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SUPPLEMENTARY MATERIAL TO

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

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ADDITIONAL EXPERIMENTAL DETAILS

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 72 h treatment, 10 mL of MTT solution (5 mg/mL), and, after 3 h, 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:

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

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.

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Antimicrobial activity and data analysis

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 48 h. 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 1.5×10^5 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 compounds ranged from 0.01 – 11 mg / mL. After incubation, during 24 or 48 h for bacteria or yeast, respectively, *MIC*s were determined visually. *MBC*s and *MFC*s were confirmed after inoculation of MHA and SA plates with 100 µL of broth, where turbidity was absent (*MIC* point). Nystatin, the antifungal drug (Hemofarm, Vršac, Serbia), andantibiotics streptomycin, kanamycin, ampicillin and chloramphenicol (Sigma), were used as positive controls (in final concentrations ranging from 0.01 – 0.45 mg / mL), while distilled water was used as negative control. Test was performed in triplicate for each compound and the average was used for getting *MIC*, *MBC* or *MFC* values

TABLE S-I. *MIC*, *MBC* and *MFC* values of tested ILs and selected antibiotics/antimicotics towards bacterial and *Candida* strains

Bacterial strains	**Str**	**Kan**	**Amp**	**Chlo**	**1**		**2**		**3**		**4**		**5**		**6**	
	mg mL^{-1}				***MIC***	***MBC***	***MIC***	***MBC***	***MIC***	***MBC***	***MIC***	***MBC***	***MIC***	***MBC***	***MIC***	***MBC***
					mg mL^{-1}											
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^{-1}				***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