Supplementary material

Anticancer and antimicrobial properties of imidazolium based ionic liquids with salicylate anion

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Determination of antiproliferative activity

Cell lines and cell culture: Antiproliferative activity of the imidazolium-and salicylate-based ILs was tested against six human cancer cell lines: two types of human breast adenocarcinoma, thus the estrogen receptor positive (ER+) MCF-7 (American Type Culture Collection–ATCC HTB22) and triple negative MDA-MB-231 (ATCC HTB26), prostate cancer PC-3 (ATCC CRL 1435), cervix adenocarcinoma HeLa (ATCC CCL2), colon cancer HT-29 (ATCC HTB38) and lung cancer A549 (ATCC CCL 185) cell lines, as well as normal fetal lung fibroblast cell line MRC-5 (ATCC CCL 171). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 4.5% glucose, supplemented with 10% of fetal calf serum (Sigma) and antibiotics: 100 IU/mL penicillin and 100 μg/mLstreptomycin (Sigma). Cells were cultured in flasks (Costar, 25 cm^2) at 37 °C in high humidity with 5% CO_2. Only viable cells were used in the assays, and cell viability was determined by trypan blue dye exclusion test.

Antiproliferative activity and data analysis: Antiproliferative activity of the imidazolium- and salicylate-based ILswas evaluated by tetrazolium colorimetric MTT assay,[13] as previously described.[14] To measure the number of viable cells in microwell plates, cells were exposed to test compounds for 72 h at five concentrations ranging from 0.01 to 100 μM (0.01; 0.1; 1; 10 and 100 μM). Reference compounds used in this assay were cisplatin (Cis) and doxorubicin (Dox), as nonselective anticancer agents[15,16] and sodium salicylate to test salicylate toxicity, respectively. Exponentially growing cells were harvested, seeded into 96-well plates at a density of 5000 cells/well and allowed to stand overnight in complete medium at 37 °C, after which the medium containing the test compound was added (10 μL/well) in all wells except in negative controls. After

72h treatment, 10 mL of MTT solution (5 mg/mL), and, after 3h, acidified 2-propanol were added to each well. After a few minutes incubation at room temperature absorbance was read on a spectrophotometric plate reader (Multiscan MCC340, Labsystems) at 540/690 nm. Wells without cells, containing complete medium and MTT only, were used as a blank. Absorbances of samples (A_{sample}) and control ($A_{control}$) were measured and antiproliferative effect, presented as percent of cytotoxicity, was calculated according to the formula:

$$CI\ (\%) = (1 - A_{sample}/A_{control}) \times 100$$

The antiproliferative activity of compounds (expressed as a percentage of cytotoxicity) was obtained by averaging values from two independent experiments conducted in quadruplicate for each administrated concentration. The IC_{50} value, defined as a dose of compound that inhibits the cell growth by 50% related to control (untreated) cells, was determined for each tested compound by median effect analysis.[17]

Antimicrobial activity and data analysis

Bacterial and Candida strains: Six bacterial strains including three Gram-positive (G^+) bacteria: *S. aureush* (human), *B. subtilis* ATCC 6633 and *E. faecalis* ATCC 19433 and three Gram-negative (G^-) bacteria: *P. mirabilis h*, *E. coli* ATCC 11229 and *P. aeruginosa* ATCC 15692, and four yeast strains: two of them (*C. albicans* L and *C. albicans* ATCC10231) were obtained from the culture collection of microorganisms from Department of Biology and Ecology, University of Novi Sad, while two human yeast isolates (*C. albicans* III *h*and *Candida* IV *h*) were obtained from the Faculty of Medicine, Clinical Centre of Vojvodina. All human isolates of microorganisms were obtained from the Faculty of Medicine, Department of Obstetrics and Gynecology, University of Novi Sad, were protocol was approved by the Institutional Ethical Board of the same Institution.

Antimicrobial assay: The antibacterial activity of ILs was evaluated as minimum inhibitory concentrations (MICs) and minimum bactericidal/fungicidal concentrations (MBCs i.e. MFCs), by double-microdilution method according to the CLSI procedure.[18,19] The strains of bacteria were obtained from the overnight cultures, grown at 37°C on the Müller-Hinton agar (MHA, Torlak, Belgrade, Serbia), while yeasts strains were grown on the Sabouraud agar (SA, Torlak, Belgrade, Serbia) during 48h. McFarland inoculum of bacteria and yeasts were prepared in the sterile saline solution; reaching the final 1.5×10^6 CFU/mL for bacteria and 1.5 x 10^5 for yeasts. Müeller Hinton broth (MHB, Torlak, Belgrade, Serbia) and Sabouraud broth (SB, Torlak, Belgrade, Serbia) were used for the antimicrobial screening. Double dilution test was performed in a 96-well microtitre plate (Spektar, Čačak, Serbia) with MHB or SB and different concentration of ILs, diluted in sterile distilled water. The final concentrations of ILs ranged from 0.01 – 11 mg/mL. After incubation, during 24h or 48h for bacteria or yeast, respectively, MICs were determined visually. MBCs and MFCs were confirmed after inoculation of MHA and SA plates with 100 μL of broth, where

71 turbidity was absent (MIC point). Nystatin, the antifungal drug (Hemofarm, Vršac, Serbia),
72 andantibiotics streptomycin, kanamycin, ampicillin and chloramphenicol (Sigma), were used as
73 positive controls (in final concentrations ranging from 0.01 – 0.45 mg/mL), while distilled water
74 without ILs was used as negative control. Test was performed in triplicate for each compound and
75 the average was used for getting MIC, MBC or MFC values.

Table SI MIC, MBC and MFC values (mg/ml) of tested ILs and selected antibiotics/antimicotics towards bacterial and *Candida* strains

	Str	**Kan**	**Amp**	**Chlo**	**1**		**2**		**3**		**4**		**5**		**6**	
Bacterial strains					**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
	mg/mL				mg/mL											
S. aureus h	0.01	0.03	0.01	0.01	4.50	9.01	9.60	↑ 9.60	8.65	↑ 8.65	11.03	↑ 11.03	4.83	9.66	9.46	9.46
B. subtilis ATCC 6633	0.01	0.01	0.01	0.01	9.01	↑ 9.01	9.60	↑ 9.60	8.65	↑ 8.65	11.03	↑ 11.03	9.66	↑ 9.66	9.46	↑ 9.46
E. faecalis ATCC 19433	0.12	0.06	0.06	0.06	9.01	↑ 9.01	9.60	↑ 9.60	8.65	↑ 8.65	11.03	↑ 11.03	9.66	↑ 9.66	9.46	↑ 9.46
P. mirabilis h	R*	R*	R*	0.23	9.01	↑ 9.01	9.60	↑ 9.60	8.65	↑ 8.65	11.03	↑ 11.03	9.66	↑ 9.66	9.46	↑ 9.46
E. coli ATCC 11229	0.01	0.01	0.01	0.01	9.01	↑ 9.01	9.60	↑ 9.60	8.65	↑ 8.65	11.03	↑ 11.03	9.66	↑ 9.66	9.46	↑ 9.46
P. aeruginosa ATCC 15692	R*	R*	R*	0.12	9.01	↑ 9.01	9.60	↑ 9.60	8.65	↑ 8.65	11.03	↑ 11.03	4.83	9.66	9.46	↑ 9.46
Fungal strains	**Nystatin** (mg/mL)				**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Candida L	0.06				4.50	9.01	9.60	↑ 9.60	8.65	↑ 8.65	8.65	11.03	4.83	9.66	4.73	9.46
C. albicans ATCC 10231	0.25				4.50	9.01	4.80	9.60	4.32	8.65	2.76	5.51	2.41	4.83	2.36	4.73
C. albicans III h	0.25				4.50	9.01	4.80	9.60	4.32	8.65	2.76	5.51	2.41	4.83	2.36	4.73
Candida IV h	0.25				4.50	9.01	4.80	9.60	4.32	9.65	2.76	5.51	2.41	4.83	4.73	9.46

Str – streptomycin; **Kan** – kanamycin; **Amp** – ampicillin; **Chlo** – Chloramphenicol; R* - resistant; ↑ - the MBC/MFC value is higher than the highest tested concentration