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Solasodine-3-*O*-β-D-glucopyranoside kills *Candida albicans* by disrupting the intracellular vacuole

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ABSTRACT

The increasing incidence of fungal infections and emergence of drug resistance underlie the constant search for new antifungal agents and exploration of their modes of action. The present study aimed to investigate the antifungal mechanisms of solasodine-3-*O*-β-D-glucopyranoside (SG) isolated from the medicinal plant *Solanum nigrum* L. In vitro, SG displayed potent fungicidal activity against both azole-sensitive and azole-resistant *Candida albicans* strains in Spider medium with its MICs of 32 μg/ml. Analysis of structure and bioactivity revealed that both the glucosyl residue and NH group were required for SG activity. Quantum dot (QD) assays demonstrated that the glucosyl moiety was critical for SG uptake into *Candida* cells, as further confirmed by glucose rescue experiments. Measurement of the fluorescence intensity of 2′,7′-dichlorofluorescin diacetate (DCFHDA) by flow cytometry indicated that SG even at 64 μg/ml just caused a moderate increase of reactive oxygen species (ROS) generation by 58% in *C. albicans* cells. Observation of vacuole staining by confocal microscopy demonstrated that SG alkalized the intracellular vacuole of *C. albicans* and caused hyper-permeability of the vacuole membrane, resulting in cell death. These results support the potential application of SG in fighting fungal infections and reveal a novel fungicidal mechanism.

1. Introduction

The application of broad-spectrum antibiotics, implantable devices and immunosuppressive agents in recent decades has led to an increase in the incidence of fungal infections (Corti et al., 2009). Invasive fungal infections are difficult to manage and are important factors in morbidity and mortality among immunocompromised patients (Gudlaugsson et al., 2003; Miller et al., 2001). A recent epidemiological survey revealed that *Candida* species were the third most common pathogen among hospital patients (Hidron et al., 2008). Current clinically used antifungal agents are mainly divided into four categories according to their modes of action: polyenes, azoles, echinocandins and flucytosine (Pappas et al., 2009). However, the latter three categories of therapeutics are often associated with drug resistance, highlighting the need for extensive efforts to develop new antifungal agents and therapeutic strategies.

Natural products, particularly traditional Chinese medicine, provide a rich pool for drug discovery due to the therapeutic efficacy and versatile structures of secondary metabolites (Harvey, 2008). *Solanum nigrum* L. (SNL), which belongs to the nightshade Solanaceae family, is traditionally used as a herbal plant, and its ripe fruits are edible. SNL produces a class of steroidal alkaloids and glycosides that possess a variety of biological activities, including antifungal, antiviral, antibacterial, molluscicidal and anticancer activities (Kumar et al., 2009; Simons et al., 2006; Sun et al., 2010; Thorne et al., 1985). Due to these diverse bioactivities, the chemical synthesis, biosynthesis and bioactivity of the steroidal alkaloids have been the focus of several recent studies (Cárdenas et al., 2015, 2016; Jiang et al., 2015; Sharpe and Johnson, 2016).

The glycosidic residues are considered crucial for the bioactivity of glycosides. For example, the steroidal glycoalkaloid α-tomatine produced by tomato exerts antimicrobial effects for self-defense. However, removal of this glycosidic residue by the tomatinase of *Septoria lycopersici* abolishes this antimicrobial activity (Bouarab et al., 2002). Moreover, attachment of the glycosidic moiety

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Abbreviations

AMB	Amphotericin B
CLSM	Confocal laser scanning microscopy
CMAC	Cell Tracker Blue CMAC Dye
CLSI	Clinical and Laboratory Standards Institute
DCFH- DA	2′,7'-dichlorofluorescin diacetate
DMSO	Dimethyl sulfoxide
MIC	Minimal inhibitory concentration
FM4-64	N-(3-triethylammoniumpropyl)-4-(6-(4-(diethylamino) phenyl) hexatrienyl) pyridinium dibromide
PI	Propidium iodide
QD	Quantum dots with streptavidin conjugate 605 nm
ROS	Reactive oxygen species
SNL	*Solanum nigrum* L.
SG	Solasodine-3-O-β-d-glucopyranoside
Spider-Glu	Spider medium with glucose instead of mannitol
Spider-Rha	Spider medium with rhamnose instead of mannitol
XTT	2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide
YPD	Yeast-peptone-dextrose

increases the hydrophilicity of the molecule, which influences pharmacokinetic properties such as circulation, elimination and concentrations in body fluids (Kren and Martínková, 2001).

We previously reported that solasodine-3-*O*-β-D-glucopyranoside (SG) displays anti-virulence activity against *C. albicans* by inhibiting cell adhesion, morphological transition and biofilm formation (Li et al., 2015b). However, little is known about the antifungal activities of SG analogues and the essential functional groups in the structure of SG. Moreover, the mode of action of SG remains unclear.

We previously isolated solasodine (**1**) and its glycoside SG (**2**) from SNL and synthesized several analogues of SG including solasodine conjugated with β-L-rhamnopyranose (**3**), β-D-xylopyranose (**4**), β-L-arabinopyranose (**5**), or β-D-ribopyranose (**6**), thio group substituted SG (**7**), and oxic group substituted SG (**8**) (Fig. 1) (Sun et al., 2010; Cui et al., 2012; Zan et al., 2014). In the present study, the in vitro antifungal activities of SG and its analogues were evaluated. SG exhibited fungicidal activity against *C. albicans* in Spider medium, whereas the other analogues were inactive. Structure-activity analysis revealed that both the NH group and glucosyl residue were necessary for SG activity. Further investigation demonstrated that SG penetrated the *C. albicans* cell membrane via the glucosyl residue. SG elicited the alkalization of vacuoles, which resulted in increased vacuole permeability and, consequently, triggered the death of *C. albicans*.

2. Materials and methods

2.1. Strains, culture and chemicals

The *C. albicans* wild type strain SC5314 and ten clinical isolates including five azole-sensitive and five azole-resistant isolates (Li et al., 2015a) were used in this study. The strains were stored routinely and propagated on yeast-peptone-dextrose (YPD) agar plates (2% tryptone, 1% yeast extract, 2% glucose and 2% agar) before each experiment. Following incubation for 24 h at 30 °C, the cells were inoculated in YPD broth (2% tryptone, 1% yeast extract, 2% glucose) for overnight culture at 30 °C and 200 rpm.

Solasodine (**1**) and SG (**2**) were previously isolated from SNL and several analogues of SG (**3**–**8**) were synthesized in our lab (Fig. 1) (Cui et al., 2012; Zan et al., 2014). To synthesize these glycosides, we first prepared glycosyl bromide as the glycosyl donor from the corresponding benzoylated monosaccharide using a solution of 45% HBr/HOAc in CH_2Cl_2. The glycosyl donors were condensed with aglycones such as solasodine via silver trifluoromethanesulfonate catalysis. The resultant intermediates were then hydrolyzed by MeOH/MeONa or CH_3NH_2 to remove the benzoyl group, finally providing the target compounds. Biotinylated-glucose (**9**) was previously synthesized in our lab (Fig. 1) (Wang et al., 2011). The vacuole-specific dyes Cell Tracker Blue CMAC Dye (CMAC) and *N*-(3-triethylammoniumpropyl)-4-(6-(4-(diethylamino) phenyl)hexatrienyl)pyridinium dibromide (FM4-64) were purchased from Invitrogen. Commercial quantum dots with streptavidin conjugate 605 nm (QD) were obtained from Invitrogen. The QD were composed of streptavidin covalently attached to a fluorescent nanocrystal (Qdot® nanocrystal). Qdot® nanocrystals are prepared from a nanometer-scale crystal of a semiconductor material (CdSe), which is coated with an additional semiconductor shell (ZnS) to improve the optical properties of the material. Streptavidin has a very high binding affinity to biotin or biotin conjugates, which permits the specific detection of a variety of proteins. Quinacrine, propidium iodide (PI), 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT), 2′,7'-dichlorofluorescin diacetate (DCFH-DA) and amphotericin B (AMB) were purchased from Sigma. Quinacrine, PI, DCFH-DA and AMB were prepared in dimethyl sulfoxide (DMSO) at 10 mg/ml and stored at −20 °C. In each assay, the content of DMSO was less than 1%.

2.2. Antifungal susceptibility test

The minimal inhibitory concentration (MIC) values of glycosides against *C. albicans* were determined using the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (M27-A3) (CLSI, 2008). Briefly, the antifungal agents were 2-fold serially diluted in cell suspensions in RPMI 1640 medium or Spider medium (1% nutrient broth, 0.5% mannitol, 0.1% K_2HPO_4, pH 7.2). Then, 100-μl aliquots were added to 96-well flat-bottomed microtitration plates. The plates were incubated at 35 °C for 24 h; zero visible growth was considered as the endpoint value.

2.3. Time-killing kinetics

To investigate the fungicidal activity of SG, time-killing curves were plotted by measuring the cell-survival rates. Overnight-cultured SC5314 cells were diluted to a final concentration of 1×10^5 cells/ml and treated with 32 or 64 μg/ml SG in Spider medium at 30 °C. At different time intervals, an aliquot was removed and plated on YPD agar plates to enumerate the surviving colonies.

2.4. QD visualization assay

Biotinylated-glucose (**9**) (2 μM) and QD (20 nM) were co-incubated at 4 °C for 40 min to form glucose-QD complexes (Wang et al., 2011). Prepared SC5314 cells were diluted to 5×10^5 cells/ml and incubated with the glucose-QD complexes or QD as a negative control at 30 °C for 1 h. After washing twice, the cells were imaged by confocal laser scanning microscopy (CLSM) (Carl Zeiss, LSM700, Germany) using a 63 × objective lens. The fluorescence of the QD upon excitation by a 555-nm laser was recorded in the emission spectrum of 590–700 nm.

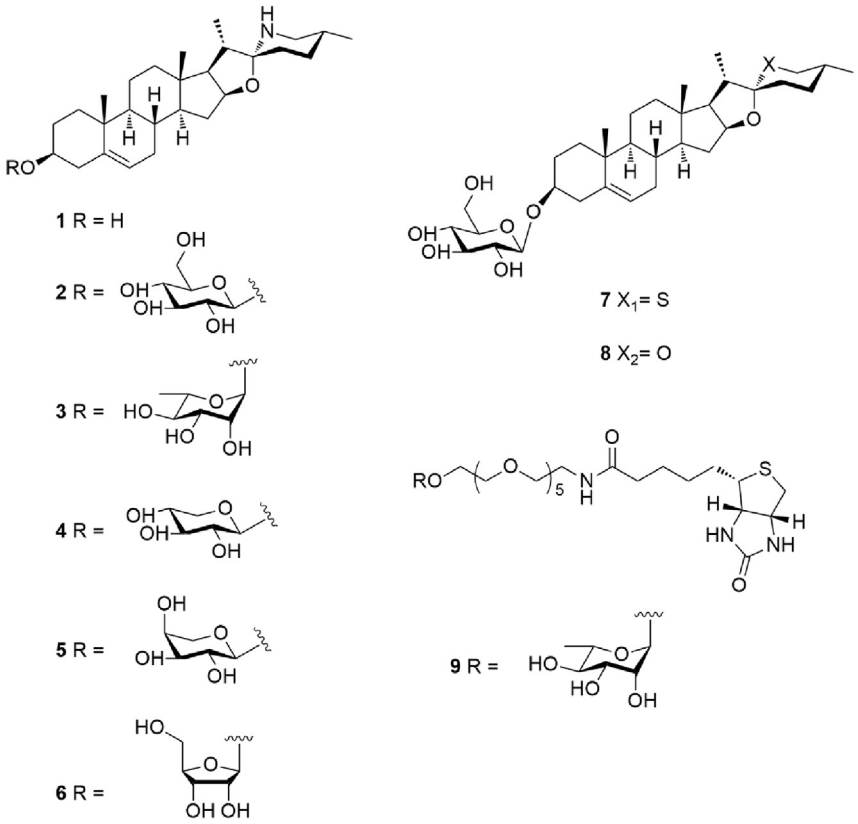

Fig. 1. The structures of the compounds used in this study: **1** solasodine; **2**–**6** solasodine glycosides; **9** biotinylated glucose. These molecules were obtained through isolation from SNL or chemical synthesis in our lab (Cui et al., 2012; Wang et al., 2011; Zan et al., 2014).

2.5. Rescuing experiments using different carbohydrates

Overnight-cultured SC5314 cells were inoculated into modified Spider medium containing different carbon sources (0.5% mannitol, 5% mannitol, 0.5% rhamnose, 5% rhamnose, 0.5% glucose, or 5% glucose) and incubated for 4 h at 30 °C and 200 rpm. The cells were then exposed to different doses of SG (32, 64, and 128 μg/ml) in the corresponding culture medium. After incubation for12 h, an XTT reduction assay (Ramage et al., 2001) was performed to determine the inhibitory effect of SG in each treated group.

2.6. Measurement of ROS formation in C. albicans

C. albicans SC5314 was cultured in Spider medium and challenged with SG. After 4 h of treatment, the cells were collected and stained with 40 μg/ml DCFH-DA. After incubation for 30 min in the dark, the samples were washed twice and analyzed using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA). The green fluorescence of DCF was excited by a 488-nm laser and collected in the FL1 channel (530 ± 15 nm). A total of 10,000 events in each sample were recorded for analysis. The resultant data were processed using WinMDI 2.9 software (Joseph Trotter, The Scripps Institute, La Jolla, CA).

2.7. Vacuolar pH measurements

Quinacrine accumulation, which exhibits green fluorescence under acidic conditions, was used to qualitatively assess the vacuolar pH in live *C. albicans* cells. Cells (1×10^5 cells/ml) were exposed to different concentrations of SG. After 4 h of incubation at 30 °C, the cells were stained with 200 μM quinacrine as previously described (Raines et al., 2013). After staining, the cells were collected, washed twice in YPD, and examined by CLSM with excitation at 488 nm and emission in the range of 490–550 nm.

2.8. Vacuole permeability measurements

To visualize vacuole membrane permeabilization, we utilized two independent staining dyes. For FM4-64 staining, log-phase *C. albicans* cells were diluted to 1×10^5 cells/ml and grown in YPD medium with or without SG for 4 h. Next, 20 μg/ml of FM4-64 was added to the cell cultures and incubated for 1 h at 30 °C. The cells were then collected, resuspended in fresh YPD medium, and incubated for 1 h at 30 °C prior to CLSM observation. For CMAC staining, cells were loaded with 5 μM CMAC and treated with SG for 4 h, followed by staining with 5 μg/ml PI staining for an additional 30 min. The cells were then collected and washed prior to CLSM observation. The fluorescence of FM4-64 was excited by a 555 nm laser and recorded in an emission spectrum of 570–700 nm. CMAC and PI were excited by 405 nm and 555 nm lasers, respectively, and emission spectra of 420–490 nm for blue fluorescence and 570–700 nm for red fluorescence were recorded.

2.9. The relationship between vacuole permeability and cell death

To determine if increased vacuole permeability was a late consequence of cell death or a prerequisite event for cell death caused by SG, *C. albicans* SC5314 cells were loaded with 5 μM CMAC, a dye that specifically accumulates in normal vacuoles. The pre-stained CMAC cells were treated with 64 μg/ml SG; 5 μg/ml PI was added at the same time as a death indicator. After treatment for 3 h at 30 °C, we chose typical cells with diffuse CMAC and without

PI staining for CLSM observation. These cells were imaged at 5-min intervals. CMAC and PI were excited by 405 nm and 555 nm lasers, respectively, and emission spectra of 420–490 nm for blue fluorescence and 570–700 nm for red fluorescence were recorded.

2.10. Statistical analysis

All experiments were performed three times on different days. The results are represented as the means±standard deviations (SDs). Statistical significances were determined by Student's *t*-test and a P value < 0.05 indicated statistical significance.

3. Results

3.1. SG exerts fungicidal activity against C. albicans in vitro

The antifungal activities of SG and its derivatives (Fig. 1) were assessed by the broth microdilution method. The susceptibility test results showed that SG (**2**) exhibited antifungal activity against the *C. albicans* wild type strain SC5314. However, when the glucosyl residue was substituted by other monosaccharides (**3**–**6**) including β-L-rhamnopyranose, β-D-xylopyranose, β-L-arabinopyranose, and β-D-ribopyranose (Cui et al., 2012), no inhibitory effect on the growth of *C. albicans* was observed, even at concentrations of 1024 μg/ml (Table 1). Moreover, the aglycone solasodine (**1**) did not show any antifungal activity, even at 1024 μg/ml (Table 1). These data suggested an essential role of the glucosyl residue in antifungal bioactivity.

Further investigation revealed that the activity of SG was enhanced in Spider medium, with an MIC value of 32 μg/ml, compared to 128 μg/ml in RPMI 1640 medium (Table 1). Based on its higher activity in the susceptibility test, SG was chosen for subsequent study. Azoles often elicit drug resistance in *C. albicans* (Canuto, 2002). We found that SG inhibited the growth of both azole-sensitive and azole-resistant clinically derived strains in Spider medium (Table 2). Moreover, time-killing curves demonstrated that SG displayed potent fungicidal activity at the concentrations of 32 μg/ml and higher, with AMB serving as the positive control (Fig. 2).

3.2. The NH group is required for SG activity

NH is required for the bioactivity of some agents (Cheng et al., 2007). When the NH group in SG was replaced with a thio or oxic group, the antifungal activity of the resultant compounds **7** and **8** (Fig. 1) was abolished (Table 1), suggesting that NH is necessary for the activity of SG.

3.3. Uptake of SG into C. albicans cells is mediated by the glucosyl moiety

We hypothesized that glucose lectins mediate the uptake of SG into *C. albicans*. To test this hypothesis, we utilized previously synthesized biotinylated-glucose (**9**) as a molecular probe bound to QD with bio-conjugated streptavidin to perform a binding assay (Wang et al., 2011). Confocal imaging revealed that the biotinylated-glucose preferentially bound to the cell membrane, as indicated by QD fluorescence around the cytoplasmic membrane, suggesting the possible existence of glucose lectins (Fig. 3). As further confirmation, we compared the susceptibility to SG of *C. albicans* cultured in Spider medium with mannitol as the carbon source to that of *C. albicans* cultured in Spider medium with glucose or rhamnose instead of mannitol (Spider-Glu or Spider-Rha). Pre-incubation of *C. albicans* in Spider-Glu for 4 h greatly reduced the susceptibility of the cells to SG (Fig. 4). However, when glucose was

Table 2
The MIC values of SG against different *C. albicans* strains cultured in Spider medium.

Strains	MICs of fluconazole (μg/ml)[a]	MICs of SG (μg/ml)
Wild type strains		
SC5314	2	32
YEM30	2	32
Clinical isolates		
23C	>128	32
23L	>128	32
24F	>128	32
28A	>128	32
28I	>128	32
18A	1	32
18B	0.5	32
18C	1	32
11E	1	32
11F	1	32

[a] The MICs of fluconazole against clinical isolates have been reported in our previous publication.

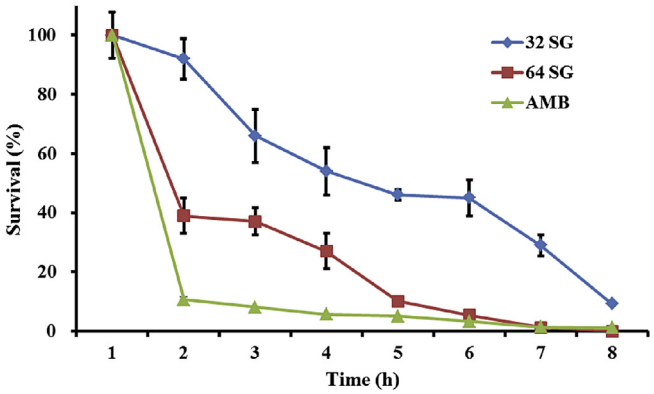

Fig. 2. The time-killing kinetics of SG against *C. albicans* SC5314. The wild type strain SC5314 was diluted with Spider medium to 1 × 10^5 cells/ml and exposed to 32 or 64 μg/ml of SG. At specific time points, the survival rates were calculated based on surviving colonies. The results are shown as the averages and standard deviations of three independent experiments.

Table 1
The MIC values of different compounds against *C. albicans* wild type strain SC5314 when cultured in RPMI1640 medium or Spider medium.

Compound	MICs in RPMI1640 medium (μg/ml)	MICs in Spider medium (μg/ml)
1	>1024	>512
2	128	32
3	>1024	>512
4	>1024	>512
5	>1024	>512
6	>1024	>512
7	>1024	>512
8	>1024	>512

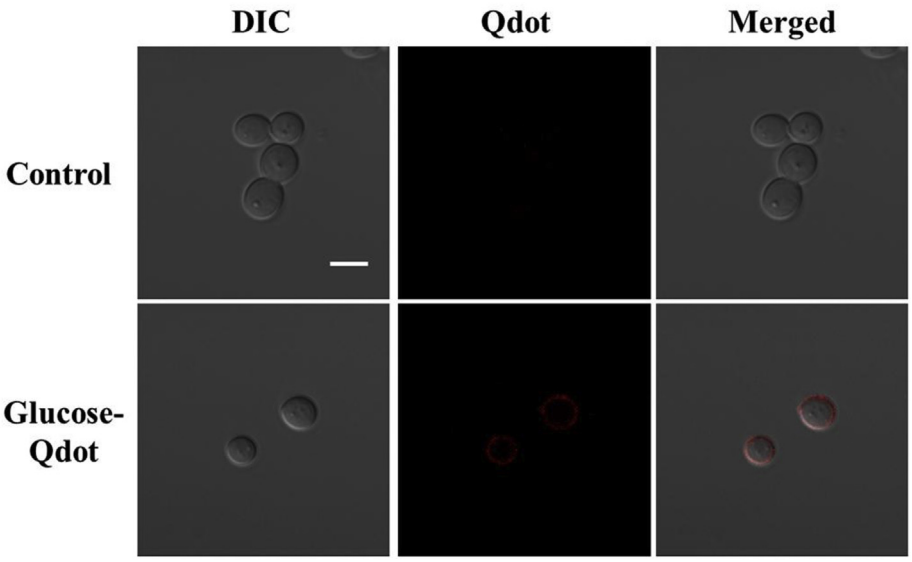

Fig. 3. Confocal microscopic observation of the binding of glucose-QD to the cell surface of *C. albicans*. *C. albicans* SC5314 cells were diluted to 5×10^5 cells/ml and incubated with preformed glucose-QD complexes or QD (negative control) for 1 h. The cellular localization of the QD was visualized by CLSM. Scale bar, 5 μm.

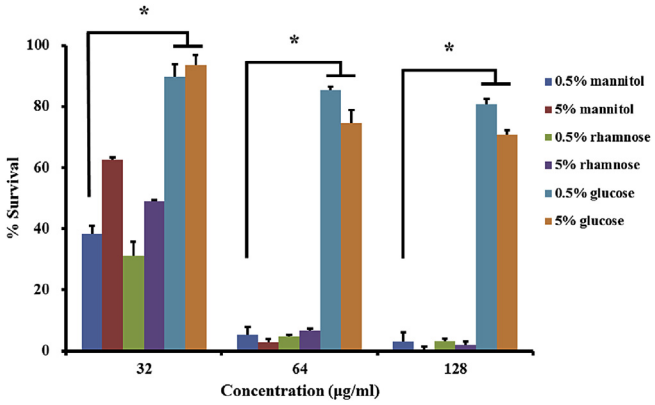

Fig. 4. The effect of different carbon sources on the fungicidal activity of SG. The wild type strain SC5314 was pre-incubated in Spider medium with different concentrations of mannitol, rhamnose, or glucose as carbon sources for 4 h. The pre-incubated cells were then exposed to different doses of SG. After 24 h, an XTT reduction assay was performed to assess the growth-inhibitory effects of SG under different culture conditions. The bars indicate standard deviations.

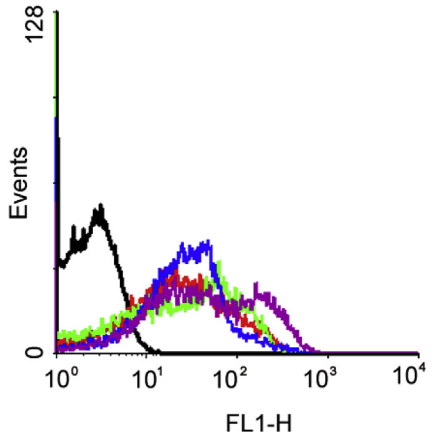

Fig. 5. ROS generation by *C. albicans* SC5314 when treated with SG. *C. albicans* cells were cultured in Spider medium and treated with SG for 4 h. The intracellular ROS contents were detected by flow cytometry based on the fluorescence intensity. Black line, negative control in which *C. albicans* cells were not stained with DCFH-DA; red line, vehicle control; green line, group treated with 16 μg/ml SG; blue line, group treated with 32 μg/ml SG; purple line, group treated with 64 μg/ml SG. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

substituted as the carbon source by other sugars such as rhamnose, the susceptibility to SG was not significantly different from that in Spider or Spider-Rha medium (Fig. 4). These results indicated that the addition of rhamnose did not inhibit the uptake of glucose into *C. albicans* cells, further confirming the existence of glucose lectins.

3.4. The fungicidal action of SG is independent of ROS generation

Fungicidal agents often cause ROS production, an important factor in cell death (Kobayashi et al., 2002; Phillips et al., 2003). The fluorescent probe DCFH-DA, which is an indicator of ROS formation (Shirtliff et al., 2009), was utilized to evaluate the effect of SG on intracellular ROS production in *C. albicans*. Flow cytometry detection showed that SG caused only a slight increase in ROS in *C. albicans* cells compared with the control (Fig. 5), suggesting that a mode of action distinct from ROS is responsible for the fungicidal activity of SG.

3.5. SG elicits the alkalization of vacuoles in C. albicans

The fungal vacuole is an acidic compartment that is involved in hydrolysis, storage, osmoregulation, homeostasis and detoxification (Teter and Klionsky, 2000). Normal vacuole function is critical for fungal cell survival. We found that the vacuolar pH of *C. albicans* became more alkaline upon treatment with SG, as assessed by quinacrine staining (Fig. 6).

3.6. SG increases vacuole permeability and cell death in C. albicans

FM4-64, a lipophilic dye that binds to the cellular membrane, is internalized by endocytosis and accumulates within the vacuole (Vida and Emr, 1995), whereas CMAC passively accumulates in the vacuolar lumen (Rane et al., 2014). FM-64 staining revealed that the

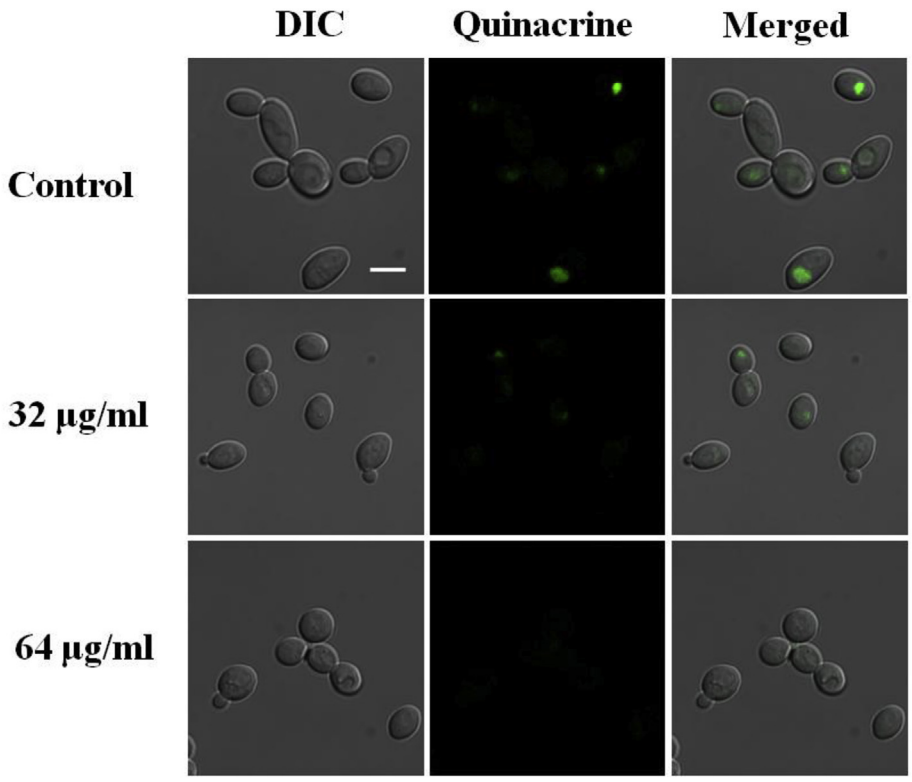

Fig. 6. The effect of SG on the vacuole acidification in *C. albicans*. The wild type strain SC5314 was treated with different concentrations of SG for 4 h and stained with quinacrine for CLSM observation. Quinacrine stains acidic compartments and is quenched as the pH increases. Scale bar, 5 μm.

vacuole membrane was disrupted by SG at a dose of 64 μg/ml in YPD medium (Fig. 7A). CMAC staining showed that the vacuole contents leaked into the cytoplasm after exposure to 64 μg/ml of SG, resulting in cell death, as indicated by intracellular PI accumulation (Fig. 7B).

To determine if the increased vacuole permeability was a late consequence of cell death or a prerequisite caused by SG, *C. albicans* cells were pre-stained with CMAC, a dye that specifically accumulates in normal vacuoles. The pre-stained CMAC cells were treated with SG, and PI was added as a cell death indicator. After 3 h of treatment, typical cells with diffuse CMAC and without PI staining were chosen for confocal microscopic observation. The time-sequence images revealed that the increased vacuole permeability occurred before cytoplasm membrane disruption, as indicated by the penetration of PI. These observations suggest that vacuole permeability was a prerequisite for cell death rather than a late consequence of cell death caused by SG (Fig. 8).

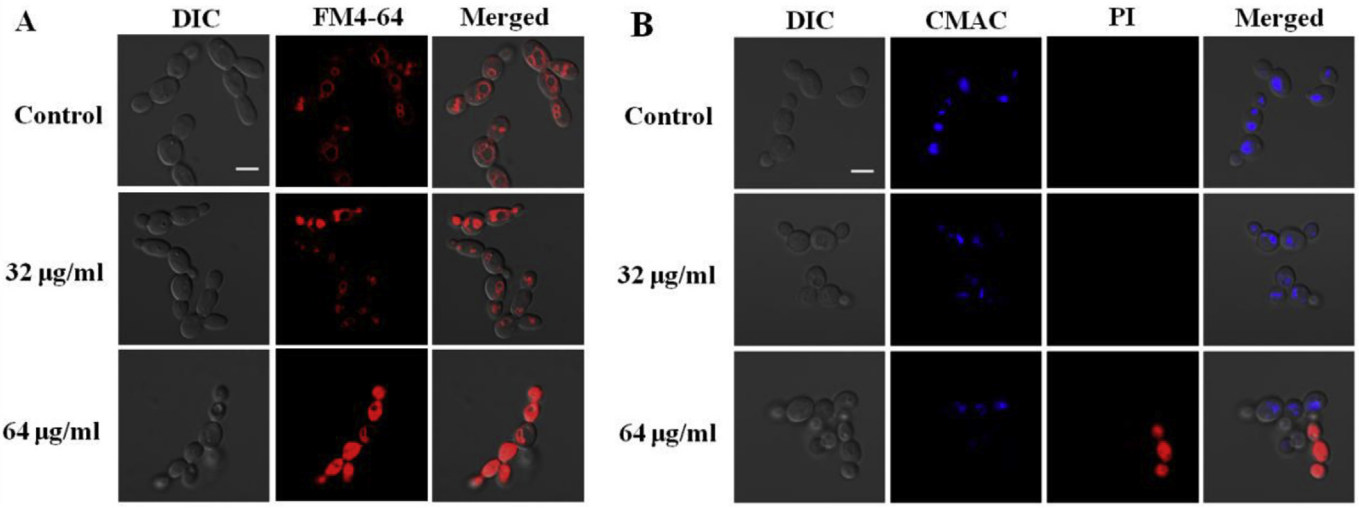

Fig. 7. The effect of SG on the vacuole membrane permeability of *C. albicans*. (A) The wild type strain SC5314 was treated with different concentrations of SG for 4 h. SG-treated cells were stained with FM4-64 for CLSM observation. (B) *C. albicans* cells pre-stained with CMAC, a dye that specifically stains vacuoles in fungi, were treated with SG for 4 h, followed by PI staining to assess the live/dead state. The double-stained cells were then observed by CLSM. Scale bar, 5 μm.

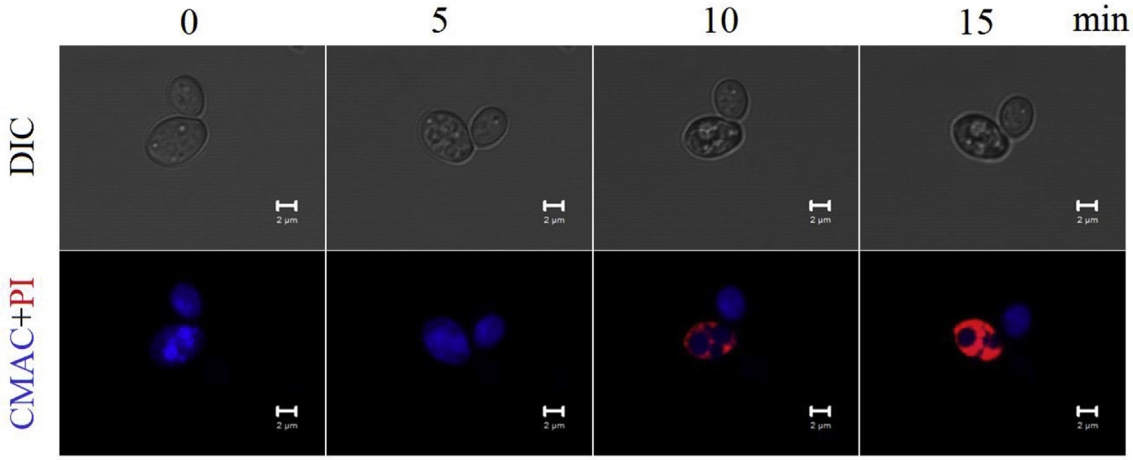

Fig. 8. Time-sequence observation of SG-treated cells. SC5314 cells were loaded with CMAC for 30 min. The preloaded cells were treated with 64 μg/ml SG for 3 h. Typical cells with diffuse CMAC and without PI staining were chosen for confocal microscopic observation. Images were obtained at 5-min intervals.

4. Discussion

Steroidal glycoalkaloids have been reported to possess antimicrobial and antitumor activities (Niño et al., 2009; Sun et al., 2010). In this study, we found that SG isolated from *Solanum nigrum* L. displayed potent antifungal activity against *C. albicans* in vitro, whereas its aglycon solasodine was inactive, suggesting a pivotal role of the glycan in bioactivity. However, when the glucosyl residue of SG was substituted by other monosaccharides, the antifungal activity was abolished (Table 1), implying a necessary role of the glucosyl moiety in SG. The QD-glucose binding assay also revealed that the glucosyl residue is a key mediator of the intracellular uptake of SG and thus probably determines the antifungal activity of SG. Similarly, dihydrodehydrodiconiferyl alcohol 9′-*O*-β-D-glucoside, which exhibits antifungal activity via disruption of the cell membrane (Choi et al., 2012), does not exhibit antifungal activity when the glucosyl moiety is removed to generate the aglycone (our unpublished data). Furthermore, when the NH group in the SG structure was replaced by a thio or oxic group, the resultant derivatives (**7**–**8**) also lacked the antifungal activity, suggesting an essential role of NH in maintaining the antifungal activity of SG. This finding is consistent with a previous study that indicated that the presence of NH is critical for the DNA-damaging activity of solasodine (Kim et al., 1996; Niño et al., 2009). The presence of an NH group is also important for the bioactivity of other steroidal alkaloids (Yang et al., 2012).

Given the critical role of the glucosyl residue in SG, we utilized biotinylated glucose as a mimic of SG to monitor its distribution in *C. albicans* cells. The QD visualization test demonstrated that the glucosyl group mediated the transport of QD through the cell membrane in *C. albicans*, suggesting the possible existence of glucose lectins (Fig. 3). Further experiments showed that the addition of glucose retarded the efficacy of SG (Fig. 4), thus confirming the existence of glucose lectins.

Increased intracellular ROS generation is a key factor in the cell death induced by some antifungal agents, such as miconazole, AMB, and farnesol (Kobayashi et al., 2002; Phillips et al., 2003; Shirtliff et al., 2009). Here, we found that ROS generation did not significantly increase in *C. albicans* cells in the presence of SG, which implies that a different mode of action is responsible for the activity of SG. Normal vacuole function is required to maintain cellular homeostasis (Kim et al., 2012; Li and Kane, 2009). We found that SG elicited vacuole alkalization which ultimately resulted in vacuole permeability (Fig. 5). Alkalization is not a prerequisite event for cell death because fluconazole, a fungistatic drug, also elicits vacuole alkalization in *C. albicans* cells (Zhang et al., 2010). We hypothesized that the increased vacuole permeability is a key event resulting in cell death. Time-sequence images revealed that the increased vacuole permeability occurred before cytoplasmic membrane disruption (Fig. 8), suggesting that SG resulted in vacuole leakage, which mediated cell death. This conclusion is consistent with findings in *Saccharomyces cerevisiae* (Kim et al., 2012).

Taken together, we found that the glucosyl residue and NH group are necessary for the activity of SG, which displays fungicidal action by disrupting vacuole function in *C. albicans* cells. Moreover, glucosylation might be employed as a strategy to transform some natural products into active antifungal agents.

Conflict of interest

The authors declare that they have no conflict of interest.

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Transparency document

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