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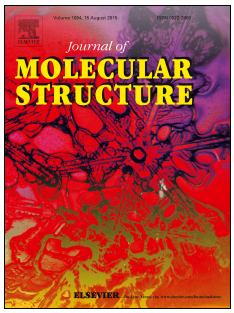

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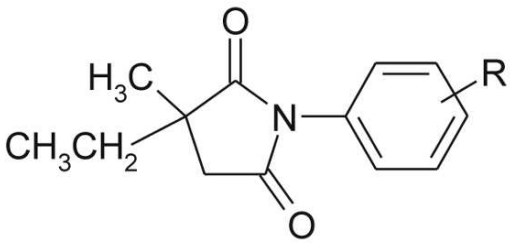

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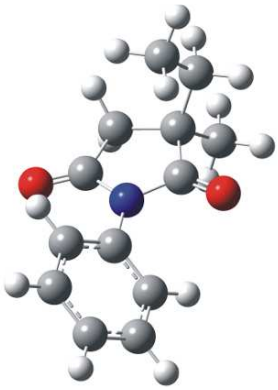

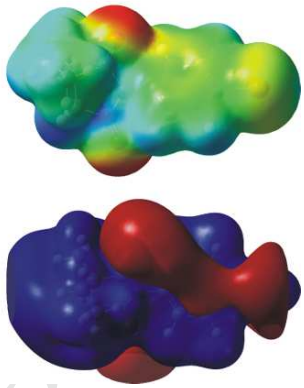

Synthesis, Antimicrobial Activity and Quantum Chemical Investigation of novel Succinimide Derivatives

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Abstract

In the present study, twelve new 1-aryl-3-ethyl-3-methylpyrrolidine-2,5-diones were synthesized and their structures were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analysis. In the final step of synthetic rout, condensation between corresponding succinic acid and substituted anilines has been improved using the microwave irradiation. Significantly higher yields compared to conventional condensation have been observed. The preliminary biological results indicated that some of the synthesized compounds showed promising *in vitro* antifungal activities towards several test fungi. 1-(4-Bromophenyl)-3-ethyl-3-methylpyrrolidine-2,5-dione (**8**) exhibited significant *in vitro* inhibitory activities against broad spectra of fungus, and on the basis of obtained data, the investigated bromo derivative has to be observed as novel potential fungicide. The density functional theory (DFT) calculations have been performed in order to study the structure-activity relationship (SAR) of the investigated molecules. In order to prediction of the chemical activity of the molecule, the molecular electrostatic potential (MEP) map was analyzed for the optimized geometry of 1-phenyl-3-ethyl-3-methylpyrrolidine-2,5-dione (**4**) and 1-(4-bromophenyl)-3-ethyl-3-methylpyrrolidine-2,5-dione (**8**).

Keyword: Succinimide; Antimicrobial activity; Antifungal activity; DFT calculation; Structure-activity relationship.

1. Introduction

In the treatment of various diseases caused by different microorganisms there are currently available several drugs [1]. Numerous side effects and toxic effects produced by their application, lead to the addition need for drug improvement or finding of novel agents. Also, results of increased use and misuse of antimicrobial drugs are development of resistant pathogens, which caused that the overcoming drug resistance has become an important issue in medicinal chemistry [2]. Moreover, it is known that the utilization of agrochemicals has led to numerous benefits and significant progress in our lifestyles. Due that, the problem of multidrug resistant microorganisms and pollution problems associated with conventional agrochemicals have reached an alarming level in the last decades. Accordingly, synthesis and design of new bioactive compounds with novel structures, biological activity, high selectivity and eco-friendly properties has become an urgent need in future [3-5].

There are number of five-member heterocyclic molecules which are very useful as pharmaceutical, chemical and agricultural agents. Succinimdes belong to the class of cyclic imides, which synthetics products exhibits wide spectrum of biological and pharmacological properties such as antimicrobial [6-10], antiepileptic [11], good electro-convulsion [12], analgesic [13], antitumor [14] etc. Moreover, cyclic imides like succinimides, maleimides and itaconimide demonstrated the defensive and restorative antifungal influence against rice blast and kidney bean stem rots [15]. The number of succinimide derivatives showed the seedling growth stimulator activities against wheat and radish [16].

The present research is directed towards the synthesis and characterization of a series of novel 1-aryl-3-ethyl-3-methylpyrrolidine-2,5-diones as potentially new heterocyclic compounds with promising bioactivity. Additionally, evaluation of antifungal and antimicrobial activity and investigation of physico-chemical properties (using the density function theory (DFT)) of newly synthesized compounds have been performed. To estimate chemical and biological activity of the molecule, different molecular descriptors were calculated for the optimized geometries of the investigated succinimides. The corresponding structure-activity relationships were proposed and discussed.

2. Experimental

2.1. Chemistry

The chemicals used in the synthesis were purchased from Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO, USA) or Merck Chemicals (Merck KGaA, Darmstadt, Germany). Microwave synthesis was performed in Anton Paar Monowave 300 (Ashland, VA, USA) microwave reactor. The FT-IR spectra of the compounds (in KBr pellets) were determined using an ABB Bomem MB Series 100 Fourier transform infrared (FT-IR) (Quebec City, Canada) spectrophotometer. The NMR spectral measurements have been performed on a Bruker AC 250 spectrometer at 200 MHz for the ^{1}H NMR and 50 MHz for the ^{13}C NMR spectra or on a Bruker 300 spectrometer at 400 MHz for the ^{1}H NMR and 100 MHz for the ^{13}C NMR spectra. The spectra were recorded at room temperature in deuterated dimethyl sulfoxide (DMSO-d_6). In this paper, chemical shifts are expressed in ppm in reference to TMS. All melting points were uncorrected and are reported here in degrees Celsius. The elemental analyses of the investigated compounds were carried out by standard analytical micromethods using an Elemental Vario EL III microanalyzer.

2.1.1. Synthesis of 1-aryl-3-ethyl-3-methylpyrrolidine-2,5-diones (1-12)

All of the investigated 1-aryl-3-ethyl-3-methylpyrrolidine-2,5-diones were synthesized from the corresponding succinic acid derivative and substituted anilines under solvent-free conditions using the microwave irradiation (MW). 3-Ethyl-3-methyl succinic acid derivative was synthesized by previously reported procedures (Scheme 1, part Results and discussion) [17-19].

Generally, the mixture of the previously synthesized 3-ethyl-3-methyl succinic acid (1 mmol, 160.2 mg) and substituted aniline (1.1 mmol) have been stirred in a 25 mL reactor tube at 180 °C (MW) for 15min under solvent-free conditions. After the completion of the reaction, the mixture was cooled to room temperature. The obtained crude mixture has been dissolved in ethyl acetate and consecutively washed with 10 mL of 5% $HCl_{(aq)}$, 10 mL of saturated $NaHCO_{3(aq)}$ and two times with 10 mL of distilled water. The organic layer was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was recrystallized from acetone.

The structures of the novel succinimides were confirmed by melting point, FT-IR, ^{1}H NMR, and ^{13}C NMR spectroscopy, as well as by elemental analysis. The data obtained during characterization are given below. The numbering of carbon atoms for ^{13}C NMR is presented in the Supplementary material (Fig. S1).

1-(4-Hydroxyphenyl)-3-ethyl-3-methylpyrrolidine-2,5-dione (**1**, $C_{13}H_{15}NO_3$). White solid; Yield: 78%; M.p.: 145-147 °C; FT-IR (KBr): |ν| cm^{-1} = 1767 (C=O), 1687 (C=O); ^{1}H NMR (200 MHz, DMSO): |δ| ppm = 9.75 (s, 1H, –OH), 7.03 (d, *J* = 8.0 Hz, 2H, –C_6H_4), 6.84 (d, *J* = 8.0 Hz, 2H, –C_6H_4), 2.80-2.56 (AB q, J_{AB} = 18.0 Hz, 2H, –CH_2–), 1.74-1.52 (m, 2H, –CH_2CH_3), 1.28 (s, 3H, –CH3), 0.86 (t, *J* = 8.0 Hz, 3H, –CH_2CH_3); ^{13}C NMR (50 MHz, DMSO): |δ| ppm = 182.37 (C6), 175.79 (C7), 157.56 (C11), 128.57 (C9, C9'), 123.80 (C8), 115.66 (C10, C10'), 43.74 (C4), 40.13 (C5), 31.15 (C2), 23.37 (C3), 8.73 (C1). Anal. calcd. for $C_{13}H_{15}NO_3$: C, 66.94; H, 6.48; N, 6.00; Found: C, 66.81; H, 6.40; N, 5.93.

1-(4-Methoxyphenyl)-3-ethyl-3-methylpyrrolidine-2,5-dione (**2**, $C_{14}H_{17}NO_3$). Black solid; Yield: 77%; M.p.: 75-77 °C; FT-IR (KBr): |ν| cm^{-1} = 1766 (C=O), 1703 (C=O); ^{1}H NMR (200 MHz, DMSO): |δ| ppm = 7.18 (d, *J* = 8,0 Hz, 2H, –C_6H_4), 7.03 (d, *J* = 8,0 Hz, 2H, –C_6H_4), 3.79 (s, 3H, –OCH_3), 2.82-2.58 (AB q, J_{AB} = 18.0 Hz, 2H, –CH_2–), 1.76-1.54 (m, 2H, –CH_2CH_3), 1.29 (s, 3H, –CH3), 0.88 (t, *J* = 8.0 Hz, 3H, –CH_2CH_3); ^{13}C NMR (50 MHz, DMSO): |δ| ppm = 182.24 (C6), 175.67 (C7), 159.17 (C11), 128.56 (C9, C9'), 125.27 (C8), 114.35 (C10, C10'), 55.57 (C12), 43.78 (C4), 40.14 (C5), 31.11 (C2), 23.27 (C3), 8.69 (C1). Anal. calcd. for $C_{14}H_{17}NO_3$: C, 68.00; H, 6.93; N, 5.66; Found: C, 67.89; H, 6.82; N, 5.60.

1-(4-Methylphenyl)-3-ethyl-3-methylpyrrolidine-2,5-dione (**3**, $C_{14}H_{17}NO_2$). White solid; Yield: 75%; M.p.: 54-56 °C; FT-IR (KBr): |ν| cm^{-1} = 1777 (C=O), 1710 (C=O); ^{1}H NMR (200 MHz, DMSO): |δ| ppm = 7.29 (d, *J* = 8.0 Hz, 2H, –C_6H_4), 7.14 (d, *J* = 8.0 Hz, 2H, –C_6H_4), 2.77-2.65 (AB q, J_{AB} = 18.0 Hz, 2H, –CH_2–), 2.34 (s, 3H, –CH3), 1.76-1.54 (m, 2H, –CH_2CH_3), 1.29 (s, 3H, –CH3), 0.88 (t, *J* = 8,0 Hz, 3H, –CH_2CH_3); ^{13}C NMR (50 MHz, DMSO): |δ| ppm = 182.16 (C6), 175.58 (C7), 138.12 (C11), 130.14 (C8), 129.64 (C10, C10'), 127.16 (C9, C9'), 43.87 (C4), 40.20 (C5), 31.16 (C2), 23.31 (C3), 20.95 (C12), 8.73 (C1). Anal. calcd. for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06; Found: C, 72.55; H, 7.33; N, 6.00.

1-phenyl-3-ethyl-3-methylpyrrolidine-2,5-dione (**4**, $C_{13}H_{15}NO_2$). White solid; Yield: 79%; M.p.: 53-54 °C FT-IR (KBr): |ν| cm^{-1} = 1776 (C=O), 1711 (C=O); ^{1}H NMR (200 MHz, DMSO): |δ| ppm = 7.54–7.42 (m, 5H, –C_6H_5), 7.28 (d, *J* = 7.0 Hz, 1H, –C_6H_5), 2.85-2.61 (AB q, J_{AB} = 18.0 Hz, 2H, –CH_2–), 1.78-1.56 (m, 2H, –CH_2CH_3), 1.31 (s, 3H, –CH_3), 0.89 (t, J = 8,0 Hz, 3H, –CH_2CH_3); 13C NMR (50 MHz, DMSO): |δ| ppm = 182.04 (C6), 175.45 (C7), 132.71 (C8), 129.14 (C9, C9'), 128.57 (C11), 127.34 (C10, C10'), 43.89 (C4), 40.21 (C5), 31.12 (C2), 23.24 (C3), 8.70 (C1). Anal. calcd. for $C_{13}H_{15}NO_2$: C, 71.87; H, 6.96; N, 6.45 Found: C, 71.77; H, 6.86; N, 6.39.

1-(4-Fluorophenyl)-3-ethyl-3-methylpyrrolidine-2,5-dione (**5**, $C_{13}H_{14}NO_2F$). White solid; Yield: 72%; M.p.: 63-64°C FT-IR (KBr): |ν| cm^{-1} = 1776 (C=O), 1704 (C=O); ^{1}H NMR (200 MHz, DMSO): |δ| ppm = 7.335 (d, *J* = 6.0 Hz, 4H, –C_6H_4), 2.79-2.65 (AB q, J_{AB} = 18.0 Hz, 2H, –CH_2–), 1.79-1.52 (m, 2H, –CH_2CH_3), 1.30 (s, 3H, –CH_3), 0.88 (t, *J* = 8.0 Hz, 3H, –CH_2CH_3); ^{13}C NMR (50 MHz, DMSO): |δ| ppm = 181.99 (C6), 175.40 (C7), 161.63 (d, *J* = 237.9 Hz, C11,11'), 129.53 (d, *J* = 8.50 Hz, C9,9'), 129.91 (d, *J* = 3.0 Hz, C8), 116.05 (d, *J* = 22.5 Hz, C10,10'), 43.88 (C4), 40.20 (C5), 31.06 (C2) 23.28 (C3), 8.82 (C1). Anal. calcd. for $C_{13}H_{14}FNO_2$: C, 66.37; H, 6.00; N, 5.95; Found: C, 66.35; H, 5.96; N, 5,92.

1-(4-Chlorophenyl)-3-ethyl-3-methylpyrrolidine-2,5-dione (**6**, $C_{13}H_{14}NO_2Cl$). White solid; Yield: 76%; M.p.: 50-52 °C FT-IR (KBr): |ν| cm^{-1} = 1775 (C=O), 1702 (C=O); ^{1}H NMR (200 MHz, DMSO): |δ| ppm = 7.57 (d, *J* = 8.0 Hz, 2H, –C_6H_4), 7.33 (d, *J* = 8.0 Hz, 2H, –C_6H_4), 2.79-2.65 (AB q, J_{AB} = 18.0 Hz, 2H, –CH_2–), 1.73-1.55 (m, 2H, –CH_2CH_3), 1.30 (s, 3H, –CH_3), 0.87 (t, *J* = 8,0 Hz, 3H, –CH_2CH_3); ^{13}C NMR (50 MHz, DMSO): |δ| ppm = 181.89 (C6), 175.29 (C7), 133.07 (C8), 131.54 (C11), 129.23 (C10, C10'), 129.17 (C9, C9'), 43.97 (C4), 40.27 (C5), 31.09 (C2), 23.13 (C3), 8.72 (C1). Anal. calcd. for $C_{13}H_{14}ClNO_2$: C, 62.03; H, 5.61; N, 5.56; Found: C, 61.92; H, 5.45; N, 5.49.

1-(3-Chlorophenyl)-3-ethyl-3-methylpyrrolidine-2,5-dione (**7**, $C_{13}H_{14}NO_2Cl$). White solid; Yield: 60%; M.p.: 58-59°C FT-IR (KBr): |ν| cm^{-1} = 1779 (C=O), 1698 (C=O); ^{1}H NMR (400 MHz, DMSO): |δ| ppm = 7.56-7.45 (m, 3H, –C_6H_4), 7.31-7.29 (m, 2H, –C_6H_4), 2.82-2.64 (AB q, J_{AB} = 16.0 Hz, 2H, –CH_2–), 1.77-1.58 (m, 2H, –CH_2CH_3), 1.32 (s, 3H, –CH_3), 0.90 (t, *J* = 8.0 Hz, 3H, –CH_2CH_3); ^{13}C NMR (100 MHz, DMSO): |δ| ppm = 181.90 (C6), 175.30 (C7), 134.26 (C8), 133.43 (C10 along –Cl), 130.97 (C10'), 128.78 (C9 close to –Cl), 127.51 (C11), 126.37 (C9'), 44.22 (C4), 40.58 (C5), 31.25 (C2) 23.32 (C3), 8.95 (C1). Anal. calcd. for $C_{13}H_{14}ClNO_2$: C, 62.03; H, 5.61; N, 5.56; Found: C, 61.90; H, 5.47; N, 5.48.

1-(4-Bromophenyl)-3-ethyl-3-methylpyrrolidine-2,5-dione (**8**, $C_{13}H_{14}NO_2Br$). White solid; Yield: 75%; M.p.: 60-63 °C FT-IR (KBr): |ν| cm^{-1} = 1775 (C=O), 1702 (C=O); ^{1}H NMR (200 MHz, DMSO): |δ| ppm = 7.70 (d, *J* = 8.0 Hz, 2H, –C_6H_4), 7.26 (d, *J* = 8.0 Hz, 2H, –C_6H_4), 2.78-2.66 (AB q, J_{AB} = 18.0 Hz, 2H, –CH_2–), 1.76-1.55 (m, 2H, –CH_2CH_3), 1.30 (s, 3H, –CH_3), 0.87 (t, *J* = 8.0 Hz, 3H, –CH_2CH_3); ^{13}C NMR (50 MHz, DMSO): |δ| ppm = 181.83 (C6), 175.24 (C7), 132.18 (C10, C10'), 131.97 (C8), 129,46 (C9, C9'), 121.56 (C11), 43.98 (C4), 40.28 (C5), 31.10 (C2) 23.13 (C3), 8.72 (C1). Anal. calcd. for $C_{13}H_{14}BrNO_2$: C, 52.72; H, 4.76; N, 4.73; Found: C, 52.59; H, 4.63; N, 4,65.

1-(3-Bromophenyl)-3-ethyl-3-methylpyrrolidine-2,5-dione (**9**, $C_{13}H_{14}NO_2Br$). White solid; Yield: 63%; M.p.: 72-73°C FT-IR (KBr): |ν| cm^{-1} = 1779 (C=O), 1698 (C=O); ^{1}H NMR (200 MHz, DMSO): |δ| ppm = 7.67-7.56 (m, 2H, –C_6H_4), 7.47 (t, *J* = 6.0 Hz, 1H, –C_6H_4), 7.35-7.30 (m, 1H, –C_6H_4), 2.84-2.59 (AB q, J_{AB} = 18.0 Hz, 2H, –CH_2–), 1.81-1.52 (m, 2H, –CH_2CH_3), 1.30 (s, 3H, –CH_3), 0.88 (t, *J* = 8.0 Hz, 3H, –CH_2CH_3); ^{13}C NMR (50 MHz, DMSO): |δ| ppm = 181.73 (C6), 175.14 (C7), 134.13 (C8), 131.44 (C10 along –Br), 131.04 (C10'), 130.06 (C9 close to –Br), 126.57 (C11), 121.38 (C9'), 43.94 (C4), 40.28(C5), 30.96 (C2) 23.18 (C3), 8.83 (C1). Anal. calcd. for $C_{13}H_{14}BrNO_2$: C, 52.72; H, 4.76; N, 4.73; Found: C, 52.56; H, 4.60; N, 4.64.

1-(4-Carboxyphenyl)-3-ethyl-3-methylpyrrolidine-2,5-dione (**10**, $C_{14}H_{15}NO_4$). White solid; Yield: 72%; M.p.: 152-154 °C FT-IR (KBr): |ν| cm^{-1} = 1779 (C=O), 1717 (C=O); ^{1}H NMR (200 MHz, DMSO): |δ| ppm = 8.06 (d, *J* = 8.0 Hz, 2H, –C_6H_4), 7.44 (d, *J* = 8,0 Hz, 2H, –C_6H_4), 2.87-2.63 (AB q, J_{AB} = 18.0 Hz, 2H, –CH_2–), 1.78-1.56 (m, 2H, –CH_2CH_3), 1.31 (s, 3H, –CH_3), 0.88 (t, J = 8,0 Hz, 3H, –CH_2CH_3); ^{13}C NMR (50 MHz, DMSO): |δ| ppm = 181.81 (C6), 175.20 (C7), 166.99 (C12), 136.51 (C8), 130.73 (C11), 130.19 (C10, C10'), 127.34 (C9, C9'), 44.05 (C4), 40.34 (C5), 31.15 (C2), 23.15 (C3), 8.73 (C1). Anal. calcd. for $C_{14}H_{15}NO_4$: C, 64.36; H, 5.79; N, 5.36; Found: C, 64.48; H, 5.89; N, 5.34.

1-(4-Cyanophenyl)-3-ethyl-3-methylpyrrolidine-2,5-dione (**11**, $C_{14}H_{14}N_2O_2$). White solid; Yield: 76%; M.p.: 93-96 °C FT-IR (KBr): |ν| cm^{-1} = 1772 (C=O), 1714 (C=O); ^{1}H NMR (200 MHz, DMSO): |δ| ppm = 7.98 (d, *J* = 8.0 Hz, 2H, –C_6H_4), 7.55 (d, *J* = 8.0 Hz, 2H, –C_6H_4), 2.87-2.63 (AB q, J_{AB} = 18.0 Hz, 2H, –CH_2–), 1.78-1.56 (m, 2H, –CH_2CH_3), 1.31 (s, 3H, –CH_3), 0.88 (t, *J* = 8.0 Hz, 3H, –CH_2CH_3); ^{13}C NMR (50 MHz, DMSO): |δ| ppm = 181.56 (C6), 174.95 (C7), 136.75 (C8), 133.26 (C10, C10'), 128.11 (C9, C9'), 118.57 (C12), 111.12 (C11), 44.07 (C4), 40.35 (C5), 31.06 (C2), 22.99 (C3), 8.68 (C1). Anal. calcd. for $C_{14}H_{14}N_2O_2$: C, 69.41; H, 5.82; N, 11.56; Found: C, 69.30, H, 5.68, N, 11.49.

1-(4-Nitrophenyl)-3-ethyl-3-methylpyrrolidine-2,5-dione (**12**, $C_{13}H_{14}N_2O_4$). Yellow solid; Yield: 73%; M.p.: 115-117 °C FT-IR (KBr): |ν| cm^{-1} = 1782 (C=O), 1713 (C=O); ^{1}H NMR (200 MHz, DMSO): |δ| ppm = 8.36 (d, *J* = 10.0 Hz, 2H, –C_6H_4), 7.63 (d, *J* = 10.0 Hz, 2H, –C_6H_4), 2.89-2.65 (AB q, J_{AB} = 18.0 Hz, 2H, –CH_2–), 1.79-1.58 (m, 2H, –CH_2CH_3), 1.32 (s, 3H, –CH_3), 0.89 (t, *J* = 8,0 Hz, 3H, –CH_2CH_3); ^{13}C NMR (50 MHz, DMSO): |δ| ppm = 181.58 (C6), 174.97 (C7), 146.85 (C11), 138.29 (C8),

128,27 (C10, C10'), 124.45 (C9, C9'), 44.12 (C4), 40.38 (C5), 31.09 (C2), 22.97 (C3), 8.70 (C1). Anal. calcd. for $C_{13}H_{14}N_2O_4$: C, 59.54; H, 5.38; N, 10.68; Found: C, 59.44; H, 5.31; N, 10.62.

2.2. Antimicrobial Activity

2.2.1. Human Pathogens

The newly synthesized succinimides were evaluated for antibacterial activity against eight bacterial species: *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240), *Listeria monocytogenes* (NCTC 7973) (gram positive bacteria), *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter cloacae* (clinical isolate), *Salmonella typhimurium* (ATCC 13311), *Escherichia coli* (ATCC 35210) (gram negative bacteria).

Antifungal activity were evaluated against eight fungal species: *Aspergillus flavus* (ATCC 9643), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus versicolor* (ATCC 11730), *Aspergillus niger* (ATCC 6257), *Penicillium ochrochloron* (ATCC 9112), *Penicillium funiculosum* (ATCC 36839), *Penicillium verrucosum var.cycopium* (food isolate), *Trichoderma viride* (IAM 5061).

2.2.2. Preparation of Cultures of Micromycetes

The micromycetes were cultivated on malt agar (MA) medium for 21 days at 26 °C. Inoculum was prepared via spores rinsing from the substrate surface using sterile solution 0.85% NaCl and 0.1% Tween 80 (v/v). The preferred concentration of spores was prepared by dissolving 100 ml of a primary culture in 900 ml of malt broth (MB) growth medium. Dilution was performed several times until the final concentration has been obtained (1.0×10^5 cells/ml). Inoculum was stored at 4 °C until further usage.

The bacterial strains were stocked in glycerol and seeded on 2 ml of Tryptic Soy Broth (TSB) medium (Biolife, Milan, Italy), then incubated in thermostat for 24 h at 37 °C. Overnight cultures have 1.0×10^9 cells/ml each. The final solution of culture and medium (1.0×10^8 cells/ml) was obtained by dissolving 100 ml of a primary culture in 900 ml of TSB. The numbers of spores were calculated under a Leica DMLS light microscope using a haemocytometer.

2.2.3. Broth Microdilution Method

The antimicrobial activity was performed by using microdilution method [20-26]. The minimal inhibitory (MICs), minimal fungicidal (MFCs) and minimal bactericidal concentrations (MBCs) of the synthesized succinimides for each species of studied microorganisms were determined. The newly synthesized compound were dissolved in 10% DMSO water solution. The concentration dissolved succinimides is 10 mg/ml. In each well of microplates ("F"-micro-plate with cover, Spektar, Čačak, Serbia), 20 µl of MB growth medium was added for fungi or TSB for bacteria, 180 µl of compound solution and different volumes in microlitres of fungal inocula for each fungal strain in particular and 10 µl of bacterial inocula for each bacterial species. Antimycotics ketoconazole and bifonazole and antibiotics streptomycin and ampicillin were used as positive controls (1 mg/ml in sterile physiological saline). Ten percent DMSO was used as a negative control. Microplates were incubated for 72 h at 28 °C (micromycetes) and for 24 h at 37 °C (bacteria).

2.2.4. MIC Determination

The minimum inhibitory concentration (MIC) is the lowest concentration of antimicrobial agent that completely inhibits bacterial and fungal without visible growth, observed under a binocular microscope [27-29]. The MIC values were determined by counting cell numbers by spectrophotometric methods [23,24]. The MICs obtained from the susceptibility testing

of various bacteria to the analyzed compound were determined likewise via a colorimetric microbial viability assay based on reduction of INT (p-iodonitrotetrazoliumviolet) [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride; Sigma-Aldrich, Munich, Germany] colour and compared to positive controls for each bacterial strain. After staining, the colonies were incubated for 2 h at 37 °C. Inhibition of growth is indicated Pale yellowish colour23. Microplate reader was used (Tecan Austria GmbH, Grödig, Austria; Eppendorf AG, Hamburg, Germany), to evaluate the intensity of yellow colour. MIC values were calculated as milligram per 100 µl, and then, the values were transferred in milligrams per milliliter [23,24].

2.2.5. MFC and MBC Determination

The minimum fungicidal (MFC) and minimum bactericidal concentration (MBC) were determined by serial subcultivation of 2 µl of inoculum into microtiter plates containing 100 µl of broth per well and the further incubation for 72 h at 26 °C for micromycetes and for 24 h at 37 °C for bacteria, according to the protocol of the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", University of Belgrade [23,24]. MFC and MBC is defined as the lowest concentration with no visible fungal/bacterial growth, indicating 99.5% killing of the original inocula31. Optical density is measurement of fungal/bacterial cultures, at the wavelength of 655 nm by a Microplate Manager (Tecan Austria GmbH, Grödig, Austria; Eppendorf AG, Hamburg, Germany) and compared to a blank (broth medium plus diluted extracts) and a positive control. MBC values were determined by a colorimetric microbial viability assay based on reduction of INT colour and were compared to positive controls for each bacterial strain. After stain was applied (40 µl per well), the stained colonies were incubated for 2 h at 37 °C. Unstained chambers of microplates have indicated the presence of a bactericidal effect (CLSI2009).The results of MFC and MBC values were calculated as milligrams per 100 µl, and then, the values were transferred in milligramme per milliliter.

2.3. Theoretical Calculations

All density functional theory (DFT) calculations were performed using the Gaussian 09 program package [30] with B3LYP method [31] and 6-311G(d,p) basis set. The default convergence criteria were used without any constraint on the geometry. The stability of the optimized geometry was confirmed by frequency calculations, which gave real values (no imaginary frequency) for all the calculated frequencies. The solvent effect was introduced by the Conductor Polarizable Continuum Model (CPCM) [32].

The frontier molecular orbital energies and energy gap of the investigated compounds are also calculated at the same level of DFT theory. For obtaining the chemical reactivity of the molecule, the Molecular Electrostatic Potential (MEP) surface is plotted over the optimized geometry. The GaussView 5.0 graphical interface was used to visualize molecular orbitals and MEP [33]. The Vega ZZ version 2.4 [34] was used to estimate physicochemical descriptors (log *P*, polar surface area and molar refractivity) from geometries previously optimized by DFT.

3. Results and discussion

3.1. Synthesis and spectral analisys

In connection with our study of the structure-property relationship of novel succinimide derivatives, we now in this paper extend our previous work [35-37] on the synthesis, structure and properties of twelve new 1-aryl-3-ethyl-3-methylpyrrolidine-2,5-diones.

The synthesis procedure of the novel compounds (**1-12**) is shown in Scheme **1**. In the last step, 1-aryl-3-ethyl-3-methylsuccinimides derivatives have been synthesized under microwave irradiation in a moderate yield, while all previous steps have been proceed by literature data [17-19]. Due to microwave irradiation, the products have been obtained in better yields (85%-89%) with a shorter reaction time (15 min), while in previously reported procedures the succinimide derivatives have been obtained in lower yields with longer reaction times (conduction heating, 180 °C, 3h).

<Scheme 1>

The chemical structures of the compounds (**1-12**) have been confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analyses. On the FT-IR spectra two intensive and characteristic stretching absorptions of the carbonyl groups (C=O) from the succinimide ring in the regions 1782-1766 cm^{-1} and 1717-1687 cm^{-1} have been observed. All aromatic and aliphatic C–H stretchings are on their characteristic absorption positions. ^{1}H NMR spectrum showed a characteristic aromatic protons belonging to 3- or 4-substituted phenyl group. Protons in *p*-substituted phenyl ring gave peaks at 8.36-7.03 and 7.63-6.84 ppm as two doublets, while for unsubstitued and *m*-substituted phenyl group multiplet signals have been observed. Moreover, aliphatic protons of $–CH_3$ and $–CH_2CH_3$ are on the characteristic positions. Methyl protons are presented as one strong singlet (from 1.32 to 1.28 ppm), while ethyl group is observed as multiplet (1.79-1.52 ppm) and triplet (0.89-0.86 ppm) signals. Also, protones from methylene group in succinimide ring ($–CH_2–$) is observed as characteristic AB quartet in the range 2.87-2.56 with coupling constant J_{AB} = 18 Hz. Additionally, ^{13}C NMR spectra of investigated compounds exhibited all necessary signals at the appropriate positions (see Experimental).

3.2. Antimicrobial Activity

The newly synthesized succinimides (**1-12**) have been tested for antibacterial activity on eight bacterial species, mentioned previously, and MIC/MBC values are given in Table **1**. Compound **2** has better MIC/MBC activity than ampicillin, except in the case of *Escherichia coli*, where it has no activity. The compound **3** has no activity to *Escherichia coli* and *Salmonella typhimurium*, but its activity (MIC/MBC) is better for other bacteria then ampicillin. The compound **10** shows activity to four bacteria: *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Salmonella typhimurium*. Other compounds did not show any activity on the tested bacteria.

<Table 1>

Also, newly synthesized succinimides have been tested for antifungal activity on eight fungal species, and MIC/MFC values are given in Table **2**. The succinimides showed great activity against different species of fungal. The compound **8**, which should be particularly prominent, have better MIC/MFC activity even then a reference commercially available compound ketoconazole and bifonazole.

<Table 2>

It can be noticed that tested compounds showed higher antifungal potential then antibacterial effect and reacted against almost all fungal species, with exception toward *Penicillium* species. Exactly these obtained antimicrobial results candidate the

investigated compounds (**1**-**12**) as promising antifungal agents, whereby compound **8** have to be specially highlighted due to its high activity against all investigated fungus even in compare to commercially available drugs.

3.3. Theoretical Calculations

3.3.1. Conformational stability

In order to find all stable conformers, a conformational analysis was carried out for the investigated compounds. The sucinimide ring of investigated compounds is very rigid so the only conformational changes are possible in the position of its substituents (methyl, ethyl and phenyl group). As the rotation of methyl group does not contribute significantly to the energy of investigated systems, only the positions of ethyl and phenyl group are investigated. The positions of phenyl group are investigated in detail in our previous work [38] and similar findings were noted in this case, too. For a better insight to the energy changes connected with rotation of ethyl group, the potential energy scans (PES) were done for two possible orientations of phenyl group. During the calculation, all the geometrical parameters were simultaneously relaxed while the torsion angle of ethyl group was varied in steps of 5° from 0-360° (Fig. **S2**, Supplementary material). Results show that six conformers for molecule with symmetric substituent and 12 conformers for asymmetric substituent are possible.

The full geometry optimizations of these conformers were performed by B3LYP [31] method with 6-311G(d,p) basis set in the vacuum and solvents (THF, ethanol, DMSO and water). The results provided from these calculations are used for different analyses in this work. The geometry of the most stable isomer for compound **4** obtained with B3LYP method is shown in Figure **1**.

<Figure 1>

The located conformers have similar energies which differ from the energy of the most stable isomer for less than 0.6 kcal/mol, depending of substituent and according to Boltzmann analysis they are simultaneously present in a mixture. Regardless of that, the properties of interest are simulated based on the geometry of the most stable conformer only.

DFT calculations indicate non-planar conformations between succinimide and phenyl rings for all investigated succinimides. The deviation from planarity (expressed in terms of dihedral angle θ between planes of succinimide and phenyl rings, Table **3** and **S1** (Supplementary material)) can serve as a rough measure for the efficient transfer of substituent's resonance effect from phenyl to succinimide ring. In investigated series of succinimides, it increases with increasing of electron-donating ability of the substituent R. The B3LYP method predicts a torsion angle θ from 38 to 45° in vacuum while with introduction of solvents (THF, ethanol, DMSO and water) predict a much larger values for this angle, i.e., from 46 to 67°. It must be noted that the angle θ increase with higher polarity of solvents.

3.3.2. Electronic analysis

The analysis of energies of frontier molecular orbitals (the HOMO and LUMO) as well as the energy gap between them, explain the kinetic stability and chemical reactivity of the molecule [39]. The energies of HOMO and LUMO are, respectively, rough measures of the electron-donating and electron-withdrawing abilities of the molecule.

In order to check possible ways of interactions for investigated succinimides the energies and electron distributions of the frontier molecular orbitals are computed in different solvents (THF, ethanol, DMSO and water) using B3LYP functional, see Tables **3** and **S1** (Supplementary material). Plots of the HOMO and LUMO molecular orbitals along with their energies and energy gaps for compounds **2**, **4**, **8** and **12** in vacuum are shown in Fig. **2**.

<Figure 2>

The electron density of HOMO orbital for all investigated molecules is dominantly populated on the aryl group and the imide part of the succinimide ring. The electron densities of the LUMO orbitals demonstrate great influence of substituents effects on the intramolecular charge transfer (ICT) process. LUMO orbital density in molecules with electron-donating substituent is localized on the succinimide ring showing big shift of electron density relative to HOMO orbital and indicates strong ICT character. Molecules with weak electron-withdrawing substituents show slight shift of electron density from the aryl group to the imide part of the succinimide ring and indicates weak ICT character. Contrary, strong electron-withdrawing substituents show slight shift of electron density from the imide part of the succinimide ring to the aryl group and indicate weak ICT character with opposite direction.

<Table 3>

In the series of succinimides investigated here, the non-substituted compound **4** has the largest energy gap with 6.03 eV in vacuum, while the presence of electron-donating substituent leads to a small decrease in the energy gap and a larger decrease for electron-withdrawing substituent. Introduction of solvents effects lead to increasing of energy gap, in absolute values, for all substituents except $-NO_2$. But, relatively to non-substituted compound **4**, introduction of the polar solvents prompts a decrease in the energy gap for all substituted succinimides. The presence of electron-donating substituent leads to a small decrease in the energy gap while electron-withdrawing substituent exhibits much larger decrease and this effect is consistent with the increase of polarity. In non-polar solvent, THF, the electron-donating substituent causes a small increase, while electron-withdrawing substituent, as in previous case, causes large decrease of the energy gap.

3.3.3. Molecular electrostatic potential

Molecular electrostatic potential (MEP) is related to the electronic density and often helps to predict the reactivity of chemical systems in electrophilic and nucleophilic reactions, the study of biological recognition process and hydrogen bonding interaction [40]. In order to predict reactive sites of electrophilic or nucleophilic attack for the investigated molecules, the MEP at the B3LYP/6-311G(d,p) optimized geometry was calculated. The negative (red and yellow) regions of the MEP are related to electrophilic reactivity and the positive (blue) region to nucleophilic reactivity, as shown in Fig. **3** (MEP).

<Figure 3>

As it can be seen from the Figure **3**, molecule of 1-phenyl-3-ethyl-3-methylpyrrolidine-2,5-dione (**4**) in vacuum has two possible sites for electrophilic and one site for nucleophilic attack. Negative regions of the studied molecule are located around the oxygen atoms of the carbonyl groups and moderate positive region is localized on the hydrogen atoms of the methylene group from the succinimide ring. Similarly, molecule of **8** has two possible sites for electrophilic attack, with elongated negative potential from carbonyl groups over phenyl group to bromide substituent on *para* position (Figs. **S3** and **S4**, Supplementary material). At the same time positive region localized on the hydrogen atoms of the methylene group from succinimide ring is elongated over H-atoms of phenyl group to σ-hole of *para*-bromide. Through such positive σ-hole, molecule

can interact attractively with negative sites to form noncovalent complex [41]. MEP maps for other investigated compounds are shown in Fig. **S5** (Supplementary material).

3.4. ADMET Factor Profiling and SAR Study

One of the most important factors in design of biologically therapeutic species is their oral bioavailability. Due that, determination which of the investigated succinimides possesses pharmacokinetic activity, their structural properties have been tested by the empirical 'rule of five' established by Lipinski [42]. In accordance with this rule, compounds which satisfied following four structural features: (1) molecular weight lower than 500; (2) maximum 5 atoms that are proton-donors of the hydrogen bonds (–OH and –NH); (3) maximum 10 atoms (N and O) that are hydrogen bond acceptors and (4) calculated value of the logarithm of the octanol-water partition coefficient (log *P*) lower than 5, will potentially exhibit good in vivo permeability [43]. Moreover, additional structural parameters which have to be included in the attempts to propose corresponding SAR model of investigated molecules are: number of rotatable bonds should be lower than 8, low polar surface area (< 140 $Å^2$) and molar refractivity in the range from 40 to 130 $Å^3$ [43-45]. All structural parameters mentioned above are presented in the Table **4**.

<Table 4>

From the Table **4** it can be noticed that all investigated compounds meet the empirical criteria 'rule of five' excluded **10** and **12** (criteria for the polar surface area). These results candidate synthesized succinimides for pharmacodynamic phase investigations. Since log P is associated with solubility and permeability of the molecule, this physicochemical parameter is one of the most important in the estimation of the molecule capability of transfer through the cell membranes. The investigated succinimide derivatives are moderately lipophilic compounds, whereby all substituents, excluding strong electron-donating hydroxyl group and the moderate electron-accepting carboxyl group, induce an increase of hydrophobic character compared to the unsubstituted molecule (**4**). Also, the investigated compounds contain a low number of the rotatable bonds (Table **4**), and thus moderate conformational changes due to binding to a receptor are possible. Additionally, it was observed that polar surface area (PSA) is a very important descriptor for drug transport properties, so the molecules which not possess corresponding PSA properties will have difficulties in such important process as its transport. In investigated series, only two molecules did not exhibit this PSA criterion which has been directly reflecting on their activity.

4. Conclusion

In this work, twelve new 1-aryl-3-ethyl-3-methylpyrrolidine-2,5-diones were synthesized in order to characterize and study the influence of electronic effects of substituents in 4-position on the phenyl group on their antimicrobial and antifungal activity. It can be concluded that tested compounds showed higher antifungal potential than antibacterial effect and reacted against almost all fungal species. Exactly these obtained antimicrobial results candidate the investigated compounds (**1**-**12**) as promising antifungal agents, whereby compound **8** have to be specially highlighted due to its high activity against all investigated fungus even in compare to commercially available drugs.

With the help of density functional theory (DFT) calculations, the transmission of substituent effects through the molecular skeletion and the nature of the HOMO and LUMO orbitals were explained. We have demonstrated that substituents on the phenyl group significantly change the conjugation effect and further affect the intramolecular charge transfer (ICT) character of the investigated succinimides. The molecular electrostatic potential (MEP) map was analyzed for the optimized geometry of 1-phenyl-3-ethyl-3-methylpyrrolidine-2,5-dione (**4**) and 1-(4-bromophenyl)-3-ethyl-3-methylpyrrolidine-2,5-dione

(**8**). The obtained results suggested that structure of compound **8** is very important for its biological recognition and therefore a higher antifungal activity. The data presented in this investigation afford guidelines for the preparation of new succinimides with greater antimicrobial activity.

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Supplementary Data

Additional Supporting Information may be found in the online version of this article.

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Figure Captions

Figure 1. The most stable conformer of compound **4** calculated by B3LYP level of theory.

Figure 2. The HOMO and LUMO molecular orbitals with their energies and the energy gaps for compounds **2**, **4**, **8** and **12** in vacuum.

Figure 3. Molecular electrostatic potential map of 1-phenyl-3-ethyl-3-methylpyrrolidine-2,5-dione (**4**) and 1-(4-bromophenyl)-3-ethyl-3-methylpyrrolidine-2,5-dione (**8**) in vacuum from B3LYP/6-311G(d,p) calculation ploted on isosurfaces of 0.002 a.u. electron density); color coding: red (very negative), orange (negative), yellow (slightly negative), green (neutral), turquoise (slightly positive), light blue (positive), dark blue (very positive).

Scheme 1. The synthetic route of novel 1-aryl-3-ethyl-3-methylsuccinimides (**1**-**12**).

Table 1. MIC/MBC activity of investigated succinimides. Values expressed in mg/ml (10% DMSO).

No.	*Staphyl. aureus*	*Bacillus cereus*	*Micrococcus flavus*	*Listeria monocytogenes*	*Pseudomonas aeruginosa*	*Enterobacter cloacae*	*Salmonella typhimurium*	*Escherichia coli*
1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2	0.125/0.25	0.125/0.25	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5	n.a.
3	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5	0.125/0.5	0.125/0.25	n.a.	n.a.
4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
7	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
8	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
9	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
10	n.a.	n.a.	n.a.	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5	n.a.
11	0.25/0.5	n.a.	n.a.	0.25/0.5	n.a.	n.a.	n.a.	n.a.
12	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Streptomycin	0.05/0.1	0.1/0.2	0.2/0.3	0.2/0.3	0.2/0.3	0.3/0.5	0.2/0.3	0.2/0.3
Ampicillin	0.3/0.4	0.3/0.4	0.3/0.4	0.4/0.5	0.8/1.25	0.4/0.8	0.3/0.5	0.3/0.5

*n.a. – no activity

Table 2. MIC/MFC activity of investigated succinimides. Values expressed in mg/ml (10% DMSO).

No.	*Aspergillus flavus*	*Aspergillus ochraceus*	*Aspergillus versicolor*	*Aspergillus niger*	*Penicillium ochrochloron*	*Penicillium funiculosum*	*Penicillium verr.var.cycl.*	*Trichoderma viride*
1	0.063/0.125	0.063/0.125	0.125/0.25	0.125/0.25	0.25/0.5	0.25/0.5	n.a.	0.25/0.5
2	0.125/0.25	0.125/0.25	0.125/0.25	0.25/0.5	0.25/0.5	0.25/0.5	0.125/0.25	0.125/0.25
3	0.125/0.25	0.25/0.5	0.25/0.5	n.a.	0.25/0.5	n.a.	n.a.	0.25/0.5
4	0.25/0.5	0.25/0.5	0.125/0.25	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5
5	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5	0.125/0.25	0.25/0.5
6	0.125/0.25	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5	0.5/0.5	0.25/0.5
7	n.a.	0.25/0.5	0.25/0.5	0.125/0.25	0.063/0.125	n.a.	n.a.	0.25/0.5
8	0.125/0.25	0.125/0.25	0.125/0.25	0.125/0.25	0.063/0.125	0.25/0.5	0.063/0.125	0.032/0.063
9	0.25/0.5	0.25/0.5	0.125/0.25	0.125/0.25	0.125/0.25	0.25/0.5	0.25/0.5	0.25/0.5
10	0.063/0.125	0.125/0.25	0.063/0.125	0.25/0.5	0.25/0.5	n.a.	n.a.	n.a.
11	0.25/0.5	0.25/0.5	0.25/0.5	0.5/0.5	n.a.	0.25/0.5	0.25/0.5	0.25/0.5
12	0.25/0.5	0.25/0.5	0.125/0.25	0.25/0.5	0.25/0.5	n.a.	0.25/0.5	0.25/0.5
Ketoconazole	0.20/0.50	0.15/0.20	0.20/0.50	0.20/0.50	1.00/1.50	0.20/0.50	0.20/0.30	1.00/1.50
Bifonazole	0.15/0.20	0.15/0.20	0.10/0.20	0.15/0.20	0.20/0.25	0.20/0.25	0.10/0.20	0.15/0.20

*n.a. – no activity

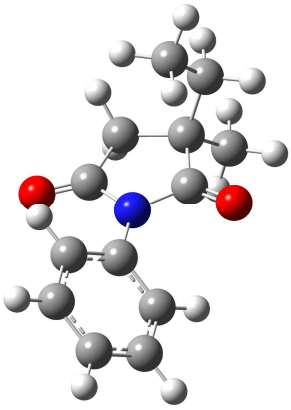
Table 3. The energies of HOMO (E_{HOMO}) and LUMO (E_{LUMO}) molecular orbital, the energy gaps (E_{gap}) in eV, torsion angle θ in ° and dipole moments (μ) in Debye for investigated compounds in vacuum and ethanol as solvent.

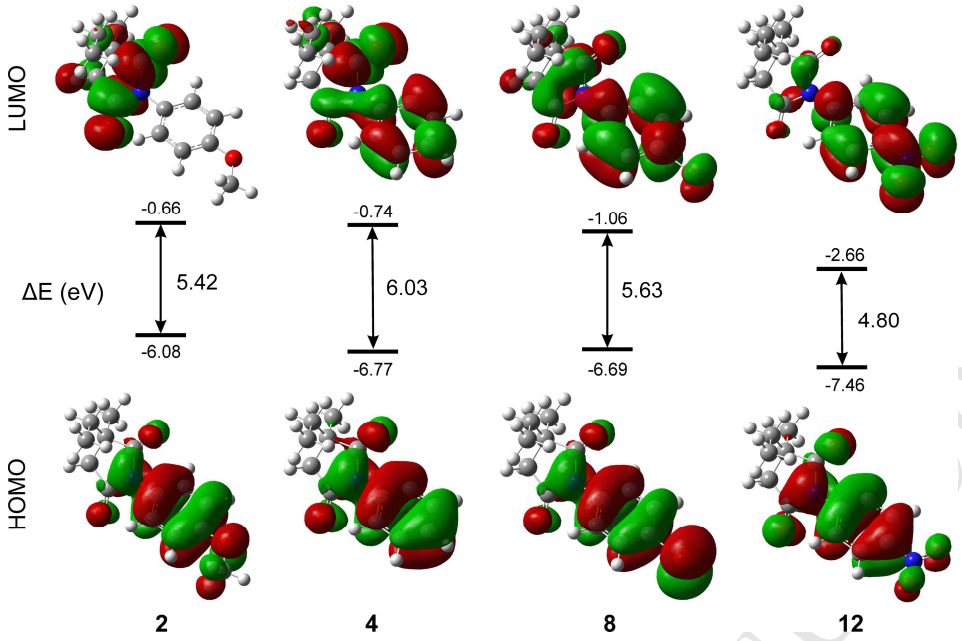
No.	R	Vacuum					Ethanol				
		E_{HOMO}	E_{LUMO}	E_{gap}	θ	μ	E_{HOMO}	E_{LUMO}	E_{gap}	θ	μ
1	4-OH	-6.18	-0.70	5.48	43.65	2.58	-6.48	-0.79	5.69	65.72	2.38
2	4-OCH_3	-6.08	-0.66	5.42	44.23	1.81	-6.39	-0.78	5.61	64.44	1.93
3	4-CH_3	-6.52	-0.69	5.83	43.67	1.36	-6.86	-0.79	6.07	63.56	1.35
4	H	-6.77	-0.74	6.03	43.91	1.84	-7.10	-0.82	6.28	61.56	2.02
5	4-F	-6.74	-0.86	5.88	42.59	3.97	-7.01	-0.86	6.15	59.21	3.97
6	4-Cl	-6.77	-1.04	5.72	40.94	4.52	-6.98	-1.09	5.89	53.38	4.70
7	3-Cl	-6.90	-1.07	5.83	41.51	3.84	-7.11	-1.12	5.99	53.26	4.19
8	4-Br	-6.69	-1.06	5.63	40.88	4.05	-6.91	-1.11	5.80	53.46	4.54
9	3-Br	-6.81	-1.06	5.75	41.80	3.44	-7.03	-1.11	5.92	55.56	4.07
10	4-COOH	-7.06	-1.74	5.32	40.65	4.18	-7.25	-1.88	5.37	50.77	4.58
11	4-CN	-7.20	-1.86	5.33	39.02	7.03	-7.28	-1.91	5.37	48.61	8.37
12	4-NO_2	-7.46	-2.66	4.80	38.63	7.91	-7.47	-2.88	4.59	47.29	8.52

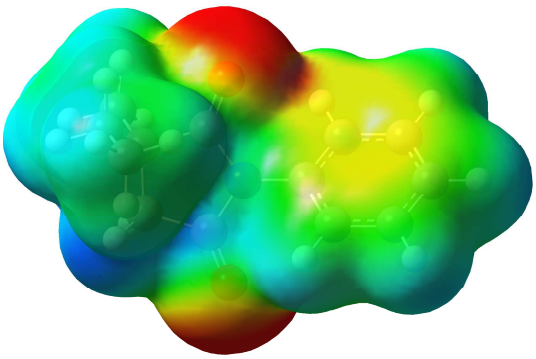
Table 4. Evaluation of drug candidates.

No.	Molecular weigh	log *P*	Hydrogen bonds		Rotatable bonds	Polar surface area/Å^2	Molar refractivity/Å^3
			Donors[a]	Acceptors[b]			
1	233.26	2.64	1	4	2	116.82	60.74
2	247.29	2.75	0	4	3	77.92	65.40
3	231.29	3.31	0	3	2	60.49	64.85
4	217.26	3.03	0	3	2	59.72	58.95
5	235.25	3.19	0	3	2	59.86	59.45
6	251.71	3.59	0	3	2	59.03	63.06
7	251.71	3.59	0	3	2	60.57	63.06
8	296.16	3.86	0	3	2	60.02	66.48
9	296.16	3.86	0	3	2	59.16	66.48
10	261.27	2.59	1	5	3	149.92	65.58
11	242.27	3.17	0	4	2	117.38	66.74
12	262.26	3.06	0	6	3	152.50	66.45
Ideal compound	<500	<5	<5	<10	<8	<140	40-130

[a] A donor indicates any O–H or N–H group.
[b] An acceptor indicates any O or N including those in donor groups.







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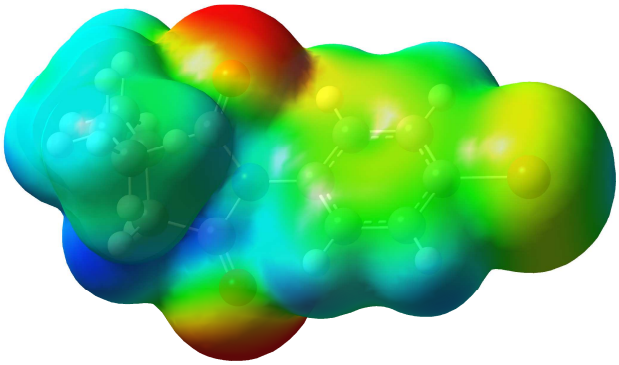

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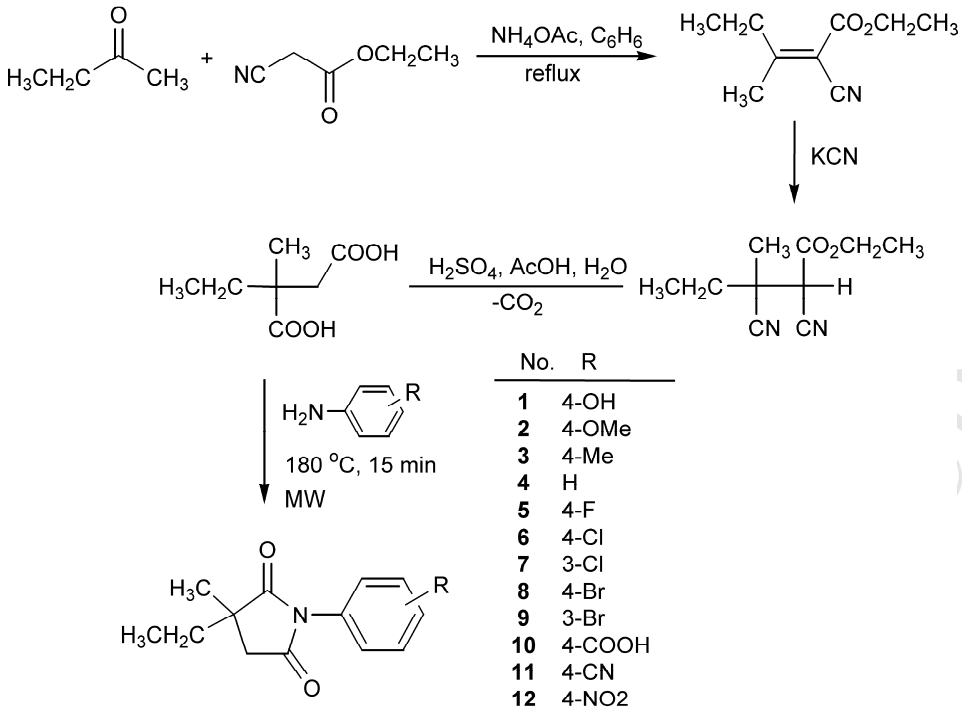

No.	R
1	4-OH
2	4-OMe
3	4-Me
4	H
5	4-F
6	4-Cl
7	3-Cl
8	4-Br
9	3-Br
10	4-COOH
11	4-CN
12	4-NO2

Highlights

1. The twelve newly synthesized succinimides were evaluated for antimicrobal activity.

2. Tested compounds showed higher antifungal potential than antibacterial effect.

3. The investigated bromo derivative was recognized as novel potential fungicide.

4. DFT calculations were performed in order to study SAR and to predict chemical activity of molecules.

5. Ten investigated compounds are candidates for pharmacodynamic phase investigations.