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Unprecedented sugar bridged bisindoles selective inhibiting glioma stem cells

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ABSTRACT

Unlike reported bisindoles linked by single bond directly, alstoniasidines A (**1**) and B (**2**), from *Alstonia scholaris* featuring unprecedented skeleton with two indole moieties bridged by a sugar, represented a novel bisindole type having strictosamide-glucopyranose-picraline scaffold. Both compounds exhibited selective cytotoxicity against human glioma stem cells (GSCs) and induced caspase-3 dependent extrinsic apoptosis by increasing the expression of interleukin 1 (IL-1), tumor necrosis factor (TNF-α), and the cleaved caspase-3, while damaged the unlimited proliferation and self-renewal capacity of GSCs. This finding might provide new type of leads for the selective killing of human glioma stem cells.

1. Introduction

Enlightened by the clinical practice of natural antitumor bisindole alkaloids, such as vinblastine, vincristine, and vindesine, pharmacists and chemists are always fascinated with their complicated architectural structures as well as potent antitumor activities.[1] Previous studies intensively investigated the cytotoxic bisindoles against general cancer cells and resulted in considerable groundbreaking discoveries.[2–5] However, the active bisindole aiming cancer stem cells (CSCs), a special cell group of important for cancer initiation and maintenances, was still unheard-of.[6] Especially, CSCs are so quiescent that the current chemotherapy drugs, acting primarily on rapidly dividing cells, failed to improve the overall survival.[7,8] Furthermore, the unlimited proliferation and self-renewal features of CSCs may make them capable of recapturing the tumor sphere and contributing to cancer recurrence.[9–12] Therefore, the treatments to target CSCs may be a prerequisite and play an increasing pivotal role for successful cancer therapeutic strategies.

Glioblastoma multiform (GBM), one of the most aggressive and common malignant brain tumor, is currently incurable and confer a poor prognosis with less than 15 months survival time.[12] The standard-of-care clinical treatment for GBM patients, including surgical resection, adjuvant radiotherapy, chemotherapy drug, and recent trials using molecular targeting agents,[13] provide only palliation with no significant effects on overall survival,[12] and to date, the clinically effective drugs against GBM remain scarce. As the incidence of GBM increases rapidly, it is urgent to develop effective compounds applied in chemotherapy for it to alleviate the public health burden.

Structurally, reported bisindoles were defined to be that two indole groups directly linked by single bond. As the first strictosamide-glucopyranose-picraline type bisindole, alstoniasidines A (**1**) and B (**2**) (Fig. 1), harboring an unprecedented sugar bridge between two indole groups, were isolated from the leaves of *Alstonia scholaris* (L.) R.Br. (Apocynaceae). Interestingly, **1** and **2** exhibited the selective cytotoxicity against glioma stem cells (GSCs), one of the first verified CSCs in 2003.[10] It was noteworthy that both the compounds activated the caspase-3 dependent extrinsic apoptotic pathway by increasing the expression of interleukin 1 (IL-1), tumor necrosis factor (TNF- α) and cleaved caspase-3. Alstoniasidi-

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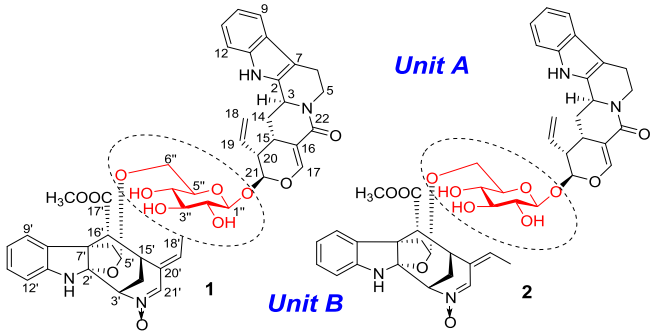

Fig. 1. Structures of **1** and **2**.

nes A and B also significantly damaged the colony spheres in the colony formation assay and further destroyed the unlimited proliferation and self-renewal capacity of GSCs.

Previous reported bisindoles connected by two units directly, such as vinblastine interfered with microtubule dynamics, prevented cell division and promoted cell death in dividing cells finally, but the separated unit (vindoline and catharanthine) were non-active, which indicated the importance of the linkage.[14–16] Nevertheless, compounds **1** and **2** inhibited glioma stem cells selectively, and in the structure two indole groups linked by sugar provided a hydrophilic moiety and more chemical modification positions, which might be new lead for further structural and pharmacological investigation in GBM treatment.

2. Experimental

2.1. General experimental procedures

Optical rotations were performed on a Jasco P-1020 polarimeter (Jasco Corp., Tokyo, Japan). UV spectra were obtained on Shimadzu UV-2401A spectrometer (Shimadzu Corp., Tokyo, Japan). ECD spectra were obtained on a Jasco 810 spectrometer (Jasco Corp., Tokyo, Japan). IR spectra were measured on a Bruker FT-IR Tensor 27 spectrometer (Bruker, Bremen, Germany) with KBr pellets. 1D and 2D-NMR spectra were recorded on Bruker AV-600 MHz spectrometer (Bruker, Bremen, Germany) with tetramethylsilane (TMS) as internal standard. HRESIMS were recorded on Waters Xevo TQS spectrometer (Waters Corp., USA). Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Ltd., Qingdao, People's Republic of China), Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd., Sweden), and MCI-gel CHP 20P (75–100 μm, Mitsubishi Chemical Co., Ltd). Thin-layer chromatography (TLC) was carried out on silica gel H-precoated plates (Qingdao Haiyang Chemical Co., Ltd.) with $CHCl_3$/MeOH (9:1, 4:1, v/v) as developing solvents and the spots were visualized by Dragendorff's reagent. High performance liquid chromatography (HPLC) was performed on Waters 600 equipment using Welch-Ultimate XB-C18 column (250 × 10 mm, 5 μm) (Welch Materials, Inc. Shanghai, People's Republic of China).

2.2. Plant material

The leaves of *A. scholaris* were collected from Puer City, Yunnan province, China, in June 2006, and identified by Dr. Zhang Jun, Kunming Plant Classification Biotechnology Co., Ltd. A voucher specimen (No. Zhang_20140612) was deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The air-dried and powdered leaves of *A. scholaris* (10 kg) were extracted with MeOH (50 L × 3) under reflux conditions at 70 °C, three hours for each time. After removal of the organic solvent under reduced pressure, the residue was dissolved in 0.3% aqueous hydrochloric acid (v/v); the solution was subsequently basified to pH 9–10 using ammonia and then extracted with EtOAc (3 L × 4) to give an alkaloidal extract. The extract (50 g) was subjected to a silica gel column ($CHCl_3$/MeOH, 1:0–0:1) to afford fractions (A-E). Fr. E (7 g) was subjected to Sephadex LH-20 column chromatography (CC) using MeOH under isocratic conditions to afford a mixture (200 mg), then further separated on MCI-gel CHP 20P CC with a gradient of MeOH/H_2O (1:4–1:0) and semi-preparative HPLC column under a gradient of CH_3CN/H_2O (1:4–4:1) to get **1** (12.2 mg) and **2** (4.5 mg).

2.3.1. Alstoniasidine A (*1*)

Colorless amorphous solid; $[\alpha]_D^{24}$ +36.9 (c 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 284 (4.36), 226 (4.58), 204 (4.67) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 202 (−30.3), 215 (−11.1), 235 (−10.8), 275 (+17.5); IR (KBr) ν_{max} 3416, 2926, 1657, 1605, 1440, and 1066 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 1; HRESIMS *m/z* 873.3320 $[M+Na]^+$ (calcd for $C_{46}H_{50}N_4O_{12}$, 873.3317).

2.3.2. Alstoniasidine B (*2*)

Colorless amorphous solid; $[\alpha]_D^{26}$ +19.2 (c 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 289 (4.21), 225 (4.47), 204 (4.58) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 202 (−29.3), 217 (−7.0), 233 (−8.6), 275 (+11.5); IR (KBr) ν_{max} 3423, 2926, 1655, 1612, 1442, and 1068 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 1; HRESIMS *m/z* 873.3325 $[M+Na]^+$ (calcd for $C_{46}H_{50}N_4O_{12}$, 873.3317).

2.4. Acid hydrolysis and GC analysis of compound *1*

The acid hydrolysis and GC analysis were performed using a previously described method (Supplementary materials).[17] By comparing the retention times of the sugar derivatives with those of the authentic sugars under the same conditions, the sugar unit of compound **1** was determined as D-glucose (22.696 min), while the retention time of standard D-glucose was 22.623 min and that of L-glucose was 22.963 min.

2.5. ECD calculation

The theoretical ECD calculations of compound **1** were performed using Gaussian 09.[18] For a detailed description see the Supplementary materials.

2.6. ^{13}C NMR calculation

For the calculations of ^{13}C NMR chemical shifts, B3LYP/6-31G (d,p) method was used to optimize the selected conformations.[19–25] For description of its experimental details see the Supplementary materials.

2.7. Cell lines and cell culture

The glioma stem cell lines (GSC-12# and GSC-18#) were isolated by Kunming Institute of Zoology.[9,11,26] Related details of this part are provided in Supplementary materials.