Bioorganic & Medicinal Chemistry Letters 28 (2018) 1652–1656

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of (aminoalkyl)cycleanine analogues: cytotoxicity, cellular uptake, and apoptosis induction in ovarian cancer cells

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ARTICLE INFO

Article history:
Received 11 January 2018
Accepted 15 March 2018
Available online 16 March 2018

Keywords:
Cycleanine
Bisbenzylisoquinoline alkaloid
Ovarian cancer
Semi-synthesis
Cellular uptake
Apoptosis

ABSTRACT

Our previous studies demonstrated that cycleanine, a macrocyclic bisbenzylisoquinoline (BBIQ) alkaloid, showed potent anti-ovarian cancer activity via apoptosis induction. Here, we synthesized two novel (aminoalkyl)cycleanine analogues (**2** and **3**) through a simple and efficient two-step reaction starting from cycleanine isolated from *Triclisia subcordata* Oliv. These analogues showed greater potency than the unmodified cycleanine in three human ovarian cancer cell lines. Both **2** and **3** induced apoptosis in ovarian cancer cells by activations of caspases 3/7, cleavage of PARP, increase in subG$_1$ cell cycle phase and in the percentage of apoptotic cells. Further confocal fluorescence microscopy analysis confirmed the cellular uptake of alkaloids in ovarian cancer cells by using the unique (alkynyl)cycleanine (**3**) via click chemistry reaction. Our results suggest that cycleanine could be a hit compound for the future development in attacking ovarian cancer.

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The macrocyclic bisbenzylisoquinoline (BBIQ) alkaloids are among different large classes of alkaloids common in plant species. BBIQ alkaloids are prevalent in the families like Menispermeaceae, Annonaceae, Berberidaceae, Monimaiaceae and Ranunuclaceae.[1–3] The BBIQ alkaloids were reported to exhibit anti-ovarian,[4–7] -lung,[8] -bladder,[9] -colorectal,[10] -gallbladder carcinoma and -prostate[11] cancer activities.[12,13] The use of isolated natural products as scaffolds to generate their analogues via chemical transformation is a promising and successful approach in drug discovery.[14,15] So far, only a small number of semi-synthetic BBIQ alkaloids (e.g. tetrandrine and fanginoline analogues,[16–19] C14-urea-tetrandrine,[20] cycleanine mono-N-oxides,[21] and berbamine derivatives.[22]) have been made, which show potent cytotoxicity or can reverse P-glycoprotein-mediated multidrug resistance in cancer cells.[17,23] The making of more potent cytotoxic BBIQ alkaloids with improved activities and investigation of their mechanisms of action (e.g. cellular uptake, target identification) by using the synthetic probes are desired.[24,25] Previously, several BBIQ alkaloids were isolated from *Triclisia subcordata* Oliv. by us and they showed potent *in vitro* anti-ovarian cancer activity.[4–6] Among them, cycleanine (**1**) is bioactive, with the highest therapeutic index and the most abundant component (1.1%).[4] Therefore cycleanine was preferred as a hit compound for chemical modification.[14] In this study, we semi-synthesized analogues of cycleanine and evaluated their cytotoxicity, induction of apoptosis, and cellular uptake.

The semi-synthesis of cycleanine analogues was achieved via a simple two-step reaction (Scheme 1). Reaction of cycleanine (**1**) under paraformaldehyde and concentrated HCl produced the intermediate 5-chloromethylcycleanine through a Blanc chloromethylation reaction.[26] Without purification 5-chloromethylcycleanine reacted with dimethylamine and propargylamine via SN2 nucleophilic substitution reaction to yield 5-[(dimethylamino)methyl]-cycleanine (**2**), and 5-[(propargylamino)methyl]cycleanine (**3**), respectively (Supporting information).

Compounds **2** and **3** were purified by silica gel chromatography to a high purity (>98%) as determined by analytical high performance liquid chromatography. The structure of 5-[(dimethylamino)methyl]cycleanine (**2**) and 5-[(propargylamino) methyl]cycleanine (**3**) was confirmed on the basis of ^{1}H NMR (Table S1, Figs. S1 and S2), ^{13}C NMR (Table S2), 2D NMR and high resolution liquid chromatography mass spectrometric (LC-MS) analysis. The symmetry of cycleanine was lost after the chemical modification, as evidenced by that the single peak for H-5 or 5′ in the ^{1}H NMR of cycleanine[4] was shifted and other peaks for aromatic protons were split into two pairs of peaks (Table S1, Figs. S1 and S2). The resulting single peak around 6.80 ppm in the ^{1}H NMR of **2** or **3** corresponds to only one proton (H-5′) which indicates the substitution at C-5 of cycleanine (**1**) by the alkylamino groups. The addition of aminoalkyl group to cycleanine could improve its water

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https://doi.org/10.1016/j.bmcl.2018.03.038
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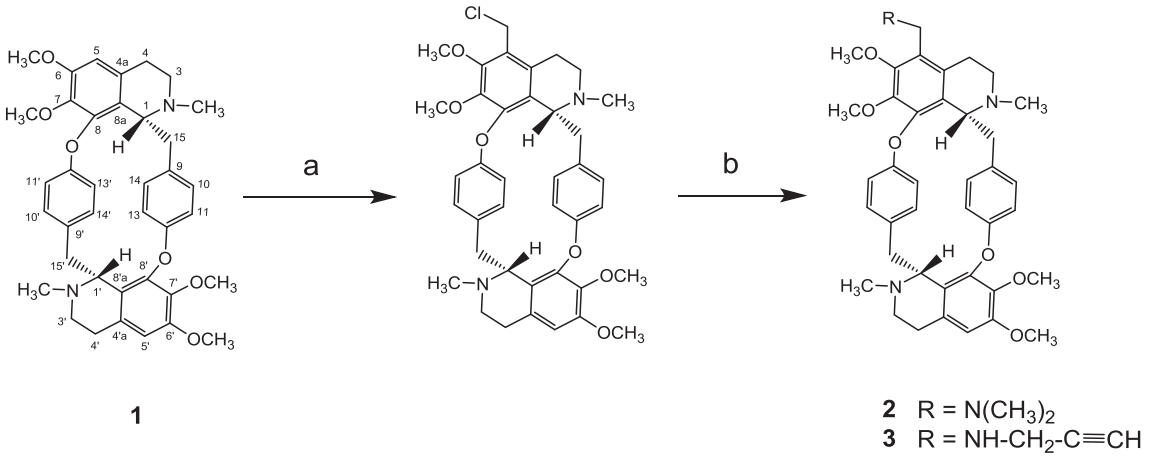

Scheme 1. Semi-synthesis of (aminoalkyl)cycleanine analogues via modification of cycleanine. Reagents and conditions: (a) paraformaldehyde, conc. HCl, 0 °C, 3 h; (b) CH_3CN, NaOH, dimethylamine or propargylamine, rt, 3 h.

Table 1
The IC_{50} of cycleanine (**1**), 5-[(dimethylamino)methyl]cycleanine (**2**) and 5-[(propargylamino) methyl]cycleanine (**3**) in ovarian cancer cells. Data represented as mean ± SEM (n = 5). SI means selectivity index. The SI values are calculated by comparing the activity against the non-cancer HOE cell line with those against OVCAR-8, A2780 and IGROV-1 ovarian cancer cells.

Compound	Ovarian cancer cell lines (μM)			HOE (μM)
	OVCAR-8	A2780	IGROV-1	
1	10 ± 0.6	7.6 ± 0.7	14 ± 1.0	35 ± 1.0
SI (1)	3	5	3	
2	5.2 ± 0.6	3.6 ± 0.5	4.4 ± 0.1	10 ± 0.2
SI (2)	2	3	2	
3	5.6 ± 0.2	6.3 ± 0.6	6.1 ± 0.5	32 ± 1.6
SI (3)	6	5	5	
Carboplatin	16 ± 1.0	8.0 ± 0.7	12 ± 0.9	–

solubility as found in the approved anticancer drug such as (aminoalkyl)camptothecin (Toptecan).[27,28]

To assay the cytotoxicity or anti-proliferative activities of **2** and **3**, the IC_{50} of these new compounds were evaluated on different ovarian cancer cell lines (OVCAR-8, A2780 and IGROV-1) and immortalized human ovarian epithelial (HOE) cell line after treatment for 72 h using Sulforhodamine B (SRB) colorimetric assay.[29,30] The results reveal the IC_{50} of **2** and **3** ranging from 3.6 ± 0.5 to 5.2 ± 0.6 μM and from 5.6 ± 0.2 to 6.3 ± 0.6 μM, respectively (Table 1; Figs. S3 and S4). The results suggest that **2** and **3**, exert about 2–3 times improved potency against three ovarian cancer cells than cycleanine (**1**) with IC_{50} ranging from 7 to 14 μM and the approved anti-ovarian cancer drug-carboplatin with IC_{50} ranging from 8 to 16 μM. Cycleanine (**1**) and **3** seem to be slightly more tolerant to HOE cells than compound **2**. Overall, compound **3** showed milder selectivity index (SI) than **1** and **2** (Table 1).

The effects of **2** and **3** on morphology of OVCAR-8 cancer cells were also found to be time- and concentration-dependent

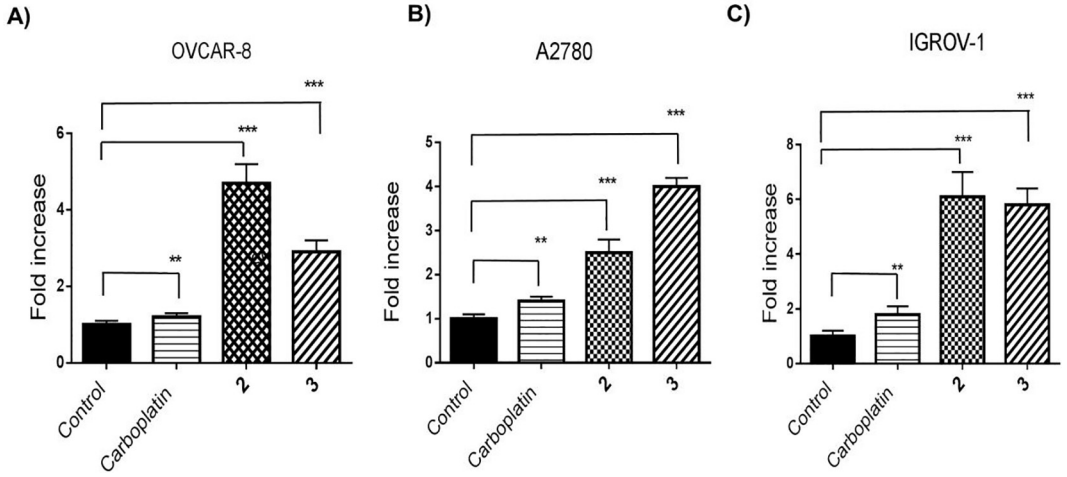

Fig. 1. The apoptotic effects (increase of caspase 3/7 activities) of carboplatin, 5-[(dimethylamino)] cycleanine (**2**) and 5-[(propargylamino) methyl] cycleanine (**3**) on three ovarian cancer cell lines. Data represented as mean ± SEM, n = 6 observations, negative control was the DMSO (0.2%), carboplatin used as positive control, **2** and **3** (20 μM). Results were significant compared to control ($^{***}P < 0.001$, one-way ANOVA) *post hoc* analysis multiple *t*-test compared with control ($^{**}P < 0.01$, $^{***}P < 0.001$).

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