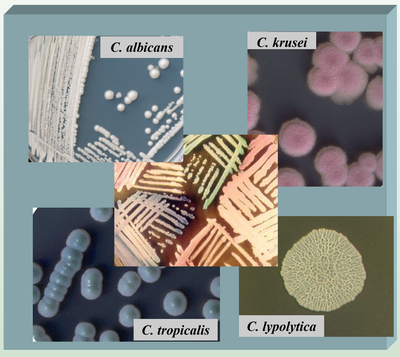
ANTIFUNGAL ACTIVITY OF DIOSGENYL 2-AMINO-2-DEOXY-β-D-GLUCOPYRANOSIDE HYDROCHLORIDE AND ITS *N,N*-DIALKYL DERIVATIVES AGAINST CLINICAL ISOLATES OF *CANDIDA SPP.*

Henryk Myszka[1], Daria Grzywacz[1] and Małgorzata Dawgul[2]

[1]*Faculty of Chemistry, University of Gdańsk, Sobieskiego 18, 80-952 Gdańsk, POLAND*

[2]*Faculty of Pharmacy, Medical University of Gdańsk, Al. Gen. Hallera 107, 80-416 Gdańsk, POLAND*

e-mail: myszka@chem.univ.gda.pl



Introduction

Candida species are common pathogens which cause opportunistic oral and genital infections in humans. Factors favoring the development of candidiasis are cancer, AIDS, treatment with antibiotics, corticosteroids or cytotoxic drugs. Strains of *Candida albicans* constitute about 60% of the strains isolated from patients suffering from candidiasis, but recent data show the increasing occurrence of strains called *non-albicans Candida*. Species belonging to this group are often characterized by reduced susceptibility to antifungal agents.

Systemic fungal infections (fungemias) have emerged as an important cause of morbidity and mortality in immunocompromised patients. In view of the fact that non-albicans infections are becoming more common and non-albicans species are more resistant to the antifungal treatment, we have made an attempt to find new substances active against those pathogens.

Material and Methods

The synthetic strategy is based on the preparation of glycosyl donors, coupling of these donors with diosgenin, deprotection of NH_2 and OH groups and finally receiving of *N,N*-dialkyl derivatives.

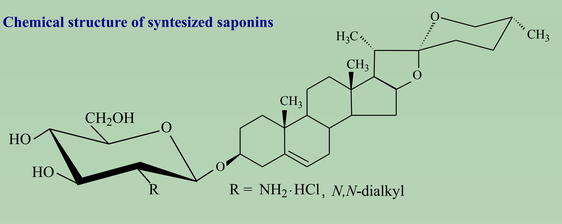
The structure of our products were confirmed by IR, ^1H, ^{13}C NMR spectroscopy and mass spectrometry.

Fungal isolates were obtained from Mycology Department of Jagiellonian University Medical Collage.

Diosgenyl glycosides are steroid saponins isolated from a variety of plants, for example *Costus*, *Dioscorea*, *Paris*, *Solanum*, *Trillium*, *Yucca*. Some of them exhibit a wide spectrum of biological activities including antifungal, antibacterial and anticancer properties.

We have synthesized a diosgenyl glycosides containing D-glucosamine derivatives as a carbohydrate residue. These glycosides have not been found in natural sources so far. We have tested their antifungal activity against clinical strains of *Candida* spp.

Chemical structure of syntesized saponins



Minimum inhibitory concentration (MIC) was determined for five steroidal saponins and six conventional antifungal agents (amphotericin B, clotrimazole, fluconazole, itraconazole, natamycin, nystatin). Antifungal activity against reference strains (*Candida albicans* ATCC 10231, *C. tropicalis* PCM 2681, *C. lipolytica* PCM 2680) and clinical isolates (*C. glabrata* - 22, *C. krusei* - 12, *C. tropicalis* - 13, *C. parapsilosis* - 19) was evaluated. MIC was determined by the broth dilution method according to the procedures recommended by CLSI (Clinical and Laboratory Standards Institute). Fungi at initial inoculum 10^3 CFU/ml diluted in Sabouraud glucose liquid medium (Sigma-Aldrich) were exposed to the saponins at adequate concentrations (range 0.025÷1024 μg/mL). Polystyrene 96-well plates (Greiner Bio-One) were incubated for 48 h at 25˚C. MIC was taken as the lowest drug concentration at which a noticeable growth was inhibited.

The experiments were performed in triplicate.

Table 1. Antifungal activity of steroidal saponins

		C. glabrata			*C. krusei*			*C. parapsilosis*			*C. tropicalis*			*C. albicans*	*C. lypolitica*	*C. tropicalis*
	R	range	50%	90%	range	50%	90%	range	50%	90%	range	50%	90%	ATCC 10231	PCM 2680	PCM 2680
S-1	$NH_2 \cdot HCl$	0.25-8	2	4	0.5-1024	16	1024	1-512	2	4	0.5-1024	4	1024	2	2	0.5
S-2	$N(CH_2CH_3)_2$	0.5-8	4	4	2-1024	64	1024	1-256	2	4	2-1024	4	512	2	2	1
S-3	$N(CH_3)_2$	0.5-8	4	4	2-1024	16	1024	0.5-128	1	2	2-1024	4	1024	2	2	1
S-4	$N(CH_2CH_2CH_3)_2$	0.5-256	4	8	4-1024	1024	1024	2-256	4	4	4-1024	8	1024	4	8	4
S-5	$N(CH_2CH_2CH_2CH_3)_2$	1-512	8	16	8-1024	1024	1024	4-256	8	16	4-1024	128	1024	8	32	8

Table 2. Antifungal activity of conventional antimicrobials

	C. glabrata			*C. krusei*			*C. parapsilosis*			*C. tropicalis*			*C. albicans*	*C. lypolitica*	*C. tropicalis*
	range	50%	90%	range	50%	90%	range	50%	90%	range	50%	90%	ATCC 10231	PCM 2680	PCM 2680
Amphotericin B	1-4	2	2	1-2	2	2	1-4	2	4	0.5-4	1	2	1	2	1
Clotrimazole	0.25-16	4	8	0.25-2	0.25	0.25	0.25-32	0.25	0.25	0.25-16	4	16	16	0.25	4
Fluconazole	1-512	128	128	16-128	32	64	2-256	4	8	32-1024	128	1024	32	8	16
Itraconazole	0.25-128	4	32	0.25	0.25	0.25	0.25-512	0.25	0.25	0.25-1024	256	1024	8	0.25	0.25
Natamycin	1-4	2	2	1-2	1	2	2-4	4	4	2-4	2	4	2	4	1
Nystatin	1-16	4	8	1-4	2	4	2-8	4	8	1-8	2	4	2	2	1

Results and Discussion

Reference fungal strains turned out to be sensitive to both saponins and antifungal agents. S-1, 2 and 3 presented slightly higher activity in comparison with remaining two compounds. Among clinical strains of *C. krusei* and *C. tropicalis* we have identified numerous isolates with decreased susceptibility to steroidal saponins. There were several *C. krusei* isolates less susceptible to fluconazole while among *C. tropicalis* strains numerous were resistant to fluconazole and itraconazole. Tested saponins exhibited very strong activity towards clinical isolates of *C. glabrata* and *C. parapsilosis* comparable or stronger than conventional antimicrobials. Azole antifungal antibiotics demonstrated decreased activity towards several strains belonging to above mentioned species. Resistance to azoles is likely due to the common use of these compounds in treatment of fungal infections and connected with the drug efflux pomp mechanism. No strains with low susceptibility to remaining antifungals were found. Results of presented work encourage to continue the study on steroidal saponins and their potential application for the treatment of candidemia.

INNOVATIVE ECONOMY
NATIONAL COHESION STRATEGY

This research was financed by the EU grant UDA-POIG.01.01.02-14-102/09-03

EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND