells MCF-7, lung cancer cells A549, and glioma cells U87 MG) and one normal cell line (mouse embryo fibroblasts NIH3T3) was evaluated using MTT assay. The IC_{50} values of the target compounds **5–10**, sparteine, and the reference drug 5-fluorouracil are listed in Table 1. The parent sparteine **3** displayed negligible cytotoxicity with IC_{50} values > 50 μM against all tested cell lines. Compound **5** bearing phenyl ring as the aryl substituent displayed minimal improvement in its antiproliferative profile compared to the parent compound **3**. However, compounds **6** and **7** with electron donating groups (EDGs) demonstrated considerable improvement in their potency against tested cancer cell lines. Regarding the substitution pattern on the phenyl ring, 3,4,5-trimethoxy substituted **7** was more active than 4-methoxy-substituted **6**. Compound **7** possessed IC_{50} values of 24.5 ± 1.9 and 28.7 ± 3.1 μM against MCF-7 and A549 cancer cell lines, respectively. Incorporation of methoxy-substituted phenyl rings (EDGs) to quinazolidine alkaloids has been reported to result in considerable improvement in their ability to block the autophagic flux and consequently enhanced antiproliferative activity (Bi et al., 2017). Interestingly, switching to electron withdrawing groups (EWGs) in **8** and **9** further increased the potency of the arylsparteine derivatives against all tested cancer cell lines. In addition, compound **9** bearing 4-nitro-aryl substituent possessed comparable potency to the reference drug 5-fluorouracil against MCF-7 and U87 MG cancer cell lines. We speculate that incorporation of EWGs to arylsparteine derivatives increase their lipophilicity which consequently alter their permeability to cellular membranes resulting in enhanced interaction with cancer cells. A similar trend has been reported for the effect of incorporation of EWGs to quinazolidine alkaloids on their antiproliferative potency (Wang et al., 2012). These results demonstrate that the substitution pattern of the aryl substituent is a crucial component of the antiproliferative profile of arylsparteine derivatives. Compound **10** bearing 4-quinolinyl moiety as the aryl substituent proved to be the most active member of this study against all tested cancer cell lines. Notably, **10** was more potent than 5-fluorouracil against U87 MG and A549 cell lines with IC_{50} values of 9.71 ± 2.2 and 5.97 ± 1.1 μM, respectively. Moreover, **10** displayed potential antiproliferative activity against Hela cell line with IC_{50} value of 9.52 ± 0.3 μM in comparison to IC_{50} value of 8.45 ± 1.4 μM for 5-fluorouracil against the same cell line. It is noteworthy to mention that **10** was less cytotoxic than 5-fluorouracil to the normal NIH3T3 cells with IC_{50} value of 35.1 ± 2.9 μM. We speculate that the selectivity of **10** to cancer cells over normal cells in comparison to 5-fluorouracil is attributed to higher sensitivity of cancer cells to autophagy inhibitors based on biochemical and metabolic differences between normal and cancer cells (Galluzzi et al., 2014).

Autophagy in cancer cells results in degradation of long-lived proteins, thus, measuring their rate of degradation is the most frequently used method to monitor the autophagic flux in cancer cells (Dupont et al., 2017). In order to investigate the ability of **10** to block autophagic flux in cancer cells, the effect of **10** on degradation of [^{14}C]-valine-labeled long-lived proteins was evaluated in Hela cancer cells. The results displayed in Fig. 2 reveal that **10** effectively reduce the degradation of long-lived proteins analogously to the autophagy inhibitor, 3-methyladenine (3-MA). Such finding validates the blockade of the autophagic flux in Hela cells by **10**. Low pH value (pH < 4.5) in the lysosome, the central platform for autophagic pathway, is crucial for its proteolytic activity. It is speculated that the basic nitrogen-containing sparteine core is protonated under physiological conditions and further accumulates in lysosomes. Incorporation of the basic 4-quinolinyl moiety contributes to inhibition of lysosomal acidification which prevents subsequent autophagic degradation as previously reported for quinoline-based compounds (Amaravadi et al., 2007; Carew et al., 2007; Golden et al., 2015).

Autophagic flux inhibition decreases the recycling of cellular fuels, which eventually results in reduced levels of adenosine triphosphate (ATP) (Mizushima and Komatsu, 2011). To further demonstrate that compound **10** blocks autophagic flux in cancer cells, the intracellular production of ATP in Hela cells was measured upon treatment with **10**. As shown in Fig. 3, the intracellular ATP level was reduced in the Hela cells treated with **10** (2.5 or 5 μM) for 48 h. These results come in good agreement with the proposed autophagic blockade mechanism for **10**.

To further validate the proposed mechanism for **10**, the effect of co-treatment of **10** and rapamycin (Rap, autophagy inducer) and CQ (autophagy inhibitor) on cancer cells viability was investigated. Interestingly, co-treatment of **10** (5 μM) with Rap (10 μM) for 48 h restored U87 MG cells viability as revealed by MTT assay (Fig. 4A). Moreover, compound **10** (10 μM) displayed enhanced cytotoxicity with



Comp. No.	Ar	Comp. No.	Ar	Comp. No.	Ar
5		6	OMe	7	OMe, OMe, OMe
8	Cl	9	NO_2	10	N

Scheme 1. Synthesis of arylsparteine derivatives **5–10**.