Med Chem Res
DOI 10.1007/s00044-016-1609-8

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Ferrocenyl chalcones with *O*-alkylated vanillins: synthesis, spectral characterization, microbiological evaluation, and single-crystal X-ray analysis

Jovana Muškinja[1] · Adrijana Burmudžija[1] · Zoran Ratković[1] · Branislav Ranković[2] · Marijana Kosanić[2] · Goran A. Bogdanović[3] · Slađana B. Novaković[3]

Received: 16 October 2015 / Accepted: 3 June 2016

Abstract *O*-alkylated vanillin derivatives **2a–f** and acetyl ferrocene react under Claisen–Schmidt conditions, resulting in good-to-high yields of the corresponding ferrocene chalcones **3a–f**. None of the resultant compounds **3b–f** has been previously described in the literature. All synthesized compounds were characterized by spectral and physical data, whereas two of them, 1-ferrocenyl-3-(4-ethoxy-3-methoxyphenyl)-prop-2-en-1-one (**3b**) and 1-ferrocenyl-3-(4-buthoxy-3-methoxy-phenyl)-prop-2-en-1-one (**3e**), were crystalline substances, suitable for single-crystal X-ray analysis, which confirmed undoubtedly their structures. Chalcones **3a–f** were tested for their biological activity and demonstrated relatively good in vitro antimicrobial activity towards different strains of bacteria and fungi. The best antibacterial activity is expressed by compounds **3b** and **3c**, while compound **3d** shows the best antifungal activity.

Keywords Vanillin · Ferrocene · Ferrocenyl chalcones · Microbial activity · Crystal structure

Electronic supplementary material The online version of this article (doi: 10.1007/s00044-016-1609-8) contains supplementary material, which is available to authorized users.

✉ Zoran Ratković
wor@kg.ac.rs

[1] Department of Chemistry, Faculty of Science, University of Kragujevac, Kragujevac, Serbia

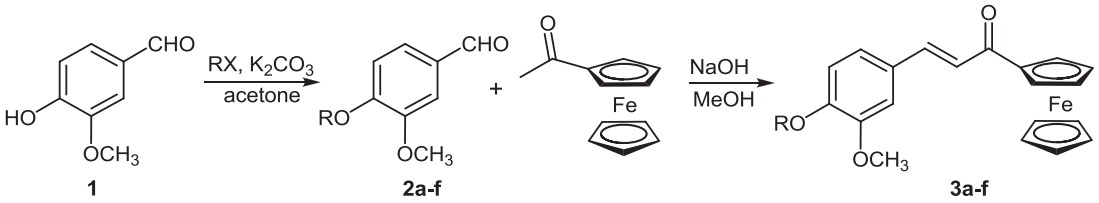
[2] Department of Biology, Faculty of Science, University of Kragujevac, Kragujevac, Serbia

[3] Vinča Institute of Nuclear Sciences, Laboratory of Theoretical Physics and Condensed Matter Physics, University of Belgrade, Belgrade, Serbia

Introduction

Chalcones are an important class of organic compounds since they often represent the core structure of many natural products and pharmaceuticals. Chalcones (1,3-diaryl-2-propen-1-ones) and their derivatives exhibit different pharmacological and biological activities, the major ones being antifungal (Bag et al., 2009; Lahtchev et al., 2008; Mostahar et al., 2007), antimicrobial (Yayli et al., 2006; Trivedi et al., 2008; Sivakumar et al., 2010; Opletalova, 2000; Katade et al., 2008), anticonvulsant (Kaushik et al., 2010), antioxidant (Vasilev et al., 2010, Sivakumar et al., 2010; Vogel et al., 2008, Cheng et al., 2008), antiprotozoal (Lunardi et al., 2003), antitrichomonal (Oyedapo et al., 2004), antimalarial (Motta et al., 2006; Awasthi et al., 2009; Lim et al., 2007; Wu et al., 2002; Liu et al., 2001), anti-inflammatory (Yadav et al., 2010; Zhang et al., 2010; Herencia et al., 1998; Nowakowska 2007), trypsin inhibition (Maliar et al., 2004) and anti-cancer activity (Achanta et al., 2006; Romagnoli et al., 2008; Echeverria et al., 2009; Szliszka et al., 2009; Ilango et al., 2010; Wattenberg et al., 1994; Edwards et al., 1988; Kumar et al., 2011; Seo et al., 2010).

Different research groups are involved with the aim to identify the fragment in chalcones molecules, synthesized or modified natural ones, responsible for previously described activities. Tsukiyama et al. (2002) reported the potent antibacterial activity of licochalcone A and licochalcone B. Following these results, it was estimated that free phenolic group in the ring at position 4 was the key factor responsible for their antibacterial activity (Chen et al., 1997). Starting from the fact that natural products, such as curcumines, show various bioactivities (Chan, 1995; Mishra et al., 2005; Dubey et al., 2008), and vanillin fragment is present in these molecules, we supposed that vanillin is a suitable aldehyde for chalcone formation due to its easy



Scheme 1 Synthesis of ferrocenyl chalcones

modification by *O*-alkylation (Loev et al., 1956, Tatsuzaki et al., 2006, Katritzky et al., 2006), by coupling reactions and forming of divanillin (Nishimura et al., 2010), formyla tions in position 5 (Blažević et al., 1979), and halogenation in position 5 (Pepper and MacDonald, 1953). On the other hand, enone system is the key part of substrates and could be easily converted into various heterocyclic derivatives (Abdel-Rahman et al., 2007, Kalirajan et al., 2009).

Ferrocenyl derivatives are among the most promising compounds, which can be used in microbiological research. The water-soluble ferrocenyl derivatives are more potent as drugs than the water-insoluble ones. In our previous work we had reported on the synthesis of different ferrocene derivatives with expressed biological activities (Damljanović et al., 2009; Damljanović et al., 2009; Ratković et al., 2010) and we expected that incorporation of vanillin pharmacophore and the ferrocene scaffold into the same molecule might have an attractive structural result for the development of novel antimicrobial agents, expecting the interesting features due to the coexistence of two kinds of promising pharmacophores.

In the present paper we wish to report on synthesis, spectral characterization and evaluation of antimicrobial activity of a series of novel chalcone derivatives containing ferrocene unit, prepared from *O*-alkylated vanillines and acetylferrocene, as well as single-crystal X-ray analysis for two of them.

Experimental

Material and methods

All the chemicals used were commercially available and used as received, except that the solvents were purified by distillation. Chromatographic separations were carried out using silica gel 60 (Merck, 230-400 mesh ASTM), whereas silica gel on Al plates, layer thickness 0.2 mm (Merck), was used for TLC. IR spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer with a KBr disc. NMR spectra were recorded on a Varian Gemini (200 MHz) spectrometer using $CDCl_3$ as the solvent and TMS as the internal standard.

Elemental microanalyses for C, H, and N were performed by standard methods on a Vario III CHNS Elemental Analyzer, Elemental Analysensysteme GmbH.

Synthesis

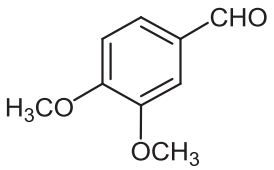
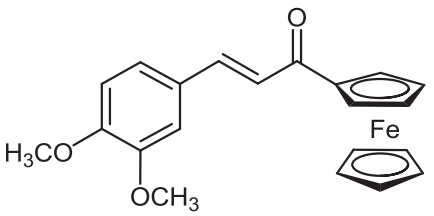
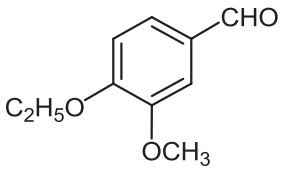
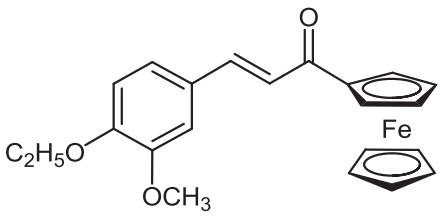
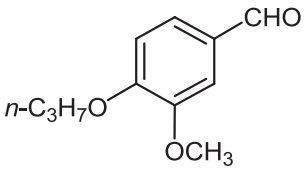
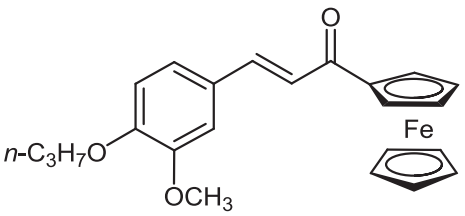
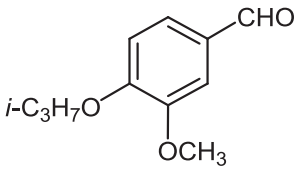
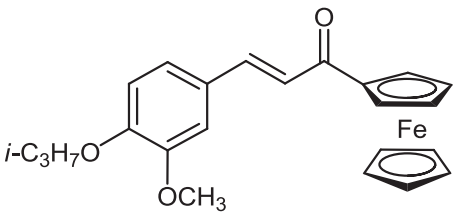
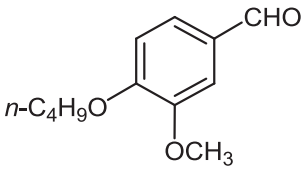
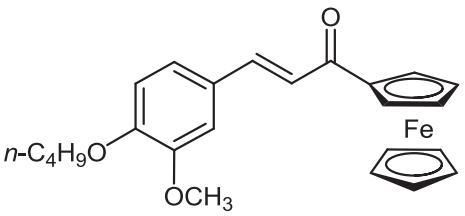
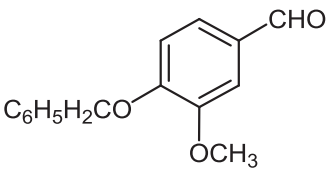
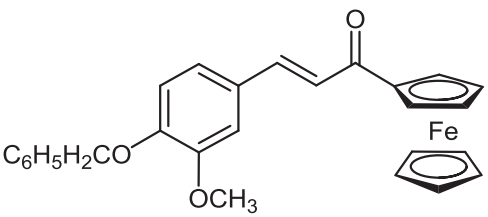
Keeping in mind the reactivity of aldehyde functional group, the synthesis of ferrocene containing chalcones was carried out as a simple Claisen–Schmidt condensation of *O*-alkylated vanilline derivatives and acetylferrocene. A set of *O*-alkyl derivatives **2a–f** (Scheme 1) was prepared by alkylation free phenolic group of vanillin **1** with corresponding alkyl halides, according to the described literature procedures (Loev and Dawson, 1956; Tatsuzaki et al., 2006; Katritzky et al., 2006). Their spectral data were listed in another paper (Ratković et al., 2016), describing synthesis and biological activity of vanillin-based 1-acetyl-5-aryl-4,5-dihydro-1H-pyrazoles.

In the next step, aldehydes **2a-f** were subjected to the reaction with acetylferrocene following the literature procedure (Cheng et al., 2008), and the desired ferrocene containing chalcone products were isolated in 59–85 % yields (Table 1). The detailed analysis of ^{1}H and ^{13}C NMR spectra (see below) revealed the structures of compounds **3a–f** (Scheme 1), whereas the structures of **3b** and **3e** were unambiguously confirmed by the single-crystal X-ray analysis.

General procedure for synthesis of chalcones

The corresponding vanillin aldehyde **2a–f** (10 mmol) and acetylferrocene (10 mmol) were dissolved in 20 mL of warm ethanol, and the mixture was stirred for 10 min and 1 mL of 40 % NaOH was added slowly. The reaction mixture was stirred overnight at 50°C. Crushed ice, 100 g, was placed in a beaker and the reaction mixture was poured onto it with stirring. The product in some cases could be isolated by filtration, if not it was necessary to extract with toluene or dichloromethane (3 ×50 mL). The organic layer was washed with water (2 ×50 mL), brine (2 ×50 mL), and dried over anhydrous Na_2SO_4. The main part of the solvent was evaporated at reduced pressure and crude concentrated solution was filtered through a short column of silica gel. The solvent was evaporated under reduced pressure and the

Table 1 Synthesis of ferrocene derivatives **3a–f**

Entry	Vanillines	Products	Yield (%)[a]
1			78
	2a	**3a**	
2			85
	2b	**3b**	
3			79
	2c	**3c**	
4			59
	2d	**3d**	
5			68
	2e	**3e**	
6			67
	2f	**3f**	

[a] Yields of isolated products based on acetylferrocene

residue was chromatographed on silica gel column using toluene-ethyl acetate (8:2) mixture as eluent for separation of reaction products **3a–f**.

*1-Ferrocenyl-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (**3a**)*

Cinnabar red crystals; m.p. 148–149 °C; yield 78 %; IR (cm^{-1}): 1648, 1586, 1517, 1263, 1081; ^1H NMR (200 MHz, $CDCl_3$): δ 3.95 (d, $J = 6.8$ Hz, 6H), 4.22 (s, 5H), 4.58 (t, $J = 2$Hz, 2H), 4.92 (t, $J = 2$Hz, 2H), 6.91 (d, $J =$ 8.4Hz 1H), 6.99 (d, $J = 15.6$Hz, 1H), 7.14–7.27 (m, 2H), 7.76 (d, $J = 15.6$Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 55.9, 69.7, 70, 72.5, 80.7, 110.6, 111.3, 121, 122.4, 128.2, 140.9, 149.2, 151.1, 192.8 (CO). Anal.: calcd. for $C_{21}H_{20}FeO_3$ (376.227): C, 67.04; H, 5.36; Fe, 14.84; O, 12.76. Found: C, 66.10; H, 5.30.

*1-Ferrocenyl-3-(4-ethoxy-3-methoxyphenyl)prop-2-en-1-one (**3b**)*

Cinnabar red crystals; m.p. 155–156 °C; yield 85 %; IR (cm^{-1}): 1650, 1588, 1513, 1267, 1076; ^1H NMR (200 MHz, $CDCl_3$): δ 1.49 (t, $J = 7$Hz, 3H), 3.96 (s, 3H), 4.16 (q, $J =$ 7.2Hz, 2H), 4.21 (s, 5H), 4.57 (t, $J = 1.8$Hz, 2H), 4.92 (t, $J = 1.8$Hz, 2H), 6.90 (d, $J = 8.4$Hz 1H), 6.99 (d, $J =$ 15.6Hz, 1H), 7.14–7.27 (m, 2H), 7.75 (d, $J = 15.6$Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.7, 56.1, 64.4, 69.6, 70, 72.5, 80.8, 110.9, 112.5, 120.9, 122.4, 128, 141, 149.5, 150.5, 192.9 (CO). Anal.: calcd. for $C_{22}H_{22}FeO_3$ (390.253): C, 67.71; H, 5.68; Fe, 14.31; O, 12.30. Found: C, 66.61; H, 5.61.

*1-Ferrocenyl-3-(3-methoxy-4-propoxyphenyl)prop-2-en-1-one (**3c**)*

Cinnabar red crystals; m.p. 104–105 °C; yield 79 %; IR (cm^{-1}): 1647, 1586, 1515, 1258, 1079; ^1H NMR (200 MHz, $CDCl_3$): δ 1.06 (t, $J = 7.6$Hz, 3H), 1.80–1.98 (m, 2H), 3.95 (s, 3H), 4.03 (t, $J = 6.8$Hz, 2H), 4.21 (s, 5H), 4.57 (t, $J = 1.6$Hz, 2H), 4.92 (t, $J = 2$Hz, 2H), 6.90 (d, $J =$ 8.4Hz 1H), 6.99 (d, $J = 15.6$Hz, 1H), 7.14–7.27 (m, 2H), 7.75 (d, $J = 15.6$Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 10.3, 22.3, 56.2, 69.6, 70, 70.5, 72.5, 80.8, 111.2, 112.7, 120.8, 122.4, 127.9, 141, 149.6, 150.8, 192.8 (CO). Anal.: calcd. for $C_{23}H_{24}FeO_3$ (404.280): C, 68.33; H, 5.98; Fe, 13.81; O, 11.87. Found: C, 67.51; H, 5.93.

*1-Ferrocenyl-3-(4-isopropoxy-3-methoxyphenyl)prop-2-en-1-one (**3d**)*

Cinnabar red crystals; m.p. 114 °C; yield 59 %; IR (cm^{-1}): 1653, 1590, 1508, 1264, 1074; ^1H NMR (200 MHz, $CDCl_3$): δ 1.41 (d, $J = 6.2$Hz, 6H), 3.94 (s, 3H), 4.21 (s, 5H), 4.57 (t, $J = 1.6$Hz, 2H), 4.92 (t, $J = 2$Hz, 2H), 6.92 (d, $J = 8.4$Hz 1H), 6.99 (d, $J = 15.6$Hz, 1H), 7.15–7.26 (m, 2H), 7.75 (d, $J = 15.6$Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 22, 56.2, 69.7, 70, 71.4, 72.5, 80.8, 111.6, 120.9, 122.2, 128.2, 129, 141, 149.6, 150.4, 192.9 (CO). Anal.: calcd. for $C_{23}H_{24}FeO_3$ (404.280): C, 68.33; H, 5.98; Fe, 13.81; O, 11.87. Found: C, 67.31; H, 5.95.

*1-Ferrocenyl-3-(4-butoxy-3-methoxyphenyl)prop-2-en-1-one (**3e**)*

Cinnabar red crystals; m.p. 128 °C; yield 68 %; IR (cm^{-1}): 1651, 1592, 1511, 1264, 1077; ^1H NMR (200 MHz, $CDCl_3$): δ 0.99 (t, $J = 7.4$Hz, 3H), 1.45–1.57 (m, 2H), 1.76–1.89 (m, 2H), 3.95 (s, 3H), 4.08 (t, $J = 6.8$Hz, 2H), 4.21 (s, 5H), 4.57 (t, $J = 2$Hz, 2H), 4.92 (t, $J = 2$Hz, 2H), 6.91 (d, $J = 8.2$Hz 1H), 6.99 (d, $J = 15.6$Hz, 1H), 7.14–7.26 (m, 2H), 7.75 (d, $J = 15.6$Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.8, 31.1, 56.2, 68.7, 69.7, 70, 72.5, 80.8, 111.2, 112.7, 120.8, 122.4, 127.9, 141, 149.6, 150.8, 192.9 (CO). Anal.: calcd. for $C_{24}H_{26}FeO_3$ (418.306): C, 68.91; H, 6.26; Fe, 13.35; O, 11.47. Found: C, 68.16; H, 6.23.

*1-Ferrocenyl-3-(4-benzyloxy-3-methoxyphenyl)prop-2-en-1-one (**3f**)*

Cinnabar red crystals; m.p. 135–136 °C; yield 67 %; IR (cm^{-1}): 1650, 1591, 1515, 1262, 1084; ^1H NMR (200 MHz, $CDCl_3$): 3.97 (s, 3H), 4.20 (s, 5H), 4.57 (t, $J =$ 2Hz, 2H), 4.91 (t, $J = 2$Hz, 2H), 5.21 (s, 2H), 6.91 (d, $J =$ 8.2Hz, 1H), 6.98 (d, $J = 15.6$Hz, 1H), 7.15–7.43 (m, 7H), 7.74 (d, $J = 15.6$Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 56.1, 69.6, 70, 70.9, 72.5, 80.7, 111.3, 113.7, 121.1, 122.1, 127.2, 127.9, 128.5, 128.6, 136.6, 140.9, 149.8, 150.2, 192.8 (CO). Anal.: calcd. for $C_{27}H_{24}FeO_3$ (452.323): C, 71.69; H, 5.35; Fe, 12.35; O, 10. 61. Found: C, 70.61; H, 5.32.

*X-ray crystallography and structural comparisons of **3b** and **3e***

Crystal data and experimental details for **3b** and **3e** compounds are summarized in Table 2. Single-crystal diffraction data were collected at room temperature on an Agilent Gemini S diffractometer equipped with Mo Kα radiation ($\lambda = 0.71073$ Å). Data integration and scaling of the reflections were performed using the CRYSALIS software (Agilent Technologies, 2013) with multi-scan absorption corrections applied using SCALE3 ABSPACK (Agilent Technologies, 2013). Crystal structure was solved by direct methods, using SIR2002 (Burla et al., 2003) and refined using SHELXL97 (Sheldrick, 2008) program both incorporated in WinGX (Farrugia, 1999) program package.

Table 2 Crystallographic data for **3b** and **3e**

Compound	**3b**	**3e**
Empirical formula	$C_{22}H_{22}FeO_3$	$C_{24}H_{26}FeO_3$
Formula weight	390.25	418.30
Temperature (K)	293(2)	293(2)
Wavelength (Å)	0.71073	0.71073
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/c$
Unit cell dimensions		
a (Å)	14.6500(6)	16.1310(6)
b (Å)	7.9537(2)	7.9881(2)
c (Å)	16.7216(5)	17.3283(6)
β (°)	106.086(3)	113.533(4)
V (Å^3)	1872.14(11)	2047.15(12)
Z	4	4
D_{calc} (Mg/m^3)	1.385	1.357
μ (mm^{-1})	0.823	0.758
Crystal size (mm^3)	0.50 × 0.37 × 0.12	0.48 × 0.24 × 0.17
θ range for data collection (°)	3.04–28.93	2.94–29.02
Reflections collected	11443	10841
Independent reflections, R_{int}	4333, 0.0244	4731, 0.0280
Completeness to $\theta = 26°$ (%)	99.8 %	99.8 %
Data/restraints/parameters	4333/0/237	4731/0/255
Goodness-of-fit on F^2	1.076	1.041
Final R_1/wR_2 indices ($I > 2\sigma_I$)	0.0437, 0.0905	0.0397, 0.0827
Final R_1/wR_2 indices (all data)	0.0633, 0.0975	0.0622, 0.0929
Largest differential density peak and hole (e Å^{-3})	0.285 and −0.218	0.285 and −0.306

All non-H atoms were refined anisotropically to convergence. All H atoms were placed at geometrically calculated positions with the C-H distances fixed to 0.93 from Csp^2, and 0.96 and 0.97 Å from methyl and methylene Csp^3, respectively. The corresponding isotropic displacement parameters of the hydrogen atoms were equal to 1.2U_{eq} and 1.5U_{eq} of the parent Csp^2 and Csp^3, respectively. Figures were produced using ORTEP-3 (Farrugia, 1997) and MERCURY, Version 2.4 (Macrae et al., 2006). The software used for the preparation of the materials for publication are WinGX (Farrugia, 1999), PLATON (Spek, 2003) and PARST (Nardelli, 1995).

Antimicrobial activity

The antimicrobial activity was estimated by determining the minimal inhibitory concentration (MIC) using the broth micro dilution method against five species of bacteria and five species of fungi. As a result of the study, the measured MIC values for tested compounds varied from 0.0352 to 0.8873 mg/ml.

The following bacteria were used as test organisms in this study: *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Bacillus cereus* (ATCC 10987), *Escherichia coli* (ATCC 25922), and *Proteus mirabilis* (ATCC 12453). All of the bacteria used were obtained from the American Type Culture Collection (ATCC). The bacterial cultures were maintained on Mueller-Hinton agar substrates (Torlak, Belgrade). The fungi used as test organisms were: *Aspergillus niger* (ATCC 16888), *Candida albicans* (ATCC 10259), *Penicillium italicum* (ATCC 10454), *Mucor mucedo* (ATCC 20094), *Trichoderma viride* (ATCC 13233). All of the fungi were from ATCC. The fungal cultures were maintained on potato dextrose (PD) agar, except for *Candida albicans* that was maintained on Sabourad dextrose (SD) agar (Torlak, Belgrade). All of the cultures were stored at 4 °C and subcultured every 15 days.

Bacterial inoculum were obtained from bacterial cultures incubated for 24 h at 37 °C on Mueller-Hinton agar substrates and brought up to approximately 10^8 CFU/ml by dilution according to the 0.5 McFarland standard. Suspensions of fungal spores were prepared from freshly mature (3- to 7-day-old) cultures that grew at 30 °C on a PD agar substrate. The spores were rinsed with sterile distilled water, used to determine turbidity spectrophotometrically at 530 nm, and were then further diluted to approximately 10^6 CFU/ml according to the procedure recommended by NCCLS (1998).

The MIC was determined by the broth microdilution method using 96-well micro-titer plates (Sarker et al., 2007). A series of dilutions with concentrations ranging from 20 to 0.0195 mg/ml of the tested compounds were used in the experiment against every microorganism tested. The starting solutions with tested compounds were obtained by measuring a certain quantity of the compounds and dissolving it in 5 % DMSO. Two-fold dilutions of the compounds were prepared in a Mueller-Hinton broth for bacterial cultures and a SD broth for fungal cultures. The MIC was determined with resazurin. Resazurin is an oxidation-reduction indicator used for the evaluation of microbial growth. It is a blue non-fluorescent dye that becomes pink and fluorescent when reduced to resorufin by oxidoreductases within viable cells. The boundary dilution without any change in the color of resazurin was defined as the MIC for the tested microorganism at a given concentration. As a positive control of growth inhibition, streptomycin was used in the case of bacteria and ketoconazole in the case of fungi. A 5 % DMSO solution was used as a negative control for the influence of the solvents. Experiment was carried out in triplicate.

Results and Discussion

Spectral characterization

All synthesized compounds were characterized by IR, ^{1}H, and ^{13}C NMR spectral data. In IR spectra of the synthesized

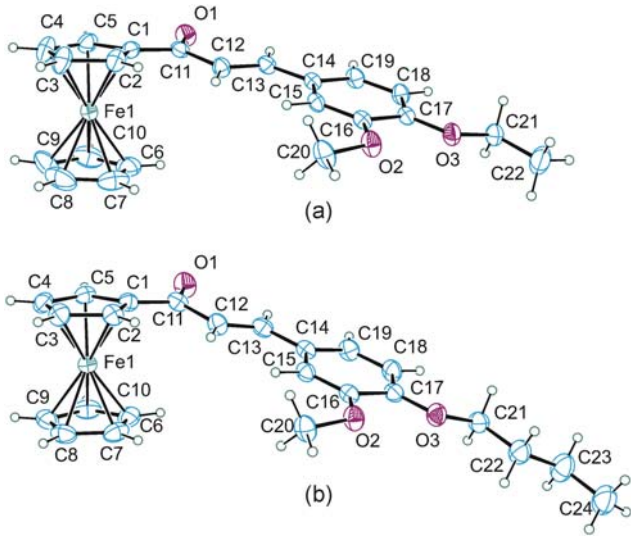


Fig. 1 Molecular structures of **3b** (a) and **3e** (b) with atom numbering scheme

compounds the most recognizable band appears in the carbonyl group region 1680 cm^{-1} and alkoxy group region 1261–1269 cm^{-1}.

^{1}HNMR spectra of compounds **2a–f** could be recognized as sharp signals, corresponding to the aldehyde proton, $\delta =$ 9.83–9.86 ppm, and strong sharp signals corresponding to three protons, approximately $\delta = 3.94$ ppm, belongs to methyl protons of methoxy group [-OC**H**$_3$] from vanillin.

^{1}HNMR spectra of compounds **3a–f** could be recognized as the most important signals, such as doublet signals, appear at $\delta = 7.75$ and $\delta = 6.99$ ppm, corresponding to protons of chalcone double bond with characteristic $J =$ 15.6 Hz, ferrocene unsubstituted ring at 4.22 area, and strong sharp signal corresponding to three protons, approximately $\delta = 3.94$–3.98 ppm, belongs to methyl protons of 3-methoxy group from vanillin ring.

X-ray structure determination

Single-crystal X-ray crystallographic studies of **3b** and **3e** show that two compounds have very similar molecular geometries (Fig. 1; displacement ellipsoids are drawn at the 30 % probability level; additional projections are shown in the Supplementary Material file, Fig. S1). The cyclopentadienyl rings (Cp) of both molecules have an almost ideal eclipsed conformation. The C1–Cg1–Cg2–C6 torsion angle is 1.8(2)° in **3b** and –0.8(2)° in **3e** (Cg1 and Cg2 are centroids of the corresponding Cp rings, the C1–C5 and C6–C10). The Cp1 and Cp2 rings show only small mutual tilting as evidenced from the corresponding Cp1/Cp2 dihedral angles which are 1.7(2) and 1.0 (2)° in **3b** and **3e**, respectively. Most importantly, two molecules are very similar in spatial orientation of the

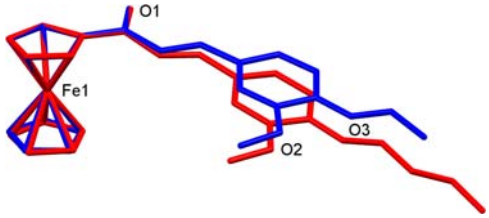


Fig. 2 The molecules of **3b** (blue) and **3e** (red) are overlapped using the C1–C5 ring as a common structural part

Table 3 Selected geometrical parameters in the crystal structures of **3b** and **3e** compounds

Compound	**3b**	**3e**
Selected bond lengths (Å)		
O1–C11	1.224(2)	1.222(2)
O2–C16	1.367(2)	1.362(2)
O2–C20	1.426(2)	1.432(2)
O3–C17	1.355(2)	1.362(2)
O3–C21	1.439(2)	1.435(2)
C1–C11	1.466(3)	1.467(3)
C11–C12	1.472(3)	1.474(3)
C12–C13	1.320(3)	1.324(3)
C13–C14	1.451(3)	1.459(3)
Selected bond angles (°)		
C2–C1–C11	128.01(19)	127.51(19)
C5–C1–C11	124.8(2)	125.10(19)
O2–C16–C15	125.40(17)	125.51(17)
O2–C16–C17	114.46(18)	114.99(18)
O3–C17–C18	125.58(18)	125.60(18)
O3–C17–C16	115.78(17)	115.24(18)
Selected torsion angles (°)		
C20–O2–C16–C17	−174.48(19)	−170.25(18)
C21–O3–C17–C16	174.15(18)	169.16(17)

C1-substituent regarding the ferrocene unit. Structure **3e** is somewhat larger deformed than **3b** and this is well illustrated in Fig. 2, where two molecules are overlapped by Cp1 rings. This perspective view illustrates similarity in spatial orientation of the C1-substituent in the two molecules.

All non-H atoms in the C1-substituent are approximately coplanar in both the molecules (Fig. 1). However, the substituent is significantly displaced from the C1–C5 (Cp1) ring plane. For example, dihedral angle between phenyl and Cp1 rings is 12.4(2) and 15.4(2)° for **3b** and **3e**, respectively, while the C21 alkyl group is bent in the direction of Cp2 ring plane. Such an orientation of the substituent is attained by rotation around the C1–C11 bond and as a consequence the C5–C1–C11–C12 torsion angle has values of −167.3(2) and −162.1(2)° for **3b** and **3e,** respectively. In the two compounds, the corresponding bond lengths and bond angles are very similar (Table 3). The C12–C13 bond

with bond distance of 1.32 Å is the shortest C–C bond in both the molecules and it could be accepted as the only localized double C–C bond.

It is interesting to analyze positions of O2 and O3 atoms. The O2–C16–C15 and O2–C16–C17 angles (which directly reflect directionality of the O2 atom regarding the phenyl ring) are extremely different (Table 3). The same could be found for the O3 atom (Table 3) and in this way two oxygen atoms are shifted close to each other. As a result, the O2 and O3 atoms attain a relatively short O…O distance (2.574(2) and 2.571(3) Å for **3b** and **3e**, respectively) with lone pair of electrons directed to each other. The present nearly coplanar orientation of O-alkyl groups (see the corresponding torsion angles in Table 3) with a short O2…O3 interatomic contact is also noticed in majority of the crystal structures (possessing similar structural fragment) that are found by searching the Cambridge Structural Database (Allen, 2002). Obviously this structural feature is not coincidental and probably it plays specific role in the stabilization of molecular conformation.

Both the compounds do not possess any significant hydrogen bond donor and as a consequence the C21–H…O1 is only the noteworthy intermolecular H-bond. The C21–H…O1 together with C21–H…π (the C14–C19 phenyl ring) interaction interconnect the molecules of **3b** and **3e** into infinite tapes where all C1-substituents are parallel to each other as illustrated in Fig. 3. It is interesting to note that in both intermolecular interactions the C21 methylene group plays the role of a C–H donor (C21–H…O1 and C21–H…π interactions are shown with dotted blue lines; H atoms not involved in intermolecular interactions have been omitted for clarity; corresponding diagram for **3e** is shown in the Supplementary Material file, Fig. S2).

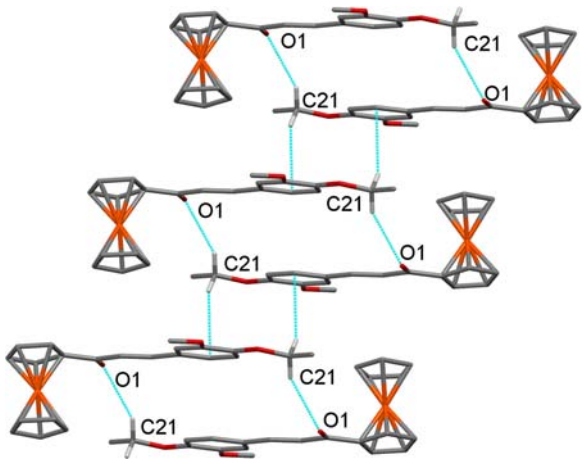


Fig. 3 The crystal packing fragment of **3b** illustrating one-dimensional association of the molecules via intermolecular interactions between C1-substituents

Antimicrobial activity

The antimicrobial activity of the investigated compounds against the test bacteria is shown in Table 4 and fungi in Table 5. The tested compounds exhibited a similar antimicrobial activity. They inhibited all the tested bacteria and antifungal activities. The MIC for bacteria ranged from 0.312 to 5 mg/ml, while MIC for fungi varied from 0.312 to 10 mg/ml. The most sensitive, among the bacteria, were *B. subtilis* and *B. cereus*, whereas the highest resistance was shown by the Gram-negative bacteria (*E. coli* and *P. mirabilis*). Among the fungi, the most sensitive appeared to be *C. albicans*, while *A. niger* was the most resistant. The antimicrobial activity was compared with the standard antibiotics, streptomycin (for bacteria) and ketoconazole (for fungi). The results showed that standard antibiotics had stronger activity than the tested samples as shown in Tables 4 and 5.

In these experiments, the compounds examined at the same concentrations showed a slightly stronger antibacterial than antifungal activity. These results could be expected due to the fact that numerous tests proved that bacteria are more sensitive to the antibiotic compared to fungi (Hugo and Russel, 1983). The reason for different sensitivities between fungi and bacteria can be found in different permeabilities of the cell wall. The cell wall of the Gram-positive bacteria consists of peptidoglycans (murein) and teichoic acids, while the cell wall of Gram-negative bacteria consists of

Table 4 Antibacterial activity of tested compounds

Bacteria	*Staphylococcus aureus*	*Bacillus subtilis*	*Bacillus cereus*	*Escherichia coli*	*Proteus mirabilis*
Test compounds			MIC (mg/ml)		
3a	1.25	0.625	0.312	1.25	2.5
3b	0.625	0.312	0.625	1.25	1.25
3c	0.625	0.312	0.312	2.5	1.25
3d	2.5	0.625	0.312	1.25	1.25
3e	2.5	1.25	0.625	2.5	1.25
3f	5	1.25	1.25	5	2.5
Streptomycin	0.031	0.016	0.016	0.062	0.062

Table 5 Antifungal activity of tested compounds

Fungi	*Mucor mucedo*	*Trichoderma viride*	*Aspergilus niger*	*Candida albicans*	*Penicillium italicum*
Test compounds			MIC (mg/ml)		
3a	1.25	2.5	2.5	0.625	2.5
3b	5	1.25	5	0.625	2.5
3c	2.5	2.5	2.5	1.25	1.25
3d	2.5	1.25	5	0.312	1.25
3e	5	2.5	5	1.25	5
3f	10	5	10	2.5	5
Ketoconazole	0.156	0.078	0.078	0.039	0.156

lipopolysaccharides and lipopoliproteins (Heijenoort, 2001), whereas the cell wall of fungi consists of polysaccharides such as chitin and glucan (Farkaš, 2003).

Conclusion

We described herein synthesis, spectral, analytical, single-crystal X-ray characterization, and in vitro microbiological activity of a series of ferrocenyl chalcones (**3a–f**). Chalcones were prepared in high yields from *O*-alkylated vanillines and acetylferrocene.

Single-crystal X-ray analysis of **3b** and **3e** compounds show that they have very similar conformation and intermolecular association via the C21–H…O1 and C21–H…π interactions. In both molecules the terminal alkyl groups attain an approximately coplanar position with phenyl ring while the methoxy O2 and alkoxy O3 atoms are mutually located at a surprisingly short interatomic distance of 2.57 Å. This structural feature could be a significant supplementary factor for antimicrobial activity of this class of molecules.

We found out that these organometallic derivatives show noticeable activity toward different strains of bacteria and fungi. Compared with our compounds, standard antibiotics, streptomycin (for bacteria) and ketoconazole (for fungi) showed stronger activity.

Acknowledgments This work was supported by the Ministry of Science and Technological Development of the Republic of Serbia, Projects No. 172034 and 172014.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

References

Abdel-Rahman AAH, Abdel-Megied AES, Hawata MAM, Kasem ER, Shabaan MT (2007) Synthesis and antimicrobial evaluation of some halcones and their derived pyrazoles, pyrazolines, isoxazolines, and 5,6-dihydropyrimidine-2-(1H)-thiones. Monatshefte 138:889–897

Achanta G, Modzelewska A, Feng L, Khan SR, Huang P (2006) A boronic-chalcone derivative exhibits potent anticancer activity through inhibition of the proteasome. Mol Pharmacol 70:426–433

Agilent Technologies (2013) CrysAlis PRO. Yarnton, Oxfordshire, UK

Allen FH (2002) The Cambridge Structural Database: a quarter of a million crystal structures and rising. Acta Crystallogr B58: 380–388

Awasthi SK, Mishra N, Kumar B, Sharma M, Bhattacharya A, Mishra LC, Bhasin VK (2009) Potent antimalarial activity of newly synthesized substituted chalcone analogs *in vitro*. Med Chem Res 18:407–420

Bag S, Ramar S, Degani MS (2009) Synthesis and biological evaluation of α,β-unsaturated ketone as potential antifungal agents. Med Chem Res 18:309–316

Blažević N, Kolbah D, Belin B, Šunjić V, Kajfež F (1979) Hexamethylenetetramine, a versatile reagent in organic syntheses. Synthesis 3:161–176

Burla MC, Camalli M, Carrozzini B, Cascarano GL, Giacovazzo C, Polidori G, Spagna R (2003) *SIR2002*: the program. J Appl Crystallogr 36:1103

Chan MM (1995) Inhibition of tumor necrosis factor by curcumin, a phytochemical. Biochem Pharmacol 49:1551–1556

Chen M, Christensen SB, Zhai L, Rasmussen MH, Theander T, Frokjaer S, Steffansen B, Davidsen J, Kharazmi A (1997) The novel oxygenated chalcone, 2,4-dimethoxy-4'-butoxychalcone, exhibits potent activity against human malaria parasite *Plasmodium falciparum* in vitro and rodent parasites *Plasmodium berghei* and *Plasmodium yoelii* in vivo. J Infect Dis 176:1327–1333

Cheng JH, Hung CF, Yang SC, Wang JP, Won SJ, Lin CH (2008) Synthesis and cytotoxic, anti-inflammatory, and anti-oxidant activities of 2',5'-dialkoxylchalcones as cancer chemopreventive agents. Bioorg Med Chem 16:7270–7276

Damljanović I, Čolović M, Vukićević M, Manojlović D, Radulović N, Wurst K, Laus G, Ratković Z, Joksović M, Vukićević RD (2009) Synthesis, spectral characterization and electrochemical properties of 1H-3-(o-, m- and p-ferrocenylphenyl)-1-phenylpyrazole-4-carboxaldehydes. J Organomet Chem 694:1575–1580

Damljanović I, Vukićević M, Radulović N, Ellmerer E, Ratković Z, Joksović M, Vukićević RD (2009) Synthesis and antimicrobial activity of some new pyrazole derivatives containing a ferrocene unit. Bioorg Med Chem Lett 19:1093–1096

Dubey SK, Sharma AK, Narain U, Misra K, Pati U (2008) Design, synthesis and characterization of some bioactive conjugates of curcumin with glycine, glutamic acid, valine and demethylenated

piperic acid and study of their antimicrobial and antiproliferative properties. Eur J Med Chem 43:1837–1846
Echeverria C, Santibanez JF, Donoso-Tauda O, Escobar CA, Tagle RR (2009) Structural antitumoral activity relationships of synthetic chalcones. Int J Mol Sci 10:221–231
Edwards ML, Stemerick DM, Sunkara SP (1988) Chalcone derivatives useful in controlling growth of tumor tissue and their preparation. European Patent Application EP 288,794
Farkaš V (2003) Structure and biosynthesis of fungal cell walls: methodological approaches. Folia Microbiol 48:469–478
Farrugia LJ (1997) *ORTEP*-3 for Windows - a version of *ORTEP*-III with a Graphical User Interface (GUI). J Appl Crystallogr 30:565
Farrugia LJ (1999) WinGX suite for small-molecule single-crystal crystallography. J Appl Crystallogr 32:837–838
Heijenoort J (2001) Formation of the glycan chains in the synthesis of bacterial peptidoglycan. Glycobiology 11:25–36
Herencia F, Ferrandiz ML, Ubeda A, Dominguez JN, Charris EJ, Lobo GM, Alcaraz MJ (1998) Synthesis and anti-inflammatory activity of chalcone derivatives. Bioorg Med Chem Lett 8:1169–1174
Hugo WB, Russell AD (1983), Pharmaceutical microbiology, 3rd edn. Blackwell Scientific Publications: Oxford, UK
Ilango K, Valentina P, Saluja G (2010) Synthesis and *in vitro* anticancer activity of some substituted chalcones derivatives. Res J Pharm Biol Chem Sci 1:354–359
Kalirajan R, Sivakumar SU, Jubie S, Gowramma B, Suresh B (2009) Synthesis and biological evaluation of some heterocyclic derivatives of chalcones. Int J ChemTech Res 1:27–34
Katade S, Phalgune U, Biswas S, Wakharkar R, Deshpande N (2008) Microwave studies on synthesis of biologically active chalcones derivatives. Indian J Chem 47B:927–931
Katritzky AR, Long Q, He H, Qiua G, Wilcox AL (2006) Preparation of 2-alkoxy-5-methoxybenzaldehyde and 2-methoxy-5-alkoxy benzaldehydes. Arkivoc vi: 868–875
Kaushik S, Kumar N, Drabu S (2010) Synthesis and anticonvulsant activities of phenoxy chalcones. Pharm Res 3:257–262
Kumar D, Kumar NM, Akamatsu K, Kusaka E, Harada H, Ito T (2011) Synthesis and biological evaluation of indolyl chalcones as antitumor agents. Bioorg Med Chem Lett 20:3916–3919
Lahtchev KL, Batovska DI, Parushev SP, Ubiyvovk VM, Sibirny AA (2008) Antifungal activity of chalcones: a mechanistic study using various yeast strains. Eur J Med Chem 43:2220–2228
Lim SS, Kim HS, Lee DU (2007) In vitro antimalarial activity of flavonoids and chalcones. Bull Korean Chem Soc 28:2495–2497
Loev B, Dawson CR (1956) Alkylphenols related to the poison ivy principle. An improved method of synthesis involving the Na-Butanol cleavage of benzyl ethers. J Am Chem Soc 78: 6095–6101
Liu M, Wilairat P, Go ML (2001) Antimalarial alkoxylated and hydroxylated chalcones: structure-activity relationship analysis. J Med Chem 44:4443–4452
Lunardi F, Guzela M, Rodrigues AT, Corre R, Eger-Mangrich I, Steindel M, Grisard EC, Assreuy J, Calixto JB, Santos ARS (2003) Trypanocidal and leishmanicidal properties of substitution-containing chalcones. Antimicrob Agents Chemother 47:1449–1451
Macrae CF, Edgington PR, McCabe P, Pidcock E, Shields GP, Taylor R, Towler M, van de Streek J (2006) Mercury: visualization and analysis of crystal structures. J Appl Crystallogr 39:453–457
Maliar T, Jedinak A, Kadrabova J, Sturdik E (2004) Structural aspects of flavonoids as trypsin inhibitors. Eur J Med Chem 39:241–248
Mishra S, Narain U, Mishra R, Mishra K (2005) Design, development and synthesis of mixed bioconjugates of piperic acid-glycine, curcumin-glycine/alanine and curcumin-glycine-piperic acid and their antibacterial and antifungal properties. Bioorg Med Chem 13:1477–1486
Mostahar S, Katun P, Islam A (2007) Synthesis of two vanillin ring containing flavones by different methods and studies of their antibacterial and antifungal activities. J Biol Sci 7:514–519
Motta LF, Gaudio AC, Takahata Y (2006) Quantitative structure–activity relationships of a series of chalcone derivatives (1,3–diphenyl–2–propen–1–one) as anti *Plasmodium falciparum* agents (anti malaria agents). Internet Electron J Mol Des 5:555–569
Nardelli M (1995) PARST95 – an update to PARST: a system of Fortran routines for calculating molecular structure parameters from the results of crystal structure analyses. J Appl Crystallogr 28:659
NCCLS (National Commitee for Clinical Laboratory Standards) (1998) Reference method for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi: proposed Standard M38-P. NCCLS, Wayne, PA, USA
Nishimura RT, Giammanco CH, Vosburg DA (2010) Green, enzymatic syntheses of divanillin and diapocynin for the organic, biochemistry, or advanced general chemistry laboratory. J Chem Ed 87:526–527
Nowakowska Z (2007) A review of anti-infective and anti-inflammatory chalcones. Eur J Med Chem 42:125–137
Opletalova V (2000) Chalcones and their heterocyclic analogs as potential therapeutic agents in bacterial diseases. Cesk Slov Farm 49:278–284
Oyedapo AO, Mankanju VO, Adewunmi CO, Iwalewa EO, Adenowo TK (2004) Antitrichomonal activity of 1,3-diaryl-2-propen-1-ones on *Trichomonas gallinae*. Afr J Trad CAM 1:55–62
Pepper JM, MacDonald JA (1953) The synthesis of syringaldehyde from vanillin. Can J Chem 31:476–483
Ratković Z, Juranić ZD, Stanojković T, Manojlović D, Vukićević RD, Radulović N, Joksović M (2010) Synthesis, characterization, electrochemical studies and antitumor activity of some new chalcone analogues containing ferrocenyl pyrazole moiety. Bioorg Chem 38:26–32
Ratković Z, Muškinja J, Burmudžija A, Ranković B, Kosanić M, Bogdanović GA, Simović Marković B, Nikolić A, Arsenijević N, Đorđević S, Vukićević RD (2016) Dehydrozingerone based 1-acetyl-5-aryl-4,5-dihydro-1*H*-pyrazoles: synthesis, characterization and anticancer activity. J Mol Struct 1109:82–88
Romagnoli R, Baraldi PG, Carrion MD, Cara CL, Cruz-Lopez O, Preti D (2008) Design, synthesis, and biological evaluation of thiophene analogues of chalcones. Bioorg Med Chem 16:5367–5376
Sarker SD, Nahar L, Kumarasamy Y (2007) Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods 42:321–324
Seo WD, Ryu YB, Curtis-Long MJ, Lee CW, Ryu HW, Jang KC (2010) Evaluation of anti-pigmentary effect of synthetic sulfonylamino chalcone. Eur J Med Chem 45:2010–2017
Sheldrick GM (2008) A short history of SHELX. Acta Crystallogr Sect A 64:112–122
Sivakumar PM, Ganesan S, Veluchamy P, Doble M (2010) Novel chalcones and 1,3,5-triphenyl-2-pyrazoline derivatives as antibacterial agents. Chem Biol Drug Des 76:407–411
Sivakumar PM, Prabhakar PK, Doble M (2010) Synthesis, antioxidant evaluation and quantitative structure activity relationship studies of chalcones. Med Chem Res 19:1–17
Spek AL (2003) Single-crystal structure validation with the program PLATON. J Appl Crystallogr 36:7–13
Szliszka E, Czuba ZP, Mazur B, Sedek L, Paradysz A, Krol W (2009) Chalcones enhance TRAIL-induced apoptosis in prostate cancer cells. Int J Mol Sci 11:1–13
Tatsuzaki J, Bastow KF, Nakagawa-Goto K, Nakamura S, Itokawa H, Lee K (2006) Dehydrozingerone, chalcone, and isoeugenol analogues as in vitro anticancer agents. J Nat Prod 69:1445–1449

Trivedi AR, Dodiya DK, Ravat NR, Shah VH (2008) Synthesis and biological evaluation of some new pyrimidines via a novel chalcone series. Arkivoc (xi): 131-141

Tsukiyama RI, Katsura H, Tokuriki N, Kobayashi M (2002) Antibacterial activity of licochalcone A against spore-forming bacteria. Antimicrob Agents Chemother 46:1226–1230

Vasilev RF, Kancheva VD, Fedorova GF, Batovska DI, Trofimov AV (2010) Antioxidant activity of chalcones: the chemiluminescence determination of the reactivity and the quantum chemical calculation of the energies and structures of reagents and intermediates. Kinet Katal 51:507–515

Vogel S, Ohmayer S, Brunner G, Heilmann J (2008) Natural and non-natural prenylated chalcones: synthesis, cytotoxicity and anti-oxidative activity. Bioorg Med Chem 16:4286–4293

Wattenberg LW, Coccia JB, Galhaith AR (1994) Inhibition of carcinogen-induced pulmonary and mammary carcinogenesis by chalcone administered after carcinogen exposure. Cancer Lett 83:165–169

Wu X, Wilairat P, Go MN (2002) Antimalarial activity of ferrocenyl chalcones. Bioorg Med Chem Lett 12:2299–2302

Yadav HL, Gupta P, Pawar PS, Singour PK, Patil UK (2010) Synthesis and biological evaluation of anti-inflammatory activity of 1,3-diphenyl propenone derivatives. Med Chem Res 19:1–8

Yayli N, Ucuncu O, Yasar A, Kucuk M, Akyuz E, Karaoglu SA (2006) Synthesis and biological activities of *N*-alkyl derivatives of *o*-, *m*-, and *p*-nitro (E)-4-azachalcones and stereoselective photochemistry in solution with theoretical calculations. Turk J Chem 30:505–514

Zhang XW, Zhao DH, Quan YC, Sun LP, Yin XM, Guan LP (2010) Synthesis and evaluation of anti-inflammatory activity of substituted chalcone derivatives. Med Chem Res 19:403–412