Kragujevac J. Sci. 44 (2022) 57–74.
doi: 10.5937/KgJSci2244057S

UDC 547.783/739:579.61
Original scientific paper

SYNTHESIS OF SERIES OF DIFFERENT IMIDAZOLIDINE-2,4-DIONE DERIVATIVES AND EVALUATION OF THEIR ANTIMICROBIAL POTENTIAL

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(Received April 12, 2022; Accepted June 07, 2022)

ABSTRACT. A series of twenty two different imidazolidine-2,4-dione derivatives, divided according to their structure into five groups, including alkyl, alkenyl or aryl 5,5-disubstituted hydantoins, spirohydantoins, and fused bicyclic and tricyclic hydantoins, was synthesized and examined for *in vitro* antimicrobial activity against 15 strains of bacteria and 4 strains of yeast. The antimicrobial activity was evaluated by the determination of the minimal inhibitory concentration (MIC) and the minimal microbicidal concentration (MMC) using the microdilution method. The assayed compounds exerted moderate antibacterial and weak antifungal activity. The antimicrobial activities were influenced by the structure and concentration of the tested compounds as well as the type of test microorganisms. The fused bicyclic hydantoin derivatives obtained by organoselenium induced intramolecular cyclization exhibited the highest inhibitory activity. The examined hydantoin derivatives seem as drug-like candidate for further evaluation of biological activities.

Keywords: hydantoins; phenylseleno derivatives; antimicrobial activity.

INTRODUCTION

Imidazolidine-2,4-dione (hydantoin) is a five-membered cyclic ureide. It is a white crystal that has a high melting point (217-218°C). Hydantoins can be synthesized from various substrates, including aldehydes and ketones (Bucherer-Bergs reaction), amino acids (Urech reaction), nitriles (Reed reaction), carbamates, olefins, etc. (KONNERT *et al*, 2017). Hydantoins are an integral part of the biologically active compounds such as anti-arrhythmic, anti-convulsive, and antitumor agents (TRIŠOVIĆ *et al*., 2008). The biological activities of different hydantoins are possible due to the interaction between the hydantoin ring, and the

various substituent groups, which are appended to the ring (OLIMPIERI *et al*., 2009). In particular, spirohydantoins and fused polycyclic hydantoin derivatives have recently attracted much attention in drug discovery due to their various biological activities. Hydantoins are also known to possess activities against viruses (RAJIC *et al*., 2006), protozoa (KEISER *et al*., 2015, MEYERS *et al*., 2015) mycobacteria (ZAIDI *et al*., 1980), and bacteria and fungi (SZYMAŃSKA *et al*., 2002; CHEN and SUN, 2006; FUJISAKI *et al*., 2010; HANDZLIK *et al*., 2011; TRIŠOVIĆ *et al*., 2011) due to the urea as an active component. Some hydantoins are an integral part of the product intended for consumer use, such as hair sprays and cosmetics as preservatives (TRIŠOVIĆ *et al*., 2008). Their functions are to prevent bacteria and fungi access as 1,3-dimethylol-5,5-dimethyl hydantoin (DMDM) which meets all microbiological, technical, and toxicological standards (SCHANNO *et al*., 1979).

Due to the increasing resistance of microorganisms to antibiotics and the extensive biological activity exhibited by hydantoins the aim of this study was to synthesize a series of 22 different hydantoin derivatives and to investigate their antibacterial and antifungal activity.

MATERIALS AND METHODS

Chemicals

Dimethyl sulfoxide (DMSO) was purchased from Acros Organics (New Jersey, USA). Resazurin was obtained from Alfa Aesar GmbH & Co. (KG, Karlsruhe, Germany). Nutrient liquid medium, Mueller–Hinton broth, and Sabouraud dextrose broth were purchased from Torlak (Belgrade, Serbia), an antibiotic, doxycycline, from Galenika A.D. (Belgrade, Serbia), while an antimycotic, fluconazole was from Pfizer Inc. (USA).

Experimental procedure

Synthesis of hydantoin derivatives

5,5-Disubstituted hydantoin derivatives and spirohydantoins were synthesized from corresponding ketones and ketoesters using multicomponent Bucherer-Bergs reaction, while annulated bicyclic and tricyclic phenylseleno hydantoin derivatives were synthesized from corresponding 5-alkenyl hydantoins and spirohydantoins by selenium induced intramolecular cyclization as previously reported by ŠMIT and PAVLOVIĆ (2015) and ŠMIT *et al.* (2016). The structures and purity of synthesized compounds are confirmed by standard spectroscopic methods. All synthesized hydantoin derivatives are divided according to the structure into five groups. For further reference, the chemical names within the group, with the marks used in this paper, are given below.

Alkenyl hidantoin derivatives (Fig. 1)

5-But-3-enyl-5-ethyl-imidazolidine-2,4-dione (**Hyd1**). White solid (0.711 g, 78%); IR (KBr, ν_{max} / cm^{-1}): 3170, 3068, 2975, 1753, 1716, 1642, 1406, 805, 765; ^{1}H NMR (200 MHz, $CDCl_3$, δ / ppm) 0.93 (t, J = 7.6 Hz, 3H), 1.59-2.29 (m, 6H), 4.94-5.10 (m, 2H), 5.66-5.88 (m, 1H), 6.42 (bs, 1H), 9.02 (bs, 1H); ^{13}C (50 MHz, $CDCl_3$, δ / ppm) 7.6, 27.8, 30.0, 35.6, 67.5, 115.7, 136.8, 157.4, 177.0; Anal. calc. for $C_9H_{14}N_2O_2$ C: 59.32, H: 7.74, N: 15.37; found C: 59.38, H: 7.72, N: 15.41.

5-But-3-enyl-5-propyl-imidazolidine-2,4-dione (**Hyd2**). White crystals (0.598 g, 61%); IR (KBr, ν_{max} / cm^{-1}) 3216, 3052, 2961, 1764, 1712, 1644, 1435, 799, 778; ^{1}H NMR (200 MHz, $CDCl_3$, δ / ppm) 0.92 (t, J = 7.2 Hz, 3H), 1.10-2.51 (m, 8H), 4.92-5.10 (m, 2H), 5.64-

5.88 (m, 1H), 6.88 (bs, 1H), 9.47 (bs, 1H); ^{13}C (50 MHz, $CDCl_3$, δ / ppm) 13.9, 16.6, 27.7, 35.9, 39.1, 67.1, 115.6, 136.8, 157.8, 177.3; Anal. calc. for $C_{10}H_{16}N_2O_2$ C: 61.20, H: 8.22, N: 14.27; found C: 61.21, H: 8.28, N: 14.24.

5-But-3-enyl-5-methyl-imidazolidine-2,4-dione (**Hyd3**). White crystals (0.697 g, 83%); IR (KBr, ν_{max} / cm^{-1}) 3231, 3051, 1766, 1710, 1643, 1431, 795, 775; ^{1}H NMR (200 MHz, $CDCl_3$, δ / ppm) 1.46 (s, 3H), 1.67-2.30 (m, 4H), 4.95-5.11 (m, 2H), 5.67-5.88 (m, 1H), 6.43 (bs, 1H), 8.96 (bs, 1H); ^{13}C (50 MHz, $CDCl_3$, δ / ppm) 23.9, 28.0, 36.8, 63.5, 115.9, 136.7, 155.8, 176.9; Anal. calc. for $C_8H_{12}N_2O_2$ C: 57.13, H: 7.19, N: 16.66; found C: 57.14, H: 7.22, N: 16.71.

5-Ethyl-5-(3-methyl-but-3-enyl)-imidazolidine-2,4-dione (**Hyd4**). White tiny crystals (0.805 g, 82%); IR (KBr, ν_{max} / cm^{-1}) 3361, 3216, 3076, 2971, 1725, 1709, 1401, 783, 758; ^{1}H NMR (200 MHz, $CDCl_3$, δ / ppm) 0.94 (t, J = 7.4 Hz, 3H), 1.72 (s, 3H), 1.61-2.21 (m, 6H), 4.67-4.76 (m, 2H), 6.29 (bs, 1H), 8.81 (bs, 1H); ^{13}C (50 MHz, $CDCl_3$, δ / ppm) 7.6, 22.3, 30.0, 31.5, 34.6, 67.5, 110.9, 144.1, 157.4, 176.9; Anal. calc. for $C_{10}H_{16}N_2O_2$ C: 61.20, H: 8.22, N: 14.27; found C: 61.24, H: 8.21, N: 14.27.

5-(3-Methyl-but-3-enyl)-5-propyl-imidazolidine-2,4-dione (**Hyd5**). White tiny crystals (0.746 g, 71%); IR (KBr, ν_{max} / cm^{-1}) 3246, 2964, 1773, 1711, 1651, 1402, 796, 776; ^{1}H NMR (200 MHz, $CDCl_3$, δ / ppm) 0.93 (t, J = 7.2 Hz, 3H), 1.11-2.20 (m, 8H), 1.71 (s, 3H), 4.66-4.75 (m, 2H), 6.49 (bs, 1H), 9.11 (bs, 1H); ^{13}C (50 MHz, $CDCl_3$, δ / ppm) 13.9, 16.7, 22.3, 31.4, 34.9, 39.1, 67.1, 110.8, 144.1, 157.3, 177.0; Anal. calc. for $C_{11}H_{18}N_2O_2$ C: 62.83, H: 8.63, N: 13.32; found C: 62.85, H: 8.67, N: 13.34.

5-Methyl-5-(pent-3-enyl)-imidazolidine-2,4-dione (**Hyd6**). White tiny crystals (0.774 g, 85%); IR (KBr, ν_{max} / cm^{-1}) 3349, 3221, 3075, 2982, 1767, 1731, 1713, 1648, 1402, 791, 778; ^{1}H NMR (200 MHz, $CDCl_3$, δ / ppm) 1.47 (s, 3H), 1.72 (s, 3H), 1.76-2.18 (m, 4H), 4.66-4.76 (m, 2H), 6.65 (bs, 1H), 9.21 (bs, 1H); ^{13}C (50 MHz, $CDCl_3$, δ / ppm) 22.3, 23.7, 31.6, 35.6, 63.5, 110.9, 144.0, 157.2, 177.7; Anal. calc. for $C_9H_{14}N_2O_2$ C: 59.32, H: 7.74, N: 15.37; found C: 59.35, H: 7.77, N: 15.32.

5-Methyl-5-(4-methyl-pent-3-enyl)-imidazolidine-2,4-dione (**Hyd7**). White amorphous solid (1.590 g, 81%); IR (KBr, ν_{max} / cm^{-1}) 3336, 3214, 3071, 2977, 1768, 1726, 1709, 1402, 768, 757; ^{1}H NMR (200 MHz, $CDCl_3$, δ / ppm) 1.45 (s, 3H), 1.58 (d, J = 1.4 Hz, 3H), 1.66 (d, J = 1.4 Hz, 3H), 1.73-2.24 (m, 4H), 5.04 (sept, J = 7.0, 1.4 Hz, 1H), 6.50 (bs, 1H), 9.00 (bs, 1H); ^{13}C (50 MHz, $CDCl_3$, δ / ppm) 17.6, 22.5, 23.9, 25.6, 37.4, 63.6, 122.3, 133.1, 157.0, 177.7; Anal. calc. for $C_{10}H_{16}N_2O_2$ C: 61.20, H: 8.22, N: 14.27; found C: 61.25, H: 8.20, N: 14.23.

Phenyl hydantoin derivatives (Fig. 2)

5-Methyl-5-phenylimidazolidine-2,4-dione (**Hyd8**). White amorphous solid (2.045 g, 86%); IR (KBr, ν_{max} / cm^{-1}) 3282, 3208, 3064, 2889, 1769, 1726, 1498, 1027, 787, 695; ^{1}H NMR (200 MHz, $CDCl_3$, δ / ppm) 1.71 (s, 3H), 7.37-7.55 (m, 5H), 8.68 (s, 1H), 10.82 (bs, 1H); ^{13}C (50 MHz, $CDCl_3$, δ / ppm) 22.76, 62.34, 122.41, 124.12, 124.48, 140.48, 157.72, 177.88; Anal. calc. for $C_{10}H_{10}N_2O_2$ C: 63.15, H: 5.30, N: 14.73; found C: 63.19, H: 5.33, N: 14.70.

5-Ethyl-5-phenethylimidazolidine-2,4-dione (**Hyd9**). White solid (0.611 g, 82%); IR (KBr, ν_{max} / cm^{-1}) 3303, 3232, 3058, 2874, 1771, 1723, 1034, 756, 690; ^{1}H NMR (200 MHz, $CDCl_3$, δ / ppm) 0.78 (t, J = 7.2 Hz, 3H), 1.62 (q, J = 7.2 Hz, 2H), 1.74-1.88 (m, 2H), 2.35-2.60 (m, 2H), 7.10-7.32 (m, 5H), 7.95 (s, 1H); ^{13}C (50 MHz, $CDCl_3$, δ / ppm) 7.54, 29.29, 29.42, 38.16, 66.06, 126.01, 128.17, 128.48, 141.08, 157.08, 177.80; Anal. calc. for $C_{13}H_{16}N_2O_2$ C: 67.22, H: 6.94, N: 12.06; found C: 67.27, H: 6.98, N: 12.02.

5-Phenethyl-5-propylimidazolidine-2,4-dione (**Hyd10**). White amorphous solid (2.045 g, 86%); IR (KBr, ν_{max} / cm^{-1}) 3298, 3219, 2895, 1765, 1715, 1035, 764, 693; ^{1}H NMR (200 MHz, $CDCl_3$, δ / ppm) 0.93 (t, J = 7.2 Hz, 3H), 1.19-1.52 (m, 2H), 1.60-2.22 (m, 4H), 2.47-2.75 (m, 2H), 6.28 (bs, 1H), 7.14-7.27 (m, 5H), 8.85 (bs, 1H); ^{13}C (50 MHz, $CDCl_3$, δ / ppm) 13.90, 16.68, 29.79, 38.62, 39.17, 67.24, 126.33, 128.28, 128.59, 140.36, 157.12, 176.75; Anal. calc. for $C_{14}H_{18}N_2O_2$ C: 68.27, H: 7.37, N: 11.37; found C: 68.30, H: 7.38, N: 11.35.

Acetylated hydantoin derivatives (Fig. 3)

2-(4-Methyl-2,5-dioxo-imidazolidin-4-yl)-pent-4-enoic acid ethyl ester (**Hyd11**). White needle crystals (0.997 g, 83%); IR (KBr, ν_{max} / cm^{-1}) 3270, 3051, 2982, 1772, 1721, 1645, 1423, 1377, 1184; ^{1}H NMR (200 MHz, $CDCl_3$, δ / ppm) 1.28 (t, J = 7.0 Hz, 3H), 1.46 (s, 3H), 2.14-2.51 (m, 2H), 2.94 (dd, J = 4.8 and 10.2 Hz, 1H), 4.20 (q, J = 7.0 Hz, 2H), 4.97-5.14 (m, 2H), 5.57-5.80 (m, 1H), 6.77 (s, 1H), 9.48 (bs, 1H); ^{13}C (50 MHz, $CDCl_3$, δ / ppm) 14.2, 23.1, 32.3, 50.5, 61.2, 63.7, 117.9, 133.3, 156.7, 172.6, 176.0; Anal. calc. for $C_{11}H_{16}N_2O_4$ C: 54.99, H: 6.71, N: 11.66; found C: 55.03, H: 6.74, N: 11.62.

4-Methyl-2-(4-methyl-2,5-dioxo-imidazolidin-4-yl)-pent-4-enoic acid ethyl ester (**Hyd12**). White needle crystals (1.03 g, 83%); IR (KBr, ν_{max} / cm^{-1}) 3330, 3181, 3075, 2987, 1783, 1717, 1651, 1408, 1208; ^{1}H NMR (200 MHz, $CDCl_3$, δ / ppm) 1.26 (t, J = 7.2 Hz, 3H), 1.46 (s, 3H), 1.73 (s, 3H), 3.07 (dd, J = 10.4 and 13.4 Hz, 1H), 4.18 (q, J = 7.2 Hz, 2H), 4.70-4.78 (m, 2H), 6.52 (s, 1H), 8.99 (bs, 1H); ^{13}C (50 MHz, $CDCl_3$, δ / ppm) 13.8, 21.4, 22.6, 36.0, 49.2, 60.9, 63.6, 113.3, 140.8, 157.3, 172.3, 176.1; Anal. calc. for $C_{12}H_{18}N_2O_4$ C: 56.68, H: 7.13, N: 11.02; found C: 56.73, H: 7.14, N: 10.99.

Ethyl-2-(4-methyl-2,5-dioxoimidazolidin-4-yl)-3-phenylpropanoate (**Hyd13**). White amorphous solid (0.672 g, 51%); IR (KBr, ν_{max} / cm^{-1}) 3295, 3098, 2975, 1778, 1720, 1660, 1398, 1212; ^{1}H NMR (200 MHz, $CDCl_3$, δ / ppm) 0.95 (t, J = 7.0 Hz, 3H), 1.36 (s, 3H), 2.67-3.02 (m, 3H), 3.89 (q, J = 7.0 Hz, 2H), 7.07-7.29 (m, 5H), 8.12 (s, 1H), 10.81 (bs, 1H); ^{13}C (50 MHz, $CDCl_3$, δ / ppm) 14.0, 22.2, 33.17, 54.8, 60.3, 62.6, 126.7, 128.6, 128.9, 138.4, 156.6, 171.2, 177.0; Anal. calc. for $C_{15}H_{18}N_2O_4$ C: 62.06, H: 6.25, N: 9.65; found C: 62.09, H: 6.28, N: 9.61.

Ethyl-2-(2,5-dioxo-4-propylimidazolidin-4-yl)-acetate (**Hyd14**). White amorphous solid (1.179 g, 73%); IR (KBr, ν_{max} / cm^{-1}) 3333, 3175, 2979, 1785, 1725, 1648, 1413, 1986, 1205; ^{1}H NMR (200 MHz, $CDCl_3$, δ / ppm) 0.93 (t, J = 7.2 Hz, 3H), 1.26 (t, J = 7.4 Hz, 3H), 1.67-2.06 (m, 6H), 4.16 (q, J = 7.2 Hz, 2H), 6.43 (bs, 1H), 8.93 (bs, 1H); ^{13}C (50 MHz, $CDCl_3$, δ / ppm) 13.9, 16.7, 22.3, 31.4, 34.91, 39.1, 67.1, 157.2, 172.9, 177.0; Anal. calc. for $C_{10}H_{16}N_2O_4$ C: 52.62, H: 7.07, N: 12.27; found C: 52.66, H: 7.09, N: 12.25.

Spiro-hydantoins (Fig. 4)

6-Allyl-1,3-diazaspiro[4.4]nonane-2,4-dione (**Hyd15**). Crystalline solid **(**0.758 g, 78%); IR (KBr, ν_{max} / cm^{-1}) 3181, 3115, 3065, 1763, 1717, 1642, 1404; ^{1}H NMR (200 MHz, $CDCl_3$, δ / ppm) 1.24-1.48 (m, 1H), 1.70-2.48 (m, 8H), 4.94-5.11 (m, 2H), 5.60-5.81 (m, 1H), 6.82 (br s, 1H), 8.58 (br s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$, δ / ppm) 22.3, 30.9, 34.1, 38.1, 47.1, 72.0, 116.7, 135.7, 157.1, 177.7. Anal. Calcd for $C_{10}H_{14}N_2O_2$: C, 61.84; H, 7.27; N, 14.42. Found: C, 61.52; H, 7.33; N, 14.54.

6-Allyl-1,3-diazaspiro[4.5]decane-2,4-dione (**Hyd16**). Crystalline solid (0.927 g, 89%); IR (KBr, ν_{max} / cm^{-1}) 3203, 2929, 2859, 1769, 1712, 1645, 1401; ^{1}H NMR (200 MHz, $CDCl_3$, δ / ppm) 0.83-1.18 (m, 1H), 1.24-1.47 (m, 2H), 1.69-2.10 (m, 8H), 4.97-5.05 (m, 2H), 5.60-5.80 (m, 1H), 6.91 (br s, 1H), 8.48 (br s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$, δ / ppm) 21.7,

25.0, 27.4, 35.1, 35.6, 40.9, 67.2, 117.1, 135.4, 157.1, 177.3; Anal. Calcd for $C_{11}H_{16}N_2O_2$: C, 63.44; H, 7.74; N, 13.45. Found: C, 63.57; H, 7.79; N, 13.51.

6-(2-Methylallyl)-1,3-diazaspiro[4.5]decane-2,4-dione (**Hyd17**). Crystalline solid; (0.856 g, 77%); IR (KBr, ν_{max} / cm^{-1}) 3289, 2926, 1767, 1714, 1406, 1256, 740, 679; ^1H NMR (200 MHz, $CDCl_3$, δ / ppm) 0.75-1.05 (m, 1H), 1.17-1.49 (m, 2H), 1.67 (s, 3H), 1.72-2.1 (m, 8H), 4.67-4.75 (m, 2H), 6.85 (br s, 1H), 8.47 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$, δ / ppm) 21.8, 21.9, 25.0, 27.3, 35.2, 38.8, 39.3, 67.3, 113.1, 142.2, 157.1, 177.2; Anal. Calcd for $C_{12}H_{18}N_2O_2$: C, 59.98; H, 8.39; N, 11.66. Found: C, 60.15; H, 8.42; N, 11.77.

Bicyclic and tricyclic hydantoin derivatives (Fig. 5)

5-Phenylselanylmethyl-7a-propyl-tetrahydro-pyrrolo[1,2-c]imidazole-1,3-dione (**Hyd18**). White tiny crystals (0.126 g, 72%); IR (KBr, ν_{max} / cm^{-1}) 3058, 2960, 2930, 2873, 1725, 1579, 1477, 1274, 737, 690; ^1H NMR (200 MHz, $CDCl_3$, δ / ppm) 0.90 (t, J = 7.2 Hz, 3H), 1.09-1.67 (m, 3H), 1.69-1.99 (m, 3H), 2.02-2.41 (m, 2H), 3.28 (dd, J = 12.5 and 8.0 Hz, 1H), 3.80 (dd, J = 12.5 and 4.0 Hz, 1H), 3.81-3.95 (m, 1H), 7.23-7.32 (m, 3H), 7.48-7.58 (m, 2H), 8.66 (bs, 1H); ^{13}C (50 MHz, $CDCl_3$, δ / ppm) 13.8, 16.9, 28.9, 31.1, 33.1, 38.4, 58.7, 74.9, 127.2, 129.2, 130.0, 132.8, 156.8, 176.5; Anal. calc. for $C_{16}H_{20}N_2O_2Se$ C: 54.70, H: 5.74, N: 7.97; found C: 54.71, H: 5.74, N: 7.93.

5-Methyl-5-phenylselanylmethyl-7a-propyl-tetrahydro-pyrrolo[1,2-c]imidazole-1,3-dione (**Hyd19**). White crystals (0.133 g, 73%); IR (KBr, ν_{max} / cm^{-1}) 3192, 3057, 2962, 1766, 1699, 1579, 1376, 735, 691; ^1H NMR (200 MHz, $CDCl_3$, δ / ppm) 0.93 (t, J = 7.2 Hz, 3H), 1.20-1.47 (m, 2H), 1.51 (s, 3H), 1.59-1.96 (m, 4H), 2.08-2.29 (m, 2H), 3.43 (d, J = 12.6 Hz, 2H), 3.82, (d, J = 12.6 Hz, 2H), 7.19-7.29 (m, 3H), 7.48-7.70 (m, 2H), 8.52 (bs, 1H); ^{13}C (50 MHz, $CDCl_3$, δ / ppm) 13.9, 16.5, 30.4, 31.7, 36.7, 39.1, 40.1, 65.1, 75.3, 127.3, 129.2, 130.6, 132.8, 156.2, 176.3; Anal. calc. for $C_{17}H_{22}N_2O_2Se$ C: 55.89, H: 6.07, N: 7.67; found C: 55.83, H: 6.01 N: 7.68.

5,7a-Dimethyl-1,3-dioxo-5-phenylselanylmethyl-hexahydro-pyrrolo[1,2-c]imidazole-7-carboxylic acid ethyl ester (**Hyd20**). Light yellow powder (0.051 g, 25%); IR (ATR, ν_{max} / cm^{-1}) 3227, 3059, 2978, 1771, 1705, 1578, 1379, 1295, 1139, 735, 692; ^1H NMR (200 MHz, $CDCl_3$, δ / ppm) 1.30 (t, J = 7.0 Hz, 3H), 1.48 (s, 3H), 1.56 (s, 3H), 2.29 (dd, J = 13.6, 7.8 Hz, 1H), 2.45 (dd, J = 14.0, 12.4 Hz, 1H), 3.50 (dd, J = 12.4, 7.8 Hz, 1H), 3.34 (d, J = 13.0 Hz, 2H), 3.90 (d, J = 13.0 Hz, 2H), 4.06-4.33 (m, 2H), 7.20-7.32 (m, 3H), 7.48-7.59 (m, 2H), 9.02 (bs, 1H); ^{13}C (50 MHz, $CDCl_3$, δ / ppm) 14.0, 20.8, 30.6, 36.8, 42.8, 46.2, 61.2, 63.4, 71.2, 127.4, 129.2, 130.2, 132.9, 155.3, 169.2, 174.5; Anal. calc. for $C_{18}H_{22}N_2O_4Se$ C: 52.82, H: 5.42, N: 6.84; found C: 52.76, H: 5.49 N: 6.87.

7a-Methyl-5-(1-methyl-1-phenylselanyl-ethyl)-tetrahydro-pyrrolo[1,2-c]imidazole-1,3-dione (**Hyd21**). White tiny crystals (0.098 mg, 56%); IR (KBr, ν_{max} / cm^{-1}) 3206, 2980, 1772, 1713, 1638, 1399, 1385, 741, 695; ^1H NMR (200 MHz, $CDCl_3$, δ / ppm) 1.41 (s, 3H), 1.44 (s, 3H), 1.52 (s, 3H), 1.68-2.00 (m, 2H), 2.20-2.55 (m, 2H), 3.91 (dd, J = 10.0, 8.0 Hz, 1H), 7.26-7.38 (m, 3H), 7.62-7.70 (m, 2H), 8.93 (bs, 1H); ^{13}C (50 MHz, $CDCl_3$, δ / ppm) 22.2, 25.7, 30.0, 30.3, 33.7, 49.1, 68.1, 71.7, 126.8, 128.8, 134.8, 138.2, 160.7, 177.1; Anal. calc. for $C_{16}H_{20}N_2O_2Se$ C: 54.70, H: 5.74, N: 7.97; found C: 54.72, H: 5.79 N: 7.90.

5-(Phenylselanylmethyl)-hexahydro-1H-cyclopenta[2,3]pyrrolo[1,2-c]imidazole-1,3(2H)-dione (**Hyd22**). Crystalline solid (0.221 g 63%); IR (ATR, ν_{max} / cm^{-1}) 3161, 3049, 2956, 1768, 1707, 1406, 761, 735; ^1H NMR (500 MHz, $CDCl_3$, δ / ppm) 1.46-1.52 (m, 1H), 1.73-1.86 (m, 4H), 1.97 (ddd, J = 2, 5.5 and 13.5 Hz, 1H), 1.99-2.06 (m, 1H), 2.18-2.25 (m, 1H), 2.79-2.85 (m, 1H), 3.22 (dd, J = 8 and 12.5 Hz, 1H), 3.68-3.76 (m, 1H), 3.78 (dd, 1H, J = 6.5 and 12.5 Hz), 7.25-7.30 (m, 3H), 7.54-7.58 (m, 2H), 7.62 (br s, 1H); ^{13}C NMR (125

MHz, $CDCl_3$, δ / ppm) 25.8, 28.5, 34.0, 37.7, 41.6, 45.3, 61.1, 80.3 127.4, 129.2, 130.2, 133.2, 156.8, 177.4; Anal. Calcd for $C_{16}H_{18}N_2O_2Se$: C, 55.02; H, 5.19; N, 8.02. Found: C, 55.08; H, 5.16; N, 7.94.

Antimicrobial activity

Tested microorganisms

In vitro antimicrobial activity of hydantoin derivatives (**Hyd1-22**) was tested against 15 strains of bacteria including 3 probiotics (*Lactobacillus plantarum, Bifidobacterium animalis* subsp. *lactis, Bacillus subtilis* IP 5832) and 12 human pathogenic bacteria - 7 clinical isolates and 5 standard strains (*Bacillus subtilis, Bacillus pumilus* NCTC 8241, *Staphylococcus aureus, Staphylococcus aureus* ATCC 25923, *Escherichia coli, Escherichia coli* ATCC 25922, *Proteus mirabilis, Proteus mirabilis* ATCC 12453, *Pseudomonas aeruginosa, Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica, Salmonella typhymirium*) and 4 strains of yeast (1 probiotic - *Saccharomyces boulardii*, 2 isolates - *Rhodotorula mucilaginosa*, *Candida albicans* and one standard strain *Candida albicans* ATCC 10231). Clinical isolates of the bacteria were a generous gift from the Institute of Public Health, Kragujevac, Serbia. The ATCC strains were provided from a collection held by the Microbiology Laboratory, Faculty of Science, University of Kragujevac.

Suspension preparation

The bacterial suspensions were prepared by the direct colony method. The turbidity of the initial suspension was adjusted using a densitometer (DEN-1, BioSan, Latvia). When adjusted to the turbidity of the 0.5 McFarland standard, the bacteria suspension contains about 10^8 colony forming units (CFU)/mL (ANDREWS, 2005). The initial suspensions were additionally diluted in a 1:100 ratio with sterile 0.85% saline.

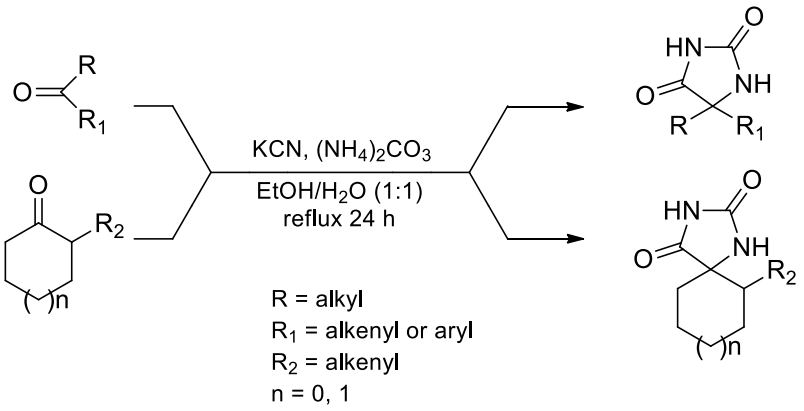
Microdilution method

Antibacterial activity was tested by determining the minimal inhibitory concentration (MIC) and the minimal microbicidal concentration (MMC) using microdilution method with resazurin (SARKER et al., 2007). The tested compounds were first dissolved in dimethyl sulfoxide (DMSO) (10% of total volume) and then into a nutrient liquid medium (up to 100% of total volume). The stock concentrations of tested compounds were 2000 µg/mL. Next, serial twofold dilutions were made in a concentration range from 1000 µg/mL to 7.81 µg/mL in sterile 96-well microtiter plates containing 100 µl of Mueller–Hinton broth. After that, 10 µl of diluted bacterial suspensions were added to appropriate wells. Finally, 10 µl of resazurin solution, as an indicator of microbial growth, was added to each well. Resazurin is a blue non-fluorescent dye that becomes pink and fluorescent when reduced to resorufin by oxidoreductases within viable cells. The inoculated plates were incubated at 37°C for 24 h. MIC was defined as the lowest concentration of the tested substance that prevented resazurin color change from blue to pink. Minimal microbicidal concentration was determined by plating 10 µl of samples from wells, where no indicator color change was recorded, on a nutrient agar medium. At the end of the incubation period, the lowest concentration with no growth (no colony) was defined as minimal microbicidal concentration (MMC).

Tetracycline and fluconazole, dissolved in a nutrient liquid medium, were used as a positive control. A solvent control test was performed to study the effect of 10% DMSO on the growth of bacteria. Each test included growth control and sterility control. All tests were performed in duplicate and MICs were constant.

RESULTS AND DISCUSSION

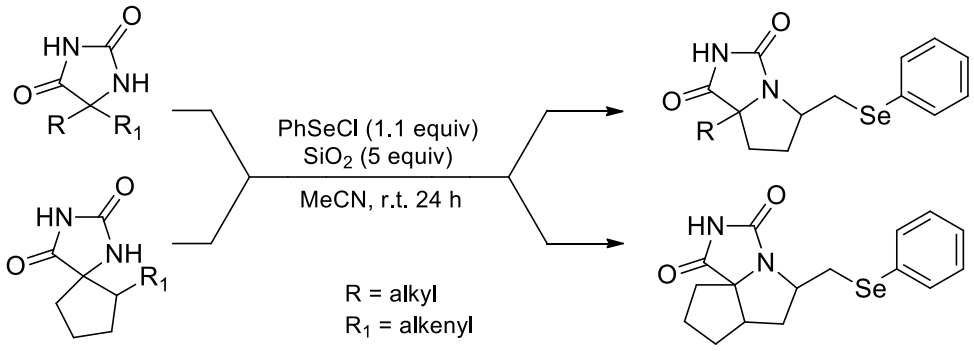
A series of 22 hydantoin derivatives are synthesized for study of their antimicrobial activity. 5,5-Disubstituted hydantoin derivatives and spirohydantoins were synthesized from corresponding ketones and β-ketoesters using multicomponent Bucherer-Bergs reaction (Scheme 1) as previously reported by ŠMIT and PAVLOVIĆ (2015) and ŠMIT *et al.* (2016).



Scheme 1. Synthesis of 5,5-disubstituted hydantoin derivatives and spirohydantoins.

Annulated bicyclic and tricyclic phenylseleno hydantoin derivatives were synthesized from corresponding 5-alkenyl hydantoins and 2-alkenyl spirohydantoins by selenium induced intramolecular cyclization (Scheme 2), as ŠMIT and PAVLOVIĆ (2015) and ŠMIT *et al.* (2016) demonstrated previously.

The results of *in vitro* antibacterial and antifungal activities expressed as MIC and MMC values are presented in Tables 1-5. For comparison, MIC and MMC values of positive controls are listed in Table 6. The solvent (10% DMSO) did not inhibit the growth of the tested microorganisms.



Scheme 2. Synthesis of bicyclic and tricyclic phenylseleno hydantoin derivatives.

The compounds showed different degrees of antimicrobial activity. The intensity of antimicrobial action varied depending on the group of microorganisms and type of the compounds. Generally, most of the tested compounds have demonstrated a moderate antimicrobial activity. In general, MIC and MMC values are in the range from <7.81 to >1000 μg/mL. Tested compounds have shown higher antimicrobial activity mainly on Gram-positive (G+) bacteria, while same compounds have shown minimal antimicrobial activity on yeasts.

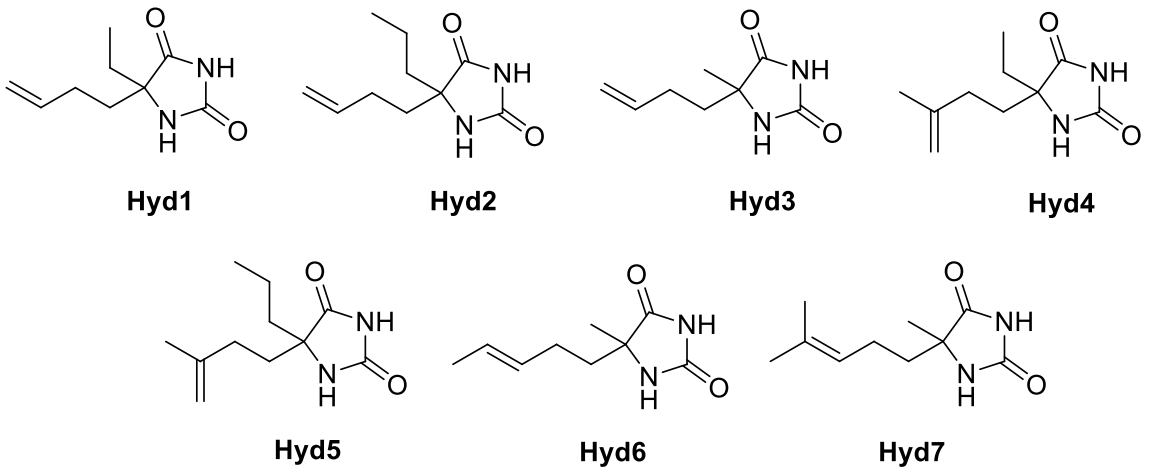


Figure 1. 5-Alkenyl hydantoin derivatives.

Table 1 shows the results of antimicrobial activity of different alkenyl hydantoin derivatives (Fig. 1) with MICs in the range from 62.5 to >1000 μg/mL. The highest sensitivity showed the species *P. aeruginosa* ATCC 27853 (MIC 62.5 μg/mL for **Hyd6**) in the group of Gram-negative bacteria, and probiotic *B. animalis* subsp. *lactis* (MIC 62.5 μg/mL for **Hyd2** and **Hyd6**) and clinical isolate of *S. aureus* (**Hyd4** MIC 62.5 μg/mL) in Gram-positive bacteria. **Hyd7** had moderate antibacterial effects towards *B. animalis* subsp. *lactis* (MIC 125 μg/mL) and *S. aureus* ATCC 25923 (MIC 250 μg/mL). *B. animalis* subsp. *lactis* was also partially sensitive to **Hyd3** (MIC 250 μg/mL), which makes this bacterial species most suitable for the action of the synthesized alkenyl hydantoin derivatives. All other microbial species used were less sensitive to tested alkenyl hydantoins (MIC 500-1000 μg/mL). The antifungal activity was examined using *C. albicans* strains and all alkenyl hydantoins showed negligible effects with MICs 1000 μg/mL and over.

Hydantoins containing a phenyl group (Fig. 2) have demonstrated significantly lower antimicrobial activity, mostly around 1000 μg/mL and more, as shown in Table 2. As was the case with the previous group of hydantoin derivatives, *B. animalis* subsp. *lactis* was the most sensitive to the action of phenyl hydantoin derivatives, particularly **Hyd8** and in a lower degree **Hyd10** (MIC 250 and 500 μg/mL, respectively). Regarding the antifungal activity of phenyl hydantoin derivatives, the standard culture of *C. albicans* showed resistance to the highest applied concentration, while the growth of the isolated strain was affected by all three hydantoin derivatives at the highest concentration (MIC 1000 μg/mL).

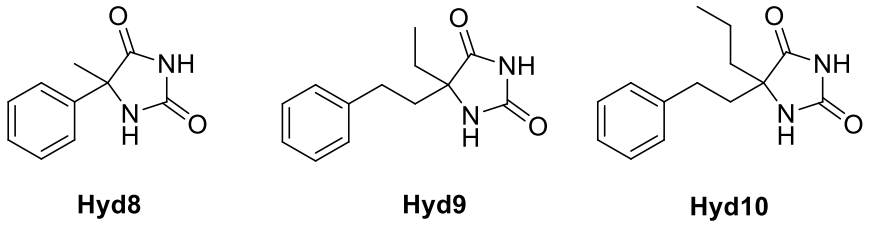


Figure 2. Phenyl hydantoin derivatives.

Table 1. Antimicrobial activity of 5-alkenyl hydantoin derivatives.

Species/Compounds	Hyd1		Hyd2		Hyd3		Hyd4		Hyd5		Hyd6		Hyd7	
	[1]MIC	[2]MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC
L. plantarum	>1000	>1000	500	500	500	1000	1000	>1000	1000	>1000	1000	>1000	1000	1000
B. animalis subsp. *lactis*	1000	>1000	**62.5**	500	250	250	125	1000	1000	1000	**62.5**	250	125	500
B. subtilis IP 5832	>1000	>1000	500	1000	1000	1000	>1000	>1000	>1000	>1000	1000	>1000	1000	1000
B.subtilis	>1000	>1000	>1000	>1000	1000	1000	>1000	>1000	>1000	>1000	>1000	>1000	1000	1000
B. pumilus NCTC 8241	>1000	>1000	>1000	>1000	1000	1000	500	>1000	1000	>1000	500	1000	500	1000
S. aureus	500	>1000	>1000	>1000	1000	1000	**62.5**	250	>1000	>1000	1000	>1000	500	>1000
S. aureus ATCC 25923	1000	>1000	>1000	>1000	500	>1000	500	1000	500	>1000	500	>1000	250	1000
E. coli	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000
E. coli ATCC 25922	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	1000	>1000	1000	>1000
P. mirabilis	>1000	>1000	1000	>1000	1000	>1000	1000	>1000	1000	>1000	1000	1000	1000	>1000
P. mirabilis ATCC 12453	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	1000	1000	>1000	>1000
P. aeruginosa	1000	>1000	1000	>1000	1000	>1000	250	>1000	1000	>1000	500	>1000	500	>1000
P. aeruginosa ATCC 27853	1000	>1000	1000	>1000	1000	>1000	500	>1000	1000	>1000	**62.5**	>1000	1000	>1000
S. enterica	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000
S. typhymirium	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000
R. mucilaginosa	500	1000	1000	1000	1000	1000	500	1000	1000	1000	1000	>1000	500	1000
S. boulardii	>1000	>1000	1000	>1000	1000	>1000	>1000	>1000	1000	1000	1000	>1000	1000	>1000
C. albicans ATCC 10231	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000
C. albicans	1000	>1000	1000	>1000	>1000	>1000	1000	>1000	>1000	>1000	1000	>1000	1000	>1000

[1]MIC values (µg/mL) – minimal inhibitory activity; [2]MMC values (µg/mL) – minimal microbicidal activity.

Table 2. Antimicrobial activity of phenyl hydantoin derivatives.

Species/Compounds	Hyd8		Hyd9		Hyd10	
	[1]MIC	[2]MMC	MIC	MMC	MIC	MMC
L. plantarum	>1000	>1000	1000	>1000	1000	>1000
B. animalis subsp. *lactis*	**250**	1000	1000	1000	500	1000
B. subtilis IP 5832	>1000	>1000	1000	>1000	1000	>1000
B.subtilis	>1000	>1000	>1000	>1000	>1000	>1000
B. pumilus NCTC 8241	>1000	>1000	>1000	>1000	500	>1000
S. aureus	1000	>1000	>1000	>1000	1000	>1000
S. aureus ATCC 25923	1000	>1000	>1000	>1000	500	>1000
E. coli	>1000	>1000	>1000	>1000	>1000	>1000
E. coli ATCC 25922	>1000	>1000	>1000	>1000	>1000	>1000
P. mirabilis	>1000	>1000	1000	>1000	>1000	>1000
P. mirabilis ATCC 12453	>1000	>1000	>1000	>1000	>1000	>1000
P. aeruginosa	1000	>1000	500	>1000	500	>1000
P. aeruginosa ATCC 27853	1000	>1000	1000	>1000	1000	>1000
S. enterica	>1000	>1000	>1000	>1000	>1000	>1000
S. typhymirium	>1000	>1000	>1000	>1000	>1000	>1000
R. mucilaginosa	1000	1000	1000	1000	1000	1000
S. boulardii	>1000	>1000	>1000	>1000	>1000	>1000
C. albicans ATCC 10231	>1000	>1000	>1000	>1000	>1000	>1000
C. albicans	1000	1000	1000	>1000	1000	>1000

[1]MIC values (μg/mL) – minimal inhibitory activity; [2]MMC values (μg/mL) – minimal microbicidal activity.

Acetylated hydantoin derivatives (Fig. 3) have weak activity against Gram-negative bacteria (MIC were mainly 1000 and >1000 μg/mL, Table 3). An exception was bacteria *P. aeruginosa*, which growth was affected by compounds **Hyd12** and **Hyd14** with MIC 500 μg/mL. **Hyd12** and **Hyd14** also showed a stronger influence on probiotic bacteria *B. animalis* subsp. *lactis* (MIC 250 μg/mL) in relation to other tested compounds and microorganisms.

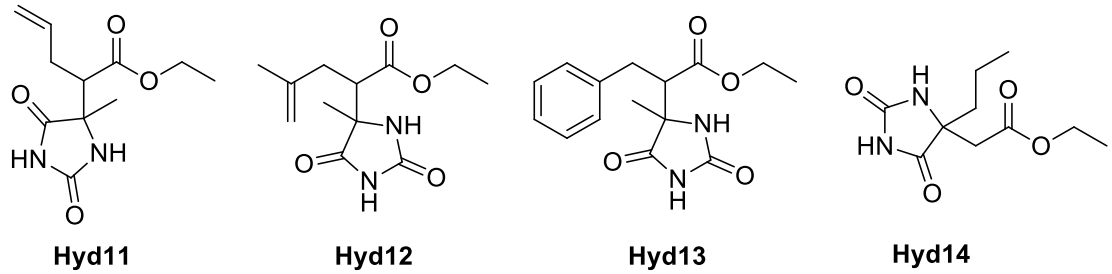


Figure 3. Acetylated hydantoin derivatives.

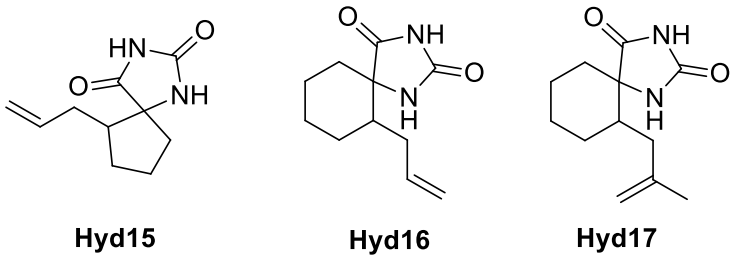
Hyd13 was able to interrupt the growth and development of *B. animalis* subsp. *lactis* and *R. mucilaginosa* at a concentration of 500 μg/mL. The acetylated hydantoin derivatives showed low antifungal potential against *C. albicans* isolate, with MIC 1000 μg/mL.

Table 3. Antimicrobial activity of acetylated hydantoin derivatives.

Species/Compounds	Hyd11		Hyd12		Hyd13		Hyd14	
	[1]MIC	[2]MMC	MIC	MMC	MIC	MMC	MIC	MMC
L. plantarum	>1000	>1000	>1000	>1000	1000	>1000	1000	>1000
B. animalis subsp. *lactis*	>1000	>1000	**250**	1000	500	1000	**250**	1000
B. subtilis IP 5832	>1000	>1000	>1000	>1000	>1000	>1000	1000	1000
B.subtilis	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
B. pumilus NCTC 8241	>1000	>1000	1000	>1000	>1000	>1000	1000	>1000
S. aureus	>1000	>1000	500	500	1000	>1000	500	>1000
S. aureus ATCC 25923	>1000	>1000	1000	>1000	1000	>1000	1000	>1000
E. coli	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
E. coli ATCC 25922	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
P. mirabilis	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000
P. mirabilis ATCC 12453	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
P. aeruginosa	1000	>1000	1000	>1000	1000	>1000	500	>1000
P. aeruginosa ATCC 27853	1000	>1000	1000	>1000	1000	>1000	1000	>1000
S. enterica	1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
S. typhymirium	1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
R. mucilaginosa	1000	>1000	1000	1000	500	1000	1000	1000
S. boulardii	>1000	>1000	1000	>1000	>1000	>1000	>1000	>1000
C. albicans ATCC 10231	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
C. albicans	1000	1000	1000	1000	1000	>1000	1000	>1000

[1]MIC values (μg/mL) – minimal inhibitory activity; [2]MMC values (μg/mL) – minimal microbicidal activity.

Spiro-hydantoins, which structures are presented in Fig. 4, have shown a stronger antibacterial effect compared to previous groups of hydantoin derivatives, especially compounds **Hyd15** and **Hyd17** against probiotic *L. plantarum* (MIC 15.75 μg/mL, Table 4). All three spiro-hydantoins **Hyd15-17** were able to inhibit the growth of *P. aeruginosa* isolate at a concentration of 500 μg/mL, while only **Hyd15** was efficient at the same concentration against *P. aeruginosa* ATCC culture. These compounds also showed low antifungal potential only against isolated *C. albicans* (MIC 1000 μg/mL).



Hyd15 **Hyd16** **Hyd17**

Figure 4. Spiro-hydantoins.

The annulated bicyclic and tricyclic phenylseleno hydantoin derivatives shown in Fig. 5 were the most efficient in the inhibition of *S. aureus* isolate and *B. animalis subsp. lactis* growth (Table 5). *S. aureus* isolate was the most sensitive to bicyclic hydantoin derivate **Hyd20,** with MIC <7.81 μg/mL and MMC 31.25 μg/mL, followed by **Hyd21** and **Hyd19** (MIC 15.75 and 62.5 μg/mL, respectively).

Table 4. Antimicrobial activity of spiro-hydantoins.

Species/Compounds	Hyd15		Hyd16		Hyd17	
	[1]MIC	[2]MMC	MIC	MMC	MIC	MMC
L. plantarum	**15.75**	**31.25**	1000	>1000	**15.75**	**31.25**
B. animalis subsp. *lactis*	-	-	500	1000	125	1000
B. subtilis IP 5832	-	-	1000	>1000	>1000	>1000
B.subtilis	-	-	>1000	>1000	>1000	>1000
B. pumilus NCTC 8241	-	-	1000	>1000	>1000	>1000
S. aureus	-	-	1000	>1000	1000	>1000
S. aureus ATCC 25923	-	-	1000	>1000	1000	>1000
E. coli	>1000	>1000	>1000	>1000	>1000	>1000
E. coli ATCC 25922	>1000	>1000	>1000	>1000	>1000	>1000
P. mirabilis	1000	>1000	1000	>1000	>1000	>1000
P. mirabilis ATCC 12453	>1000	>1000	>1000	>1000	>1000	>1000
P. aeruginosa	500	>1000	500	>1000	500	>1000
P. aeruginosa ATCC 27853	500	>1000	1000	>1000	1000	>1000
S. enterica	>1000	>1000	>1000	>1000	>1000	>1000
S. typhymirium	>1000	>1000	>1000	>1000	>1000	>1000
R. mucilaginosa	1000	1000	1000	1000	1000	1000
S. boulardii	>1000	>1000	>1000	>1000	>1000	>1000
C. albicans ATCC 10231	>1000	>1000	>1000	>1000	>1000	>1000
C. albicans	1000	>1000	1000	>1000	1000	>1000

[1]MIC values (μg/mL) – minimal inhibitory activity; [2]MMC values (μg/mL) – minimal microbicidal activity

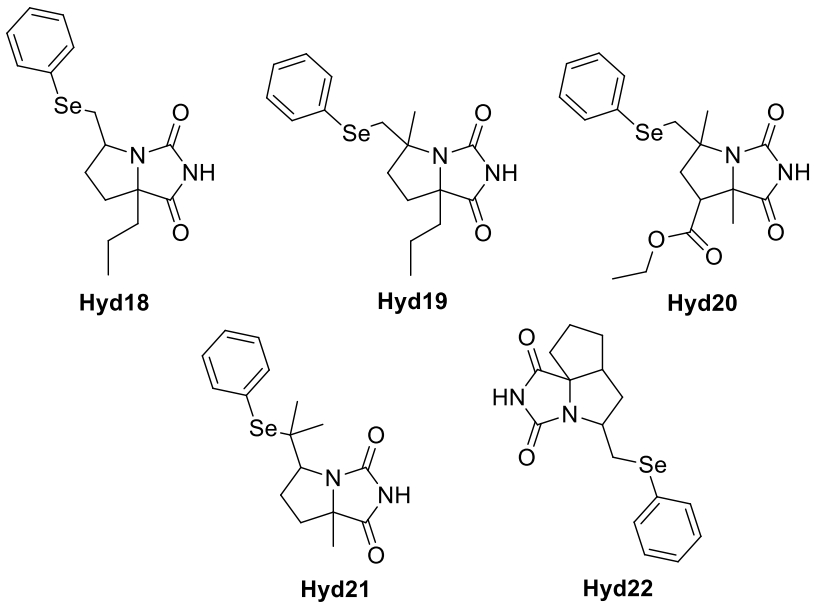


Figure 5. Bicyclic and tricyclic phenylseleno hydantoin derivatives.

Hyd21 also showed high antibacterial potential against probiotic *B. animalis subsp. lactis* (MIC 15.75 μg/mL) followed by moderate activity of **Hyd20** with MIC value 62.5 μg/mL. The tricyclic hydantoin derivate **Hyd22** also showed moderate antibacterial activity

against *B. animalis subsp. lactis* (MIC 62.5 μg/mL), while other microorganisms are significantly less sensitive to treatment with all synthesized bicyclic and tricyclic compounds.

TRIŠOVIĆ *et al.* (2011) tested the antibacterial activity of a series of hydantoins on isolates of *P. mirabilis, P. aeruginosa, Enterococcus faecalis, E. coli,* and standard culture *E. coli* ATCC 25922. The results showed significant antibacterial activity of certain compounds against a standard strain and clinical isolate of *E. coli*. The 3-*iso*-propyl and 3-benzyl hydantoin derivatives showed the weakest activity. CHEN and SUN (2006) have tested compounds from the hydantoin group, 1-chloro-3-alkyl-5,5-dimethyl hydantoins (CADMH), on *E. coli* and *S. aureus* and the result was the complete elimination of these bacteria within 30 min., which contributed to CADMH being used as an antimicrobial additive for polymeric materials.

In the presented work, the synthesized hydantoin derivatives showed low activity against *E. coli* and *E. coli* ATCC 25922 strains. In fact, the MIC value was 1000 μg/mL for *E. coli* only for 5-alkenyl hydantoin derivative **Hyd6**, while all remaining compounds had a MIC above 1000 μg/mL. In accordance with the results of the previous two studies (TRIŠOVIĆ *et al.*, 2011 and CHEN and SUN, 2006) it can be concluded that the antibacterial activity of hydantoin derivatives against *E. coli* and *E. coli* ATCC 25922 depends on their structure and substitution on position C5 and nitrogen atoms.

In our study, only bicyclic phenylseleno hydantoins **Hyd20**, **Hyd21** and **Hyd19**, showed strong antibacterial activity on *S. aureus* ATCC 25923 with MICs <7.81, 15.75, and 62.5 μg/mL, respectively. FUJISAKI *et al.* (2010) also reported the significant antibacterial activity of certain hydantoin derivatives against *S. aureus*. Taking into account the effect of bicyclic phenylseleno hydantoins on *S. aureus* in our study, it can be pointed out that the additional substitution of the imidazole ring with methyl as well as ester group leads to a stronger antibacterial effect of these compounds. The bioactivity of bicyclic and tricyclic hydantoin derivatives may also be attributed to the existence of selenium in their structure. Moreover, several studies demonstrated notable antimicrobial properties of organoselenium compounds against *S. aureus* (JAMAL ABDUL NASSER *et al.*, 2010; SANCINETO *et al.*, 2016). All other synthesized compounds showed low potential to inhibit the growth of *S. aureus* ATCC 25923 and isolated strain (MIC >1000-250 μg/mL), except **Hyd4** activity towards isolated strain with MIC 62.5 μg/mL.

Studies of antimicrobial activity of spirohydantoins (4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) have shown no antimicrobial activity on clinical isolates of *E. coli* and *S. aureus* (MARINOVA *et al.*, 2013). In the mentioned study, the disc-diffusion method on agar medium was used to test the antibacterial activity, and the substance was added in an amount of 360 ppm. Spirohydantoins in our work had a higher MIC than the maximal applied concentration (>1000 μg/mL) for these bacteria, so the results for this group of compounds coincide with the results of MARINOVA and coworkers.

Table 5. Antimicrobial activity of bicyclic and tricyclic phenylseleno hydantoin derivatives.

Species/Compounds	**Hyd18**		**Hyd19**		**Hyd20**		**Hyd21**		**Hyd22**	
	[1]MIC	[2]MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC
L. plantarum	1000	1000	1000	>1000	500	1000	**62.5**	250	1000	>1000
B. animalis subsp. *lactis*	1000	1000	250	1000	**62.5**	250	**15.75**	**62.5**	**62.5**	1000
B. subtilis IP 5832	>1000	>1000	>1000	>1000	500	1000	250	1000	>1000	>1000
B.subtilis	>1000	>1000	>1000	>1000	1000	1000	>1000	>1000	>1000	>1000
B. pumilus NCTC 8241	1000	>1000	>1000	>1000	250	500	500	>1000	>1000	>1000
S. aureus	500	>1000	>1000	>1000	500	1000	125	1000	500	>1000
S. aureus ATCC 25923	1000	>1000	**62.5**	>1000	**<7.81**	**31.25**	**15.75**	**62.5**	1000	>1000
E. coli	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
E. coli ATCC 25922	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
P. mirabilis	>1000	>1000	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000
P. mirabilis ATCC 12453	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
P. aeruginosa	1000	>1000	500	>1000	500	>1000	500	>1000	1000	>1000
P. aeruginosa ATCC 27853	1000	>1000	1000	>1000	1000	>1000	1000	>1000	>1000	>1000
S. enterica	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
S. typhymirium	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
R. mucilaginosa	1000	1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
S. boulardii	1000	1000	>1000	>1000	>1000	>1000	1000	>1000	1000	>1000
C. albicans ATCC 10231	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
C. albicans	>1000	>1000	>1000	>1000	1000	>1000	1000	>1000	>1000	>1000

[1]MIC values (µg/mL) – minimal inhibitory activity; [2]MMC values (µg/mL) – minimal microbicidal activity.

Table 6. Antimicrobial activity of positive control (tetracycline and fluconazole).

Species/Compounds	Tetracycline/Fluconazole	
	[1]MIC	[2]MMC
L. plantarum	0.45	7.81
B. animalis subsp. *lactis*	31.25	62.50
B. subtilis IP 5832	1.95	15.63
B.subtilis	0.11	1.95
B. pumilus NCTC 8241	0.11	7.81
S. aureus	0.22	3.75
S. aureus ATCC 25923	0.45	7.81
E. coli	7.81	15.63
E. coli ATCC 25922	15.63	31.25
P. mirabilis	250	>250
P. mirabilis ATCC 12453	15.63	62.50
P. aeruginosa	250	>250
P. aeruginosa ATCC 27853	62.50	125
S. enterica	15.63	31.25
S. typhymirium	15.63	125
R. mucilaginosa	62.50	1000
S. boulardii	31.25	1000
C. albicans ATCC 10231	31.25	1000
C. albicans	62.50	1000

[1]MIC values (μg/mL) – minimal inhibitory activity; [2]MMC values (μg/mL) – minimal microbicidal activity.

DYMEK *et al.* (2012) demonstrated that a specific hydantoin compound (PI8a) may have the potential to treat methicillin-resistant infections caused by *S. aureus*. Given the shown sensitivity of this bacterial species to certain groups of hydantoins, it can be concluded that they can be successful in the fight against it. MACHADO *et al.* (2011) found that the hydantoin derivative affects the change in the activity of *S. enterica*, while in our experimental study hydantoins showed a very weak effect on *S. enterica* (MIC 1000 μg/mL for alkenyl hydantoins **Hyd3** and **Hyd6**, as well as acetylated hydantoin derivative **Hyd11**, while for all other compounds the MIC was higher than 1000 μg/mL. In a study using two strains of *Enterobacter aerogenes*, hydantoins showed low to medium antibacterial potential (HANDZLIK *et al*., 2011).

DMDM hydantoins as well as the preservatives phenoxyethanol and methylparaben have been shown as very effective against *Burkholderia cepacia* when evaluated on growing medium at the maximum concentration prescribed for non-rinsing body care products in EU countries. *B. cepacia* can contaminate and survive in a variety of industrial products. Pollution can lead to economic loss for producers and potentially pose a risk to the health of vulnerable consumers (THOMAS, 2011).

Some studies have shown that hydantoin-based compounds exert equally good bactericidal and fungicidal activity (DUPAIPANDI, S. and BALAKRISHNAN, V., 2013). The most important fungicidal hydantoin is iprodione, which simultaneously stops the germination of spores and the growth of fungal mycelium. Fungicidal hydantoins are environmentally safe due to their decomposition into biologically inactive components in the soil (BURGAUD *et al.*, 1975). Thiohydantoins have shown high antifungal activity on *Alternaria tennuis* and *Botrytis alli*, which cause grapevine disease and wheat rust (CREMLYN *et al.*, 1988). In our study, yeasts *C. albicans* ATCC 10231 and isolated strain of *C. albicans* were used as test organisms

from the group of fungi. The tested hydantoins showed low antifungal activity towards both *C. albicans* strains, which is also the result of a study examining the effect of hydantoins on *C. albicans* (MARINOVA *et al*., 2013).

CONCLUSION

The tested hydantoin derivatives have shown moderate antibacterial and weak antifungal activity, with MIC and MMC values in a broad range of <7.81 do >1000 μg/mL. The intensity of influence on the applied microorganisms varied depending on the structure and concentration of the tested compounds and the type of microorganisms.

Hydantoin derivatives generally showed a stronger action against Gram-positive bacteria (especially probiotics) than Gram-negative ones. The bicyclic phenylseleno derivatives of hydantoin have shown the highest inhibitory activity, probably due to the presence of selenium in their structure. They were most effective against *S. aureus* strain.

The tested compounds appeared promising for a fragment-based drug design approach and further bioactivity studies.

Acknowledgments

The financial support for this research was provided by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Agreements Nos. 451-03-68/2022-14/200378 and 451-03-68/2022-14/200122).

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