Med Chem Res (2018) 27:980–988
DOI 10.1007/s00044-017-2121-5

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Antimicrobial and cytotoxic activities of short carbon chain unsaturated sucrose esters

Krasimira T. Petrova[1] · **M. Teresa Barros**[1] · **Ricardo C. Calhelha**[2] · **Marina Soković**[3] · **Isabel C. F. R. Ferreira**[2]

Received: 8 August 2017 / Accepted: 18 November 2017 / Published online: 27 November 2017

Abstract A library of C3–C5 unsaturated 6-*O*-sucrose esters have been investigated for their antibacterial, antifungal, and cytotoxic activities. Most of the target compounds showed good inhibitory activity against a variety of clinically and food contaminant important microbial pathogens. In particular, 6-*O*-methacryloyl sucrose **2** and 1′,2,3,3′,4,4′,6′-hepta-*O*-acetyl-6-*O*-methacryloyl sucrose **9** were the most active bactericides against all the tested bacteria with minimal inhibitory concentrations (MICs) ranging between 0.24 and 1.40 µM. The compound **9** showed also the highest antifungal activity with MICs from 0.28 to 1.10 µM. The synthesized compounds possessed low cytotoxicity against human breast, lung, cervical, and hepatocellular carcinoma cell lines without showing toxicity for non-tumor liver cells. Thus, this library of short carbon chain unsaturated sucrose esters represent promising leads for the development of new generation of sucrose-based antimicrobial agents.

Keywords Unsaturated esters · Sucrose · Antibacterial activity · Antifungal activity · Cytotoxic activity

✉ Krasimira T. Petrova
k.petrova@fct.unl.pt

[1] LAQV, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

[2] Mountain Research Centre (CIMO), ESA, Polytechnic Institute of Bragança, Campus de Santa Apolónia, 1172, 5301-855 Bragança, Portugal

[3] Department of Plant Physiology, University of Belgrade, Institute for Biological Research, "Siniša Stanković", Bulevar Despota Stefana 142, Belgrade 11000, Serbia

Introduction

The alarming rates of emerging and reemerging microbial threats coupled with the growing antimicrobial resistance to current antibiotics are major concerns to the public health and scientific communities worldwide (He et al. 2010; Butler and Cooper 2011). These trends have emphasized the urgent need for designing and developing new classes of antimicrobial agents with different chemical structures and mechanism of action compared with traditional drugs, in order to improve their activities while retaining good bioavailability and safety profiles (Ziemska et al. 2013).

Antitumor agents for chemotherapy also attract a lot of attention, since cancer is responsible for many lethal outcomes worldwide (Avendaño and Menéndez 2008). Various sugar esters, isolated from natural sources were tested as cancer inhibitors and could be applied as therapeutic or preventive compounds (Xu 2016). For example, saccharide–fatty acid esters were investigated for their antimicrobial, antitumor, and anti-human immunodeficiency virus (HIV) activity (Shen et al. 2012; Ye et al. 2016); and have been employed to form drug-delivery systems (Kitaoka et al. 2014). Several *O*-decanoyl sucrose esters were isolated from the natural sticky coating of tomatillo fruits and showed antiinflammatory activity as confirmed by in vitro cyclooxygenase enzymes inhibitory assays (Zhang et al. 2015).

Various glycosides can be found in natural resources, mainly in the form of glycoconjugates, such as glycopeptides, glycolipids, and nucleic acids, where the saccharide moiety plays important role for their biological activity (Crucho et al. 2015). Considering that sugar moieties with multiple hydroxyl groups have been extensively employed in drug design with the view to improve water solubility and to increase the interaction between receptors and guests for

molecular recognition (Dwek 1996; Peça et al. 2012; Cardoso et al. 2016) various novel saccharide-derived compounds were synthesized and their antimicrobial properties were tested (Potewar et al. 2013; Petrova et al. 2015b; Raposo et al. 2016).

Sucrose esters of fatty acids have found applications as emulsifiers, which have been approved for use in the food industry under the code E437 (Aguilar et al. 2010), and their antimicrobial properties were well documented in the literature (Marshall and Bullerman 1986; Yang et al. 2003). Their use have been extended to oral care, as they represents a non-toxic and non-allergenic means of controlling the acidogenic organisms associated with dental caries (Iwami et al. 1995). All this have lead to the synthesis of novel analogues and more extensive study of their properties and structure–activity relationships (SAR) (Xin 2014; Zhao et al. 2015). Also, it was shown that esters of sucrose with short-chain branched carboxylic acids-like 2-methylpropanoic, 2-methylbutyric, and 8-methylnonanoic acids, are produced by some plants to increase their resistance to insects (Neal et al. 1990). C6–C12 aliphatic acid sucrose esters, analogues to the natural sucrose esters isolated from various *Nicotiana* species were prepared and were shown to be potent whitefly insecticides (Chortyk et al. 1996).

Another widespread class of sucrose esters are bearing phenyl groups (Panda et al. 2011). Some examples are niruriside (1′,2,4,6-tetra-*O*-acetyl-3′,6′-*O*-cinnamoyl sucrose), which was a HIV REV/RRE-binding inhibitor (Duynstee et al. 1996; Qian-Cutrone et al. 1996); lapathoside D (3′,6′-*O*-coumaroyl sucrose) (Panda et al. 2012a); helonioside A (3′,6′-di-*O*-feruloyl sucrose), 3′,4′,6′-tri-*O*-feruloyl sucrose; and lapathoside C (6-*O*-feruloyl-3′,6′-*O*-coumaroyl sucrose) (Panda et al. 2012b), studied for their antitumor activity, demonstrating the interest in the pharmacological properties of these compounds.

Saccharide-containing synthetic polymers have attracted great attention because of their potentials as biotechnological, pharmacological, and medical materials (Kobayashi et al. 1985; Carneiro et al. 2001). The most widely used method for the synthesis of poly (vinylsaccharide)s was based on the free radical polymerizations of vinyl sugars (Klein et al. 1990). An extensive review of the preparation and applications of this type of polymers is available (Varma et al. 2004). Synthetic carbohydrate-based polymers having pendant sugar residues are of great interest, not only as simplified models for biopolymers bearing oligosaccharides, but also as artificial glycoconjugates in biochemistry and medicine.

The introduction of sugars into polymeric molecules can bestow new properties, such as increased polarity, chirality, biodegradability, and biocompatibility. Sucrose-containing polymers, having a polyvinyl backbone and pendant sucrose moieties, have been obtained by polymerization or copolymerization of sucrose derivatives—esters, ethers, and acetals, bearing a carbon–carbon double bond (Patil et al. 1991; Fanton et al. 1992; Jhurry et al. 1992; Ferreira et al. 2000). The monomers have been prepared either by multistep synthesis, leading to defined compounds and subsequently a well-defined polymerization processes, or by direct functionalization of unprotected sucrose, leading to mixtures of isomers and therefore to more complex polymers (Crucho et al. 2008; Petrova et al. 2014a).

Sucrose, being a biorenewable, biocompatible, and biodegradable raw material with relatively low cost (Lichtenthaler and Peters 2004), is a promising starting material for the synthesis of new compounds with biological activity (Queneau et al. 2008). Our research group has been focused on the applications of sucrose for the synthesis of new compounds with potential applications either industrial or in academia. In this sense, we have developed chemoselective methods for the derivatization of sucrose (Petrova et al. 2014a; Raposo et al. 2014), the synthesis of sucrose-based biodegradable polymers (Barros and Petrova 2009; Barros et al. 2010; Petrova et al. 2014b) and nanoparticles(Petrova et al. 2015a; Raposo et al. 2015). To the best of our knowledge, the biological activities of the short-carbon-chain unsaturated sucrose esters have not been tested.

Based on these literature data and the features described previously, we have created a small library of C3–C5 unsaturated 6-*O*-sucrose esters, as previously described (Barros et al. 2011), to be screened for their biological activities. Their antimicrobial and antifungal activities were tested and compared with the ones of some commercial antibiotics. Cytotoxicity against a number of human tumor cell lines and non-tumor liver cells primary culture was studied as well.

Materials and methods

Standards and reagents

Ampicillin, bifonazole, and ketoconazole were purchased by Panfarma (Belgrade, Serbia), Srbolek (Belgrade, Serbia) and Zorkapharma (Šabac, Serbia), respectively. Fetal bovine serum (FBS), L-glutamine, Hank's balanced salt solution (HBSS), trypsin–EDTA (ethylenediaminetetraacetic acid), penincillin/streptomycin solution (100 U/mL and 100 mg/mL, respectively), and RPMI-1640 were from Hyclone (Logan, USA). Streptomycin, acetic acid, ellipticine, sulforhodamine B (SRB), trypan blue, trichloroacetic acid (TCA), and Tris were purchased from Sigma Chemical Co. (Saint Louis, USA).

Chemistry

Compounds **1–14** were synthesized as previously described (Barros et al. 2011). Their structures and purity were confirmed by common analytical techniques.

Stock solutions of the compounds were prepared in 5% DMSO and kept at −20 °C. Prior to the assays, appropriate dilutions were prepared.

Antimicrobial activity

Antibacterial activity

The Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240), and *Listeria monocytogenes* (NCTC 7973), and the Gram-negative bacteria *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Escherichia coli* (ATCC 35210), and *Enterobacter cloacae* (human isolate), were used. The antibacterial assay was carried out by a microdilution method (Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, 8th ed. CLSI publication M07-A8. Clinical and Laboratory Standards Institute 2009; Tsukatani et al. 2012). The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0×10^5 CFU/mL. Compound solutions were added to the Tryptic Soy broth (TSB) medium (100 µL) with bacterial inoculum (1.0×10^4 CFU per well). The lowest concentrations without visible growth (at the binocular microscope) were defined as concentrations that completely inhibited bacterial growth (minimal inhibitory concentrations (MICs)). The MICs obtained from the susceptibility testing of various bacteria to tested extracts were determined also by a colorimetric microbial viability assay based on reduction of an INT ((p-iodonitrotetrazolium violet) [2-(4-iodophenyl)-3-(4-nitrphenyl)-5-phenyltetrazolium chloride; Sigma]) color and compared with positive control for each bacterial strains. The minimum bactericidal concentrations (MBCs) were determined by serial sub-cultivation of 2 µL into microtitre plates containing 100 µL of broth per well and further incubation for 24 h. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum. The optical density of each well was measured at a wavelength of 655 nm by Microplate manager 4.0 (Bio-Rad Laboratories) and compared with a blank (broth medium plus diluted extracts) and the positive control. Streptomycin and ampicillin were used as positive controls. Five percent DMSO was used as a negative control.

Antifungal activity

Aspergillus fumigatus (human isolate), *Aspergillus versicolor* (ATCC 11730), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus niger* (ATCC 6275), *Trichoderma viride* (IAM 5061), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112), and *Penicillium verrucosum* var. *cyclopium* (food isolate), were used. In order to investigate the antifungal activity of the compounds, a modified microdilution technique was used (Espinel-Ingroff 2001). The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v) and spore suspension was adjusted with sterile saline to a concentration of 1.0×10^5. Compound solutions were added to the broth Malt medium with inoculum. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs. The minimal fungicidal concentrations (MFCs) were determined by serial subcultivation of a 2 µL of tested compounds dissolved in medium and incubated for 72 h at 28 °C. The lowest concentration with no visible growth was defined as MFC indicating 99.5% killing of the original inoculum. Five percent DMSO was used as a negative control, and commercial fungicides, bifonazole, and ketoconazole were used as positive controls.

Cytotoxic activity

Cytotoxicity in human tumor cell lines

Four human tumor cell lines were used: MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung carcinoma), HeLa (cervical carcinoma), and HepG2 (hepatocellular carcinoma) from DSMZ (Leibniz-Institut DSMZ—Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH). Cells were routinely maintained as adherent cell cultures in RPMI-1640 medium containing 10% heat-inactivated FBS and 2 mM glutamine at 37 °C, in a humidified air incubator containing 5% CO_2. Each cell line was plated at an appropriate density (1.0×10^4 cells/well) in 96-well plates and allowed to attach for 24 h. Cells were then treated for 48 h with various concentrations of the compounds. Following this incubation period, the adherent cells were fixed by adding cold 10% TCA (100 µL) and incubated for 60 min at 4 °C. Plates were then washed with deionized water and dried; SRB solution (0.1% in 1% acetic acid, 100 µL) was then added to each plate well and incubated for 30 min at room temperature. Unbound SRB was removed by washing with 1% acetic acid. Plates were air dried, the bound SRB was solubilized with 10 mM Tris (200 µL) and the absorbance was measured at 540 nm in ELX800 Microplate Reader (Bio-Tek Instruments, Inc.;

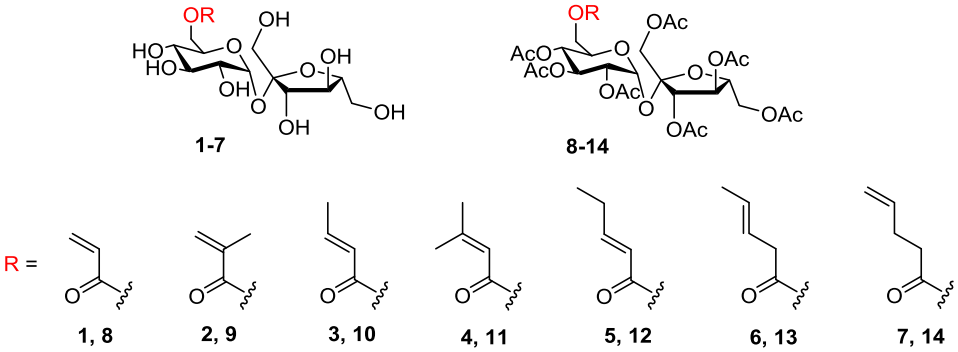

Fig. 1 General structure and library of the synthesized C3–C5 unsaturated 6-*O*-sucrose esters

Winooski, USA) (Abreu et al. 2011). The results were expressed in GI_{50} values (compound concentration that inhibited 50% of the net cell growth). Ellipticine was used as positive control. The absence of DMSO toxicity was confirmed by treating cells with the maximum concentration of DMSO used in the assays (0.25%).

Cytotoxicity in a porcine liver primary cell culture (PLP2)

A cell culture was prepared from a freshly harvested porcine liver obtained from a local slaughter house, and it was designed as PLP2. Briefly, the liver tissues were rinsed in HBSS containing 100 U/mL penicillin, 100 µg/mL streptomycin and divided into $1 \times 1\ mm^3$ explants. Some of these explants were placed in $25\ cm^2$ tissue flasks in DMEM medium supplemented with 10% FBS, 2 mM nonessential amino acids and 100 U/mL penicillin, 100 mg/mL streptomycin and incubated at 37 °C with a humidified atmosphere containing 5% CO_2. The medium was changed every 2 days. Cultivation of the cells was continued with direct monitoring every 2–3 days using a phase contrast microscope. Before confluence was reached, cells were subcultured and plated in 96-well plates at a density of 1.0×10^4 cells/well, and cultivated in DMEM medium with 10% FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin. SRB assay was performed according to a procedure previously described (Abreu et al. 2011). The results were expressed in GI_{50} values (compound concentration that inhibited 50% of the net cell growth). Ellipticine was used as positive control. The absence of DMSO toxicity was confirmed by treating cells with the maximum concentration of DMSO used in the assays (0.25%).

Statistical analysis

For all the experiments three solutions were prepared from each compound concentration, and all the assays were carried out in triplicate. The results were analysed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$. This analysis was carried out using SPSS v. 22.0 program (IBM Corp., Armonk, NY, USA).

Results and discussion

Chemistry

The library of C3–C5 unsaturated 6-*O*-sucrose esters is presented in Fig. 1 and has been synthesized as previously described (Barros et al. 2011). The antimicrobial and cytotoxic activities of the unsaturated 6-*O*-sucrose esters have been studied in their peracetylated form as well (compounds **8–14**). There are indications in the literature that the presence of hydrophobic groups as acetyls increases the molecule's tendency to aggregate on the cell membrane and facilitate its permeability. On the other hand, the presence of the acetyl groups can influence the enzymatic activity, triggering higher or lower affinity of the compound towards various enzymes involved in the processes (Liu et al. 2004).

Antibacterial activity

The results of the antibacterial activity, evaluated by the microdilution method, of the synthesized C3–C5 unsaturated 6-*O*-sucrose esters and standard antibiotics are presented in Table 1. All derivatives showed antibacterial activity against all the tested bacteria with MICs ranging between 0.24 and 10.60 µM and bactericidal concentrations (MBCs) from 0.44 to 14.00 µM. In general, the antibacterial inhibitory activity of the tested compounds could be presented as follows: **2 > 3 > 1 > 9 > 10 > 8 > 11 > 13 > 14 > 12 > 5 > 4 > 7 > 6**, but lower than the tested commercial drugs streptomycin and ampicillin. Regarding SAR, it was possible to conclude that the shorter carbon-chain esters with C3 and C4 (**1–3** and **8–10**) were more active than the longer ones with C5 (**4–7** and **11–14**). For the shorter carbon-chain esters with C3 and C4 (**1–3**) the free-hydroxyl groups form was more active than the acetylated (**8–10**),

Table 1 Antibacterial activity of compounds **1–14** (MIC and MBC in μM)

Compound	[μM]	*Bacillus cereus*	*Micrococcus flavus*	*Staphylococcus aureus*	*Listeria monocytogenes*	*Escherichia coli*	*Enterobacter cloacae*	*Pseudomonas aeruginosa*	*Salmonella typhimurium*
1	MIC	0.45	1.40	0.45	1.40	1.80	0.45	0.45	1.40
	MBC	0.90	1.80	0.90	1.80	2.50	0.90	0.90	1.80
2	MIC	1.36	0.24	0.44	0.24	0.24	0.24	1.36	1.36
	MBC	1.75	0.44	0.88	1.75	0.44	0.44	1.75	1.75
3	MIC	0.44	0.68	0.24	0.88	0.68	0.44	0.44	0.88
	MBC	0.88	0.88	0.44	1.75	0.88	0.88	0.88	1.75
4	MIC	3.50	5.30	3.50	7.00	2.25	3.50	3.50	7.00
	MBC	7.00	3.00	7.00	14.00	7.00	7.00	7.00	14.00
5	MIC	1.00	2.25	1.50	3.00	2.25	0.75	1.50	3.00
	MBC	1.50	3.00	3.00	4.50	3.00	1.50	3.00	4.50
6	MIC	2.35	5.30	2.35	7.00	10.60	10.60	5.30	7.00
	MBC	3.50	7.00	3.50	14.00	14.00	14.00	7.00	10.60
7	MIC	3.50	5.30	350	7.00	5.30	5.30	3.50	7.00
	MBC	7.00	7.00	7.00	10.60	7.00	7.00	7.00	10.60
8	MIC	1.10	2.20	1.10	2.20	3.25	1.45	1.45	2.20
	MBC	2.20	4.40	2.20	4.40	4.40	4.40	3.25	4.40
9	MIC	1.10	0.80	1.10	1.10	1.40	0.55	0.55	0.55
	MBC	2.20	1.10	2.20	2.20	2.20	1.10	1.10	1.10
10	MIC	0.70	1.10	1.10	2.20	1.40	1.40	1.10	2.20
	MBC	2.50	2.20	2.20	4.40	2.20	2.20	2.20	4.40
11	MIC	1.05	2.10	1.40	2.10	2.80	0.78	0.78	1.05
	MBC	2.10	4.20	2.10	4.20	4.20	1.05	1.05	2.10
12	MIC	1.05	2.80	2.10	4.20	4.20	1.05	0.78	2.10
	MBC	2.10	4.20	4.20	4.50	6.30	2.10	1.05	4.20
13	MIC	0.78	2.80	2.10	2.10	2.10	1.05	0.40	0.50
	MBC	1.05	4.20	4.20	4.20	4.20	2.10	0.50	1.05
14	MIC	1.40	2.10	2.80	2.80	2.80	1.05	1.05	2.10
	MBC	2.10	4.20	4.20	4.20	4.20	2.10	2.10	4.20
15. Streptomycin	MIC	0.043	0.086	0.17	0.25	0.17	0.043	0.17	0.17
	MBC	0.086	0.17	0.34	0.50	0.34	0.086	0.34	0.34
16. Ampicillin	MIC	0.25	0.25	0.25	0.37	0.37	0.25	0.74	0.25
	MBC	0.37	0.37	0.37	0.74	0.50	0.37	1.25	0.50

MIC minimum inhibitory concentration, *MBC* minimum bactericidal concentration, expressed in μM

Table 2 Antifungal activity of compounds **1–14** (MIC and MFC in μM)

Compound	[μM]	*Aspergillus fumigatus*	*Aspergillus versicolor*	*Aspergillus ochraceus*	*Aspergillus niger*	*Trichoderma viride*	*Penicillium funiculosum*	*Penicillium ochrochloron*	*Penicillium verrucosum*
1	MIC	1.40	0.90	0.75	2.50	0.45	0.90	0.90	1.40
	MFC	1.80	1.80	1.80	3.60	0.90	1.80	1.80	1.80
2	MIC	3.50	2.40	1.75	2.40	1.36	1.75	1.75	1.75
	MFC	7.00	3.50	3.50	3.50	1.75	3.50	3.50	3.50
3	MIC	1.75	0.88	0.88	1.75	0.44	1.36	0.88	1.36
	MFC	3.50	1.75	3.50	3.50	0.88	3.50	1.75	3.50
4	MIC	5.30	3.50	3.50	7.00	2.35	5.30	3.50	5.30
	MFC	7.00	7.00	7.00	14.00	3.50	7.00	7.00	7.00
5	MIC	7.00	5.30	5.30	7.00	1.75	5.30	5.30	7.00
	MFC	10.60	7.00	7.00	10.60	3.50	7.00	7.00	10.60
6	MIC	5.30	2.35	3.50	7.00	2.35	3.50	3.50	2.35
	MFC	7.00	5.30	7.00	10.60	3.50	7.00	7.00	3.50
7	MIC	5.30	2.35	2.35	5.30	2.35	2.35	2.35	2.35
	MFC	7.00	3.50	3.50	7.00	3.50	3.50	3.50	3.50
8	MIC	2.20	1.10	1.45	2.20	0.80	1.45	1.10	1.45
	MFC	4.40	2.20	2.20	4.40	2.20	2.20	2.20	2.20
9	MIC	1.10	0.28	0.55	0.55	0.40	0.28	0.28	0.55
	MFC	2.20	0.55	1.10	1.10	0.55	0.55	0.55	1.10
10	MIC	2.20	1.10	1.10	2.20	1.10	1.40	1.40	1.40
	MFC	4.40	2.20	2.20	4.40	2.20	2.20	2.20	2.20
11	MIC	2.10	1.05	1.05	2.10	0.78	1.40	1.40	2.10
	MFC	4.20	2.10	2.10	4.20	2.10	2.10	2.10	4.20
12	MIC	2.10	1.05	0.78	1.05	0.50	1.05	0.78	1.05
	MFC	4.20	2.10	1.05	2.10	1.05	2.10	1.05	2.10
13	MIC	2.25	1.05	2.10	2.25	1.05	1.40	1.40	1.40
	MFC	3.00	2.10	4.20	3.00	2.10	3.10	2.10	2.10
14	MIC	2.10	1.05	0.78	1.05	0.50	0.78	0.78	1.05
	MFC	4.20	2.10	1.05	2.10	1.05	1.05	1.05	2.10
15. Ketoconazole	MIC	0.38	2.85	0.38	0.38	4.75	0.38	3.80	0.38
	MFC	0.95	3.80	0.95	0.95	5.70	0.95	3.80	0.57
16. Bifonazole	MIC	0.48	0.48	0.48	0.48	0.64	0.64	0.48	0.48
	MFC	0.64	0.64	0.80	0.64	0.80	0.80	0.64	0.64

MIC minimum inhibitory concentration, *MFC* minimum fungicidal concentration, expressed in μM

while for the longer ones with C5 this relation was inverse and the acetylated forms (**11–14**) were more active than the free-hydroxyl groups forms (**4–7**). Between the esters with C5 carbon chain, the most active was 6-*O*-(2-pentenoyl) sucrose ester **5**. The methacryloyl esters, both with free hydroxyl groups **2** and acetylated **9**, were the most active compounds in this library, even more efficient than Ampicillin against *M. flavus*, *L. monocytogenes*, *E. coli*, and *En. cloacae*.

The most resistant bacteria to these compounds were *L. monocytogenes* and *S. typhimurium*, while the most susceptible bacteria were *B. cereus* and *S. aureus*. According to the bactericidal activities (MBC values), the most active compounds were **1**, **2**, and **3**, while the least active were **4**, **6**, and **7**.

Antifungal activity

All the synthesized sucrose esters **1–14** showed antifungal activity, which was also evaluated by the microdilution method, with MICs from 0.28 to 7 μM and MFCs ranging between 0.55 and 14 μM (Table 2). The antifungal activity could be presented as follows: **9 > 1 > 3 > 12 > 14 > 11 > 8 > 10 > 13 > 2 > 7 > 6 > 4 > 5**, which was in some cases higher than the tested standards, bifonazole and ketoconazole. The highest activity was verified against *T. viride*,

Table 3 Cytotoxicity and hepatotoxicity of the synthesized compounds 1–14 (GI_{50} values in μM)

Compounds	MCF7	NCI-H460	HeLa	HepG2	PLP2
1	188 ± 15.20	>400	>400	201.13 ± 13.21	>400
2	92.56 ± 4.93	>400	>400	274.78 ± 17.40	>400
3	252.86 ± 10.73	>400	>400	306.94 ± 18.96	>400
4	247.21 ± 10.52	>400	>400	233.53 ± 15.46	>400
5	62.70 ± 1.90	>400	>400	84.64 ± 1.76	>400
6	240.89 ± 8.49	>400	>400	212.36 ± 21.38	>400
7	215.79 ± 9.27	>400	277.73 ± 26.31	>400	>400
8	>400	>400	>400	>400	>400
9	305.49 ± 19.02	>400	>400	>400	>400
10	236.93 ± 14.42	286.82 ± 10.28	250.29 ± 8.56	180.52 ± 15.48	>400
11	61.30 ± 0.46	304.04 ± 7.86	318.30 ± 15.21	67.97 ± 3.30	>400
12	70.95 ± 3.60	>400	>400	82.65 ± 4.20	>400
13	248.20 ± 15.90	320.21 ± 12.29	283.31 ± 7.57	241.99 ± 23.15	>400
14	>400	>400	>400	>400	>400
Ellipticine	3.69 ± 0.16	7.96 ± 0.25	4.75 ± 0.05	13 ± 1	3.69 ± 0.16

GI_{50} values correspond to the compound concentration achieving 50% of growth inhibition in human tumor cell lines or in liver primary culture PLP2

while *A. fumigatus* and *A. niger* were the most resistant fungi. Fungi were in general more sensitive to the tested compounds than bacteria species.

According to the obtained results, the compound **9** (acetylated 6-*O*-methacryloylsucrose ester) showed the highest antifungal activity, with MICs higher than the both standard fungicides against the species *A. versicolor, T. viride, P. funiculosum*, and *P. ochrochloron*. All of the tested sucrose esters **1–14** were more potent fungi inhibitors than ketoconazole against *A. versicolor, T. viride*, and *P. ochrochloron*.

As it was the case for the antibacterial activity, again the shorter carbon-chain esters with C3 and C4 (**1–3**) were more active than the longer ones with C5 (**4–7**), for which the acetylated forms (**11–14**) were more active than the free-hydroxyl groups forms (**4–7**).

Cytotoxicity

The compounds cytotoxicity was evaluated through the SRB assay against four human tumor cell lines (breast—MCF7, non-small cell lung—NCI-H460, cervical—HeLa and hepatocellular—HepG2 carcinomas), and in a PLP2, established by some of us (Table 3). The tested compounds showed low activity against human breast, colon, and cervical carcinoma cell lines, being the first (MCF7) the most susceptible one, followed by the latter (HepG2). The obtained GI_{50} values were higher (lower antitumor activity) than the ones of the ellipticine (used standard). Nevertheless, it was noted that the tested compounds did not show toxicity for non-tumor cells (PLP2), while the standard proved to be strongly hepatotoxic (3.69 μg/mL).

At the maximum tested concentration (400 μg/mL), compounds **8** and **14** did not present any activity; but the compounds **10, 11**, and **13** inhibited the growth of all the tested tumor cell lines. Compound **11** was the most efficient inhibitor against MCF7 and HepG2.

Looking into compounds' structure, only the per-acetylated derivatives showed activity against NCI-H460 and HeLa (with exception of **7**), while the compounds with free hydroxyl groups were not active against these cell lines.

Conclusions

In summary, a small library of C3–C5 unsaturated sucrose esters has been synthesized as previously reported and screened for their antibacterial, antifungal, and cytotoxic activities in order to identify lead compounds for the pharmacology. The experimental results showed that all the derivatives showed antibacterial activity against all the tested bacteria with MICs ranging between 0.24 and 10.60 μM and antifungal activity with MICs from 0.28 to 7 μM. The shorter carbon-chain esters with C3 and C4 (**1–3** and **8–10**) were more active than the longer ones with C5 (**4–7** and **11–14**). For the shorter carbon-chain esters with C3 and C4 (**1–3**) the free-hydroxyl groups form was more active than the acetylated (**8–10**), while for the longer ones with C5 this relation was inverse, and the acetylated forms (**11–14**) were more active than the free-hydroxyl groups forms (**4–7**). The methacryloyl esters, both with free hydroxyl groups **2** and

acetylated **9**, were the most active compounds in this library. In general, the tested compounds exhibited a wide spectrum of activity, depending on the bacterial and fungal species.

The compounds showed low antitumor potential (especially towards human breast and hepatocellular carcinoma cell lines, MCF7 and HepG2 cells, respectively), but without hepatotoxicity towards non-tumor porcine liver primary cells, PLP2.

Acknowledgements This work has been supported by Fundação para a Ciência e a Tecnologia through grant nos. PEst-C/EQB/LA0006/2013 and PEst-OE/AGR/UI0690/2014. The authors thank Serbian Ministry of Education, Science, and Technological Development for financial support (grant number 173032).

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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