Digest Journal of Nanomaterials and Biostructures Vol. 9, No. 4, October - December 2014, p. 1537 - 1546

SYNTHESIS, QSAR ANALYSIS AND MECHANISM OF ANTYBACTERIAL ACTIVITY OF SIMPLE 2′-HYDROXY CHALCONES

J. BASIC[a], M. KALINIC[b], B. IVKOVIC[b*], S. ERIC[b], M. MILENKOVIC[c], S. VLADIMIROV[b], Z. VUJIC[b]

[a]*Highly-Health School of Professional Studies in Belgrade, Zemun*

[b]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade, , 450 Vojvode Stepe, 11221 Belgrade, Serbia*

[c]*Department of Microbiology and Immunology, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, Belgrade, Serbia*

Twelve 2′-hydroxy chalcones were synthesized and their *in vitro* antibacterial activity was tested using eight standard strains of bacteria: *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *B. subtilis* (ATCC 6633), *M. luteus* (ATCC 10240), *M. flavus* (ATCC 9341), *E. faecalis* (ATCC 29212), *K. pneumoniae* (NCIMB 9111) and *P. aeruginosa* (ATCC 27853). All 2′-hydroxy chalcones have shown moderate to good antimicrobial activity, determined by microdilution method. QSAR analysis was performed for all the cases, R^2 = 0.918 - 0.997. The results of our QSAR analysis indicate that an alternative and complementary mechanism of action is a major determinant of 2'-hydroxy chalcone antibacterial efficiency. These chalcone derivatives posses the ability to act as bidendate chelating agents whereby the ketone moiety forms a coordinate bond and the 2'-hydroxy group forms a covalent bond with a corresponding metal ion. Chelate formation can disrupt the function of bacterial metalloproteins wich may affect the further growth of bacterial cells.

(Received August 22, 2014; Accepted November 26, 2014)

Keywords: Synthesis, Chalcones **,** QSAR, antybacterial activity

1. Introduction

Chalcones are one of the major classes of natural products with widespread distribution in edible plants. Chemically they consist of an open-chain flavonoid (1,3-diaryl-2-propen-1-one) skeleton in which the two aromatic rings are joined by a three carbon α,β- unsaturated carbonyl system [1]. Chalcones are known to display a large number of different biological activities such as anti-inflammatory [2], antibacterial [2–4], antifungal [5,6], antiviral [7], antiproliferative [8], tuberculostatic [9], antimalarial [10] etc. An-easy-to synthesize template from differently substituted acetophenones and benzaldehydes makes chalones an attractive drug scaffold.

The antimicrobial activity of chalcones has been studied by several groups and is believed to present significant therapeutic potential that is still under-explored [11–13]. As demonstrated by the combinatorial synthesis and antibacterial evaluation of 120 chalcones [4], different substitution patterns of the two aromatic rings result in a wide range of inhibitory activities – from negligible to highly potent. Several ways of rationalizing the antimicrobial activity of chalcones have been proposed so far. In part, this activity can be attributed to the presence of phenolic groups, which have high affinity for proteins and thus may be responsible for the inhibition of bacterial enzymes [14]. Furthermore, antibacterial effects have been related to the ability of the α,β-unsaturated ketone to undergo a conjugated addition to a nucleophylic group like a thiol group in an essential protein [2].

*Corresponding author: blucic@pharmacy.bg.ac.rs

Due to the rapid development of bacterial resistance to antibacterial agents, it is vital to discover novel scaffolds for the design and synthesis of new low-molecular antibacterial agents to help in the battle against resistant pathogenic microorganisms. In the present study, twelve simple 2′-hydroxychalcones bearing mono substituent in their ring B (hydroxyl, methyl, metoxy, chloro, fluoro and trifluoromethyl) were synthesized by Claisen-Schmidt method and evaluated for their antibacterial activity *in vitro,* against six Gram-positive and two Gram-negative bacterial strains. Quantitative structure-activity relationship (QSAR) analysis of the evaluated chalcones was performed for better understanding of their mechanism of antibacterial action, using simple and interpretable molecular descriptors.

The choice of the synthesised chalcones was made since 2′-hydroxyl group, a very common feature of natural chalcones, is always present in the active compounds and may be considered a crucial group for the structure stability [14]. Moreover, the importance of 2′-hydroxy halcones is also emphasized by the fact that they are intermediates in the synthesis and biosynthesis of several types of flavonoids [1].

2. Experimental

2.1 Instruments

The melting points of the products were determined on a Kofler melting point apparatus. The IR spectra, expressed in cm^{-1}, were recorded on a Nicolet 6700 FT instrument using ATR technique. The NMR spectra were recorded on a Varian Gemini 200, ^{1}H NMR at 200 MHz, ^{13}C NMR at 50 MHz, for samples in deuterated chloroform. Chemical shifts are expressed in ppm using tetramethylsilane as the internal standard; coupling constants (*J)* are in Hz. Splitting patterns are described as singlet (s), doublet (d), triplet (t) and multiplet (m).

ESI-TOF analysis of the chalcones was carried out on a 6210 time-of-flight LC/MS system (G1969A, Agilent Technology, Santa Clara, CA, United States). The mobile phase was a 50:50 mixture of solvent A (0.2% formic acid in distilled water) and solvent B (acetonitrile) at a flow-rate of 0.2 ml min^{-1}. The mass spectrometer was run in positive or negative electron spray ionisation (ESI) mode with the mass/charge (m/z) ratio in the range 100–2500 m/z. The sample was introduced *via* a 1200 Series HPLC system (Agilent Technologies). Agilent MassHunter Workstation software was used for data acquisition.

2.2 Chemicals

All the used chemicals: 2-hydroxyacetophenone, differently substituted benzaldehide, methanol, sodium hydroxide, toluene, dichloromethane, ethanol are analytical grade and commercially available from Sigma-Aldrich (Steinheim, Germany) and Merck (Hohenbrunn, Germany). The procedure for the synthesis of chalcones 1, 3, 7, 9, 11 and 12 has been described previously in greater detail [15]. The reaction was monitored by TLC on silicagel $60F_{254}$ using toluene or ciclohexane-etylacetate (4:1) as eluent.

2.3 General procedure for the synthesis of chalcones (2, 8 and 10)

2-hydroxyacetophenone (1.4 g) and substituted benzaldehyde (1.2 g) were dissolved in 96% ethanol (10 mL) with stirring. Solution of sodium hydroxide (30%, 20 g) in water was added in portions to give a blood-red solution that was stirred overnight during which chalcones precipitated as the sodium salts. The solution/suspension was kept 24 h at 0 ºC with occasional shaking, diluted with ice water and acidified with cold 1M HCl (pH about 3). The resulting yellow precipitate was filtered, washed with water (to neutral reaction) and crude mixtures were purified by flash column chromatography with toluene as eluent. After removal of toluene under vacuum the crude products were precrystallized from ethanol.

2.4 General procedure for the synthesis of chalcones (4-6)

2-hydroxyacetophenone (1.4 g) and substituted benzaldehyde (1.2 g) were dissolved in 96% ethanol (10 mL) with stirring. Solution of potassium hydroxide (30%, 20 g) in water was added in portions to give a blood-red solution that was stirred overnight during which chalcones remained as orange-red solutions. The solution was kept 24 h at 0º C with occasional shaking, diluted with ice water and acidified with cold 1M HCl (pH about 3). The resulting mixture was extracted with dichloromethane three times. The combined organic layers were dried with anhydrous sodium-sulfate and evaporated under vacuum. The crude products were purified by flash column chromatography with ciclohexane-ethylacetate (4:1) as eluent. Finally, eluent was removed under vacuum.

Chalcone 2: (*E*)-3-(3-fluorophenyl)-1-(2-hydroxyphenyl) prop-2-en-1-one

IR (ATR) (cm^{-1}): $\overline{\nu}$ = 1644.8, 1583.5, 1486.3, 1449.4, 1439.4, 1367, 1346.7, 1319, 1288, 1221.4, 1147.8, 1023.6, 1001, 968.8, 860.9, 847, 801.5, 750.9, 663.3; **^{1}H NMR data (δ, ppm):**12.72 (s, 1H), 7.93 (dd, 1H, J_1=8.20, J_2=1.60), 7.90 (d, 1H, J=15.40), 7.67 (d, 1H, J=15.44), 7.56-7.26 (m, 4H, ArH), 7.18-6.91 (m, 3H, ArH); **^{13}C NMR data (δ,** ppm):193.45; 165.52; 163.63; 160.61; 143.91; 143.86; 136.90; 136.63; 130.68; 130.51; 129.66; 124.79; 124.74; 121.37; 119.88; 118.93; 118.68; 117.93; 117.51; 114.84; 114.40; **HRMS:** Measured mass for [M-H]- is 241.06673, calculated mass for $C_{15}H_{10}FO_2$ is 241.06703.

Chalcone 4: *(E)*-1-(2-hydroxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one

IR (ATR) (cm^{-1}): $\overline{\nu}$ = 3155.8, 1665.6, 1594.2, 1451.4, 1385.7, 1314.4, 1282.7, 1215.6, 1156.7, 1112.7, 858.1, 830.8, 788.4, 701.7, 641.1, 601.9; **^{1}H NMR data (δ, ppm):** 12.99 (s, 1H), 7.96-7.80 (m, 4H, a, b, ArH), 7.61-7.46 (m, 2H, ArH), 7.05-6.90 (m,4H, ArH); **^{13}C NMR data (δ, ppm**):191.54; 161.92; 145.59; 136.28; 132.28; 130.84); 129.66; 129.55; 119.80, 118.57; 117. 49; 116.07; **HRMS:** Measured mass for [M-H]- is 239.07149, calculated mass for $C_{15}H_{11}O_3$ is 239.07137.

Chalcone 5: *(E)*- 1-(2-hydroxy-phenyl)-3-(3-hydroxy-phenyl)- -prop-2-en-1-one

IR (ATR) (cm^{-1}): $\overline{\nu}$ = 3355.3, 1636.8, 1580.1, 1488.2, 1446.3, 1344.6, 1262.7, 1153, 1029.6, 971.4, 856.9, 817.6, 741.3, 664.5, 621**; ^{1}H NMR data (δ, ppm):** 12.79 (s, 1H), 7.94 (dd, 1H, J_1=7.86, J_2=1.40), 7.90 (d, 1H, J=15.44), , 7.66 (d, 1H, J=15.44), 7.59-7.47 (m, 1H, ArH), 7.36-7.22 (m, 2H, ArH), 7.06-6.89 (m, 4H, ArH); 5.09 (s, 1H); **^{13}C NMR data (δ, ppm):**193.76; 163.59; 156.04; 145.06; 136.52; 136.23; 130.29; 129.67; 121.59; 120. 55; **HRMS:** Measured mass for [M-H]- is 239.07188, calculated mass for $C_{15}H_{11}O_3$ is 239.07137.

Chalcone 6: *(E)*- 1,3-*Bis*-(2-hydroxy-phenyl)-prop-2-en-1-one

IR (ATR) (cm^{-1}): $\overline{\nu}$ = 3262.3, 1626, 1557.6, 1487.4, 1458.7, 1338.5, 1302.3, 1260.9, 1229.6, 1196.7, 1152.4, 1091.6, 1021.8, 987.8, 863.1, 822.9, 787.9, 742.8, 723.7, 660.6; **^{1}H NMR data (δ, ppm):** 12.92 (s, 1H), 8.24 (d, J=15.72), 7.97 (dd, J_1=8.14, J_2=1.40), 7.89 (d, J=15.73), 7.64-7.46 (m, 2H, ArH), 7.35-7.26 (m, 1H, ArH), 7.05-6.84 (m, 4H, ArH), 5.63 (s, 1H); **^{13}C NMR data (δ, ppm):**194.49; 163.58; 155.55; 140.99; 136.34; 132.06; 130.22; 129.84; 122.06; 121.30; 121.23; 120.17; 118.88; 118.59; 116.53; **HRMS:** Measured mass for [M-H]- is 239.07113, calculated mass for $C_{15}H_{11}O_3$ is 239.07137.

Chalcone 8: *(E)*-1-(2-hydroxyphenyl)-3-m-tolylprop-2-en-1-one

IR (ATR) (cm^{-1}): $\overline{\nu}$= 3015.3, 2919.4, 1641.6, 1573.8, 1485.7, 1439.8, 1362.8, 1234.2, 1200, 1154, 1026, 973.6, 858.4, 819, 759.1, 665.1; **^{1}H NMR data (δ, ppm):** 12.85 (s, 1H), 7.94 (d, 1H, J=15.80), 7.96 (dd, 1H, J_1=7.86, J_2=1.68), 7.68 (d, 1H, J=15.20), 7.54-7.46 (m, 1H, ArH), 7.37-6.91 (m, 6H, ArH), 2.41 (s, 3H); **^{13}C NMR data (δ, ppm):**193.80; 163.61; 145.71; 138.74; 136.36; 134.54; 131.82; 129.67; 129.22; 128.93; 125.98; 120.02; 119.84; 118.82; 118.60; 21.30; **HRMS:** Measured mass for [M-H]- is 237.09294, calculated mass for $C_{16}H_{13}O_2$ is 237.09210.

Chalcone10: *(E)*-1-(2-hydroxyphenyl)-3-(2-methoxyphenyl) prop-2-en-1-one

IR (ATR) (cm^{-1}): $\overline{\nu}$ = 1637.1, 1599.4, 1570.5, 14872, 1463.2, 1338.9, 1301.8, 1250.1, 1161.4, 1107.8, 1022.7, 865.5, 804.2, 726.7, 662.8; **^{1}H NMR data (δ, ppm):** 12.97 (s, 1H), 8.27 (d, 1H, J=15.80), 7.95 (dd, 1H, J_1=8.00, J_2=1.80), 7.82 (d, 1H, J=15.60), 7.65 (dd, 1H, J_1=7.40, J_2=1.80), 7.50-7.36 (m, 2H, ArH), 7.04-6.90 (m, 4H, ArH), 3.94 (s, 3H); **^{13}C NMR data (δ, ppm):**194.29; 163.56; 159.01; 141.13; 136.16; 132.22; 129.69; 129.62; 123.54; 120.77; 120.68; 120.15; 118.75; 118.51; 111.27; 55.53; **HRMS:** Measured mass for [M-H]- is 253.08759, calculated mass for $C_{16}H_{13}O_3$ is 253.08702

2.5 Antimicrobial assay

Gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Micrococcus luteus* (ATCC 10240), *Micrococcus flavus* (ATCC 9431), *Enterococcus faecalis* (ATCC 29212) and *Bacillus subtilis* (ATCC 6633); Gram-negative bacteria: *Klebsiella pneumoniae* (NCIMB 9111) and *Pseudomonas aeruginosa* (ATCC 27853) were obtained from the Institute of Virology and Immunology Torlak, Belgrade, Serbia.

Active cultures for experiments were prepared by transferring a loopful of cells from the stock into tubes that contained 10 mL of Müller-Hinton broth (MHB) for the bacteria. After incubation for 24 h at 37 °C and 25 °C, respectively, the cultures were diluted with fresh MHB in order to achieve optical densities corresponding to 2×10^6 colony forming units per millilitre (CFU mL^{-1}) and were then used in inoculums.

The broth microdilution method was used to determine the minimal inhibitory concentration (MIC) following the Clinical and Laboratory Standards Institute guidelines [16]. All tests were performed in MHB. Overnight broth cultures of each strain were prepared and the final concentration in each well was adjusted to 1.5×10^6 CFU mL^{-1} for the bacteria. Compounds were dissolved in 1% dimethylsulfoxide and serial doubling dilutions (over the range 6.25-500 μg mL^{-1}) were prepared, in Müller–Hinton broth for bacteria in a 96-well microtiter plate. In the tests, triphenyltetrazolium chloride (TTC) (Aldrich Chemical Company Inc., USA) was also added to the culture medium as growth indicator. The final concentration of TTC after inoculation was 0.05% The microbial growth was determined by the absorbance at 600 nm using a universal microplate reader after incubation at 37 °C for 24 h for each bacteria. The MIC is defined as the lowest concentration of the compound at which the microorganism does not demonstrate visible growth. The MIC values of the tested chalcones were expressed as mM.

2.6 Computational analysis

2.6.1 Preparation of structures and calculation of descriptors

All structures were drawn using ChemDraw X.X, exported in MOL format and opened in VegaZZ (version 2.4.0) [17]. Hydrogen atoms were added; Gasteiger charges and atom types assigned using the SP4 force field. A 3000 step initial molecular mechanics optimization was carried out using AMMP conjugate gradients algorithm. Subsequently, an AMMP systematic conformational search was conducted with 10 rotation steps for each flexible torsion and a 1000 step conjugate gradients optimization of each generated conformation. The best conformation was finally minimized using the AM1 semi-empirical method implemented in MOPAC 7.1. Minimized structures were merged into a single SDF file that served as input for descriptors calculations. A large number of molecular 2D and 3D descriptors were calculated using the freely-available PaDEL-Descriptor suite (version 2.12) [18] and were used in subsequent QSAR analysis.

2.6.2 Data analysis and QSAR modeling

The initial statistical analyses of measured activity were performed using SPSS. All other calculations on descriptor values and activity data were performed using RapidMiner 5.2 [19, 20]. Namely, the selection of statistically most significant descriptors was carried out by the following process. The PaDEL-Descriptor generated .csv file and activity data were imported into RapidMiner's repository, descriptors with missing and zero values were removed, values were then range normalized, and descriptors whose relative standard deviation did not exceed 5% were excluded. Descriptors most highly correlated to activity were subsequently analyzed.

To further explore the structure-activity relationship, two-parameter equations were established. Continuing from the previously described procedure, highly correlated descriptors (absolute $r > 0.95$) were removed from the pool and selection of two descriptors was performed using a genetic algorithm (GA). Population size per generation was set to 100, GA ran for a maximum of 200 generations, with early stopping after 50 generations if no improvement in performance was achieved. Selection was optimized by evaluating the performance of a multiple linear regression (MLR) model in leave-one-out cross-validation.

3. Results and discussion

3.1 Chemistry

Chalcones used in this study were prepared by Claisen-Schmidt condensation of 2-hydroxylacetophenon and differently substituted benzaldehydes, as illustrated in Figure 1.

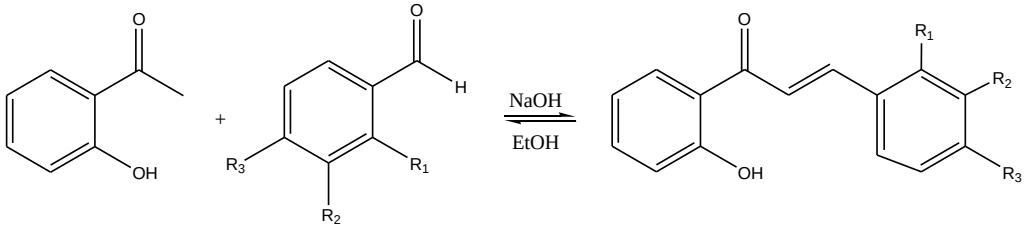
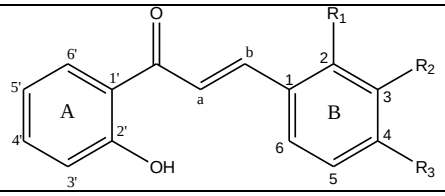


Fig. 1. Synthesis of investigated 2′-hydroxy chalcones

The purity of the compounds was checked using HPLC and TLC methods. After purification, chalcones were obtained in 50.62 - 98.82% yield. The structures of investigated chalcones are presented in Table 1. All compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, and ESI-TOF analysis. The spectral data for six compounds (1, 3, 7, 9, 11, 12) were previously reported by Ivkovic et al [15].

Table 1. The structure and physicochemical properties of investigated 2′-hydroxylchalcones



N^{0}	R_1	R_2	R_3	Mol. formula	Mol .wt	mp/C°	Yield %
1	H	H	F	$C_{15}H_{11}FO_2$	242.25	84-88	55.96
2	H	F	H	$C_{15}H_{11}FO_2$	242.25	107-108	98.92
3	F	H	H	$C_{15}H_{11}FO_2$	242.25	82-83	83.30
4	H	H	OH	$C_{15}H_{12}O_3$	240.25	138-139	50.91
5	H	OH	H	$C_{15}H_{12}O_3$	240.25	132	50.62
6	OH	H	H	$C_{15}H_{12}O_3$	240.25	166	71.59
7	H	H	CH_3	$C_{16}H_{14}O_2$	238.28	117-118	93.79
8	H	CH_3	H	$C_{16}H_{14}O_2$	238.28	117-119	95.00
9	CH_3	H	H	$C_{16}H_{14}O_2$	238.28	153-156	90.79
10	OCH_3	H	H	$C_{16}H_{14}O_3$	254.28	112-114	60.85
11	CF_3	H	H	$C_{16}H_{11}F_3O_2$	292.25	111-114	88.50
12	Cl	H	H	$C_{15}H_{11}ClO_2$	258.70	50-53	82.49

3.2 Antimicrobial activity

Antibacterial activity of chalones is well documented [11–14]. Many research groups reported that isolated chalcones as well as synthesized and modified natural chalcones possess antibacterial activity. We contribute to this field by synthesising twelve low-molecular 2′-hydroxychalcones, some of which can be found in literature and some that are new, and by testing them on different bacterial strains, as summarized in Table 2.

Table 2. Antimicrobial activity of investigated 2′-hydroxychalcones expressed as MIC in mM

Chalcone	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
S. aureus	0.515	0.515	0.515	0.052	0.052	0.052	1.050	1.050	1.050	0.983	0.428	0.483
S. epidermidis	0.258	0.515	0.258	0.052	0.052	0.052	1.050	1.050	1.050	0.983	0.428	0.483
B. subtilis	0.515	0.515	0.515	0.052	0.052	0.052	1.050	1.050	1.050	0.983	0.855	0.483
M. luteus	0.515	0.515	0.515	0.104	0.104	0.104	1.050	1.050	1.050	0.983	0.855	0.966
M. flavus	0.515	0.515	0.515	0.104	0.104	0.104	1.050	1.050	1.050	0.983	0.855	0.483
E. faecalis	1.030	1.030	1.030	0.104	0.104	0.104	2.100	2.100	2.100	1.966	0.855	0.966
K. pneumoniae	1.030	1.030	1.030	0.104	0.104	0.104	1.050	1.050	1.050	0.983	0.855	0.966
P. aeruginosa	1.030	1.030	1.030	0.104	0.104	0.104	1.050	1.050	1.050	0.983	0.855	0.966

Chalcones attained antimicrobial activity with MIC values ranging from 0.052 to 2.10 mM.

According to results in Table 2, it is possible to make comparative efficacy ranking of chalcones on tested bacterial strains:

S. aureus: 4=5=6 > 11> 12 > 1=2=3 > 10 > 7 = 8=9
S. epidermidis: 4=5=6 > 1=3 > 11 > 12 > 2 > 10 > 7= 8=9
B. subtilis: 4=5=6 > 12 >1=2=3 > 11 > 10 >7=8=9
M. luteus: 4=5=6 > 1=2=3 > 11 > 12 > 10 > 7=8=9
M. flavus: 4=5=6 > 12 > 1=2=3 > 11 > 10 >7= 8=9
E. faecalis: 4=5=6 > 11 > 12 > 1=2=3 > 10 > 7=8=9
K. pneumoniae: 4=5=6 > 11 > 12 > 10 > 1=2=3 > 7=8=9
P. aeruginosa: 4=5=6 > 11 > 12 > 10 > 1=2=3 > 7=8=9

Dihyhydroxylated chalcones (Compounds **4**, **5** and **6**) have shown the best antimicrobial activity with MICs values between 0.052 and 0.104 mM. Methylchalcones (Compounds **7, 8** and **9**) have shown the lowest antimicrobial activity with MIC between 1.05 and 2.10 mM. Halogenated chalcones (Compounds **1, 2, 3, 11** and **12**) and *ortho* methoxy chalcones (Compound **10**) showed moderate antimicrobial activity (MIC between 0.428 to 1.966 mM). Activity of trifluoromethylated (**11**) and chlorinated (**12**) chalcones is slightly better compared to the fluorinated chalcones (**1, 2, 3**), which may be caused by both steric and electronic properties of these substituents.

These results are mostly in accordance with previous studies of antimicrobial activity of chalcones, which are tested on the identical bacterial ATCC strains [14]. Certain derivatives showed even better antibacterial activities.

Levene test of homogeneity of variances indicated that the obtained MIC values were heteroscedastic (p=0.034). Correspondingly, MIC values were first logarithmically transformed and one-way analysis of variance (ANOVA) was then conducted. The results indicated that at 0.05 level of significance, no differences existed in the susceptibility of different bacterial strains to the tested chalcones (p=0.651).

Correspondingly, QSAR analysis was conducted using mean inhibitory values, specifically the logarithmically transformed reciprocal MIC values ($\log(1/MIC_{mean})$). Although the tested Gram-negative bacteria were not statistically more resistant than Gram-positive species, as confirmed by contrast analysis (p=0.304), in order to elucidate the observed differences in inhibitory concentrations, separate analyses were also conducted using mean MIC values for Gram-positive and Gram-negative bacteria, respectively.

3.3 QSAR analysis

Statistically most significant descriptors influencing the antimicrobial activity of the studied chalcones are summarized in Tables 3 and 4.

Table 3. Five molecular descriptors most highly correlated to log(1/MIC_{mean}) for all bacterial species tested

Symbol	**Squared correlation coefficient (R^2)**	**Best linear fit (coefficients for range normalized descriptor values)**
ETA_dEpsilon_D	0.990	*log(1/MIC_{mean})* = 1.098 *ETA_dEpsilon_D* - 0.017
SHBd	0.961	*log(1/MIC_{mean})* = 1.088 *SHBd* + 0.040
SHsOH	0.961	*log(1/MIC_{mean})* = 1.088 *SHsOH* + 0.040
MLFER_A	0.956	*log(1/MIC_{mean})* = 1.018 *MLFER_A* + 0.055
SsOH	0.954	*log(1/MIC_{mean})* = 1.046 *SsOH* + 0.051

ETA_dEpsilon_D – Extended topochemical atom descriptor indicating the measure of contribution of hydrogen bond donor atoms; *SHBd* – Sum of electrotopological states (E-States) for strong Hydrogen Bond donors; *SHsOH* – Sum of atom-type hydrogen atom E-State of –OH groups; *MLFER_A* – Overall or summation solute hydrogen bond acidity; *SsOH* – Sum of atom-type E-State of –OH groups.

Table 4. Five molecular descriptors most highly correlated to log(1/MIC_{mean}) for Gram-positive and Gram-negative bacteria, respectively

Gram-positive species		**Gram-negative species**	
Symbol	**Squared correlation coefficient (R^2)**	**Symbol**	**Squared correlation coefficient (R^2)**
ETA_dEpsilon_D	0.970	*MLFER_A*	0.997
SHBd	0.929	*SsOH*	0.996
SHsOH	0.929	*SHBd*	0.994
MLFER_A	0.922	*SHsOH*	0.994
SsOH	0.918	*ETA_dEpsilon_D*	0.989

As can be seen, 5 most significant descriptors all relate to hydrogen-bond donor capabilities of the studied molecules, indicating a strong influence of the acidity of the 2'-phenol group on the observed activities. Although there is some reordering in significance of these closely related descriptors between Gram-positive and Gram-negative species (Table 4), there are no significant differences in the activity determinants suggesting a mechanism of action that is not specific for either Gram type.

The antimicrobial activity of the chalcone scaffold is most frequently attributed to the reactivity of the enone moiety i.e. its affinity for nucleophiles such as sulfhydryl groups found in proteins [2]. Within the series of 2'-hydroxychalcone derivatives presented in this study, systematic variation of electron withdrawing and donating ring B substituents was carried out to study the effects of varying electron density at the enone β-carbon on antimicrobial activity. None of the large number of molecular and atom-type reactivity indices were, however, found to correlate well with the observed differences in antimicrobial activity. Notably, presence of hydroxyl groups in ring B tends to have a minor effect or to decrease the electrophilicity of β-carbon, yet these derivatives were found to be most active. Some authors have proposed that thiol-alkylating reactivity of related compounds is increased by the presence of *o*-hydroxy ring B substituents through a mechanism independent of β-carbon electron density [20]. However, comparable activity was observed in derivatives **4-6**, which suggests that ring B phenol functionalities greatly enhance chalcone antibacterial activity irrespective of their positioning. Similar findings were reported by other authors for antibacterial chalcones [21], as well as for an extensive series of anti-Candidal chalcones [5]. It should be noted that even when QSAR analysis was repeated without these 3 hydroxy analogues, reactivity indices were not found to be

significant predictors of activity (results not shown). Somewhat surprisingly, it would seem that due to extensive electron delocalization, ring B substituent effects influence phenol acidity more considerably than electrophilic reactivity of the enone moiety directly.

The results of our QSAR analysis indicate that an alternative and complementary mechanism of action is a major determinant of 2'-hydroxychalcone antibacterial efficiency. These chalcone derivatives posses the ability to act as bidendate chelating agents whereby the ketone moiety forms a coordinate bond and the 2'-hydroxy group forms a covalent bond with a corresponding metal ion. Previous studies have explored the conformation of the phenolate form of a related 2'-hydroxychalcone in water by means of molecular dynamics simulations [22]. Energetically most favorable conformers seem to posses the stereochemistry necessary for successful complex formation. Thus, the descriptors found to be most important for activity of the studied chalcones can be rationalized in terms of their ability to quantify the acidity of the phenol group which needs to be deprotonated to facilitate complex formation. Chelate formation can disrupt the function of bacterial metalloproteins. Furthermore, *in situ* complex formation with free intracellular metal ions (e.g. Mg^{2+}) can favor electron delocalization and increase the electrophilic reactivity of the resultant chalcone chelate.

Molecular descriptors that were found to correlate with differences in activity between Gram-positive and Gram-negative bacteria, respectively, are listed in Table 5.

Table 5. Five molecular descriptors most highly correlated to the ratio of log(1/MIC_{mean}) values of Gram-negative versus Gram-positive bacteria

Symbol	**Squared correlation coefficient (R^2)**	**Best linear fit (coefficients for range normalized descriptor values)**
apol	0.870	*Ratio* = -0.966 *apol* + 1.714
WA.polar	0.717	*Ratio* = -0.944 *WA.polar* + 1.670
Wlambda2.eneg	0.709	*Ratio* = 0.708 *Wlambda2.eneg* + 0.977
Wnu2.eneg	0.693	*Ratio* = 0.676 *Wnu2.eneg* + 0.985
WA.unity	0.674	*Ratio* = -0.920 *WA.unity* + 1.677

Ratio = log(1/MIC_{mean}(G-))/log(1/MIC_{mean}(G+)); apol – Sum of the atomic polarizabilities (including implicit hydrogens); *WA.polar* – Non-directional WHIM, weighted by atomic polarizabilities; *Wlambda2.eneg* – Directional WHIM, weighted by Mulliken atomic electronegativites; *Wnu2.eneg* – Directional WHIM, weighted by Mulliken atomic electronegativites; *WA.unity* – Non-directional WHIM, weighted by unit weights.

These results suggest that with increasing chalcone polarity susceptibility of Gram-negative bacteria increases, while conversely, increasing electronegativity diminishes chalcone efficiency against Gram-negative species studied. These results can clearly be rationalized in terms of differential permeation into the intracellular environment of the bacteria. Increasing polarity likely facilitates porin-mediated permeation through the outer-membrane of Gram-negative bacteria while increasing electronegativity hinders permeation due to stronger repulsion with the negatively charged membrane constituents such as lipopolysaccharides.

Finally, the GA-based selection of two descriptors offering the best linear relation to antibacterial activity resulted in Equation 1. In addition to the hydrogen-donor capabilities, polarity can be seen to have a weak, negative impact on activity, which is in agreement with good lipophilicity being a prerequisite for chalcone antibacterial efficiency.

QSAR equation representing the best linear fit between chalcone antibacterial activity and two of the available descriptors.

$$\boldsymbol{log(1/MIC_{mean})} = -\,(0.050 \pm 0.011)\,\boldsymbol{apol} + (1.545 \pm 0.059)\,\boldsymbol{SHBd} + (1.128 \pm 0.432)$$

$$R = 0.995,\ R^2 = 0.990,\ F = 448.66$$

t_{apol} = -4.564 (p = 0.001); t_{SHBd} = 26.067 (p < 0.001); $t_{intercept}$ = 2.608 (p = 0.03)

The results of QSAR analysis of 2'-hydroxychalcones can be summarized as follows:

- antibacterial activity is strongly dependent on the hydrogen-donor capabilities of the hydroxy substituent in ring A – increasing phenol acidity favours antibacterial action;
- introduction of additional phenol groups in ring B strongly contributes to activity irrespective of position and despite their electron donating characteristics;
- chalcone polarity and overall electronegativity are important determinants of the susceptibility of Gram-negative bacteria;
- in addition to thiol-alkylation, a likely mechanism of antibacterial action of 2'-hydroxychalco
- nes also involves metal chelation.

4. Conclusion

These results suggest that the simple 2´-hydroxi chalcones showed promising antibacterial activity and that amenable to further improvement guided by the results of the presented QSAR analysis.

Acknowledgements

Financial support from the Serbian Ministry of Science (Grants 172041 and 172009) is greatly appreciated. We also thank the Faculty of Chemistry, Belgrade University for their assistance

The manuscript has been read and approved by all of its authors. The authors are not in conflict of interest and there are no pertinent financial.

References

[1] D.N. Dhar. The chemistry of chalcones and related compounds 1st ed., John Wiley, New York, 1981.
[2] Z. Nowakowska, Eur. J. Med. Chem.**42**, 125 (2007).
[3] P.M. Sivakumar, S. Priya, M.Chem. Biol. Drug Des. **73**, 403 (2009).
[4] F.L. Ansari, S. Nazir, H. Noureen, B.Mirza, Chem. Biodivers. **2**, 1656 (2005).
[5] D. Batovska, S. Parushev, A. Slavova, V. Bankova, I. Tsvetkova, M. Ninova, H.Najdenski, Eur. J. Med. Chem. **42**, 87 (2007).
[6] K.L. Lahtchev, D.I. Batovska, S.P. Parushev, V.M. Ubiyvovk, A.A. Sibirny, Eur. J. Med. Chem. **43**, 2220 (2008).
[7] N. Lall, A.A. Hussein, J.J. Meyer, Fitoterapia **77**, 230 (2006).
[8] A. Boumendjel, J. Boccard, P-A Carrupt, E. Nicolle, M. Blanc, A. Geze, L. Choisnard, D. Wouessidjewe, E-L Matera, C.Dumontet, J. Med. Chem. **51**, 2307 (2008).
[9] Y.M. Lin, Y. Zhou, M.T. Flavin, L.M. Zhou, W. Nie, F.C. Chen, Bioorg. Med. Chem. **10**, 2795 (2002).
[10] M. Liu, P. Wilairat, M.L. Go, J. Med. Chem. **44**, 4443 (2001).
[11] L. Ni, C.Q. Meng, J.A. Sikorski. Expert Opin. Ther. Patents **14**, 1669 (2004).
[12] D.I. Batovska, I.T. Todorova, Curr. Clin. Pharmacol. **5**, 1 (2010).
[13] J.R. Dimmock, D.W. Elias, M.A. Beazely, N.M. Kandepu, Curr. Med. Chem. **6**, 1125 (1999).
[14] H.P. Ávila, Ede F. Smânia, F.D. Monache, A. Jr Smânia, Bioorg. Med. Chem. **16**, 9790 (2008).
[15] B.M. Ivković, K. Nikolic, B.B. Ilić, Ž.S. Žižak, R.B. Novaković, O.A. Čudina, S.M. Vladimirov, Eur. J. Med. Chem. **63**, 239 (2013).
[16] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial

susceptibility testing. Fifteenth informational supplement. CLSI document M100-S15. 2005.
[17] A. Pedretti, L. Villa, G.Vistoli .J. Mol. Graph. Model. **21**, 47 (2002).
[18] C.W.Yap, J. Comput. Chem., **32**, 1466 (2011).
[19] I. Mierswa, M. Wurst, R. Klinkenberg, M. Scholz, T.Euler YALE: rapid prototyping for complex data mining tasks. Proceedings of the 12th ACM SIGKDD international conference on Knowledge discovery and data mining, New York, NY, USA: ACM; 2006, pp. 935–940.
[20] RapidMiner version 5.2. Dortmund, Germany: Rapid-I GmbH; 2012.
[21] A.T. Dinkova-Kostova, M.A. Massiah, R.E. Bozak, R.J. Hicks, P.Talalay, Proc Natl Acad Sci U S A, **98**, 3404 (2001).
[22] C.M. Devia, N.B. Pappano, N.B.Debattista, Revista De Microbiologia, **29**, 307 (1998).
[23] S. Hur, Z.E.Newby, T.C.Bruice, Proc Natl Acad Sci U S A, **101**, 2730 (2004).