

ORIGINAL PAPER

Synthesis, biological evaluation, quantitative-SAR
and docking studies of novel chalcone derivatives
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In the present study, a series of chalcone derivatives including 17 new compounds were synthesised; their antibacterial activities against eleven bacteria, and their free radical-scavenging activities using DPPH were evaluated. All compounds showed significant antibacterial activities against both Gram-positive and Gram-negative bacteria. In particular, compound *III*f strongly inhibited *Staphylococcus aureus* (JMC 2151) and *Enterococcus faecalis* (CARS 2011-012) with MIC values of 6.25 $\mu\text{g mL}^{-1}$ and 12.5 $\mu\text{g mL}^{-1}$, respectively, which are comparable to that of the standard antibiotic, nalidixic acid. Compound *III*g also inhibited *S. aureus* with a MIC value similar to that of nalidixic acid (6.25 $\mu\text{g mL}^{-1}$). Furthermore, like nalidixic acid (MIC value of 25 $\mu\text{g mL}^{-1}$), compounds *III*a, *III*c and *III*d inhibited *Listeria monocytogenes* (ATCC 43256) with MIC values of 25 $\mu\text{g mL}^{-1}$, 12.5 $\mu\text{g mL}^{-1}$ and 25 $\mu\text{g mL}^{-1}$, respectively. Quantitative structure–activity relationship (Q-SAR) studies using physicochemical calculations indicated that the antibacterial activities of chalcone derivatives correlated well with predicted physicochemical parameters ($\log P$ and PSA). Docking simulation by positioning the most active compound *III*f in the active site of the penicillin-binding protein (PBP-1b) of *S. aureus* was performed to explore the feasible binding mode. Furthermore, most of the compounds synthesised exhibited significant DPPH radical-scavenging activity, although compounds *II*c and *III*c exhibited the greatest antioxidant activity with IC_{50} values of 1.68 μM and 1.44 μM , respectively, comparable to that of the standard antioxidant, ascorbic acid (1.03 μM).

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Keywords: chalcones, antibacterial, antioxidant, physicochemical properties, docking study

Introduction

In recent decades, the treatment of microbial infectious diseases has become a crucial and challenging problem due to the increasing incidence of bacterial resistance to existing drugs, with particular relevance for Gram-positive bacteria such as *Staphylococcus* species (Muroi et al., 2004; Tenover & McDonald, 2005). Extensive use of antibacterial drugs and their resistance against bacterial infections has led to severe health problems. Moreover, as the multidrug-resistant pathogens have become more widespread in both com-

munities and hospitals, the frequency of discovery of new antimicrobial agents has declined (Walsh, 2000). Accordingly, there is a critical need for new antimicrobial agents with a potent, wide therapeutic window, broad-spectrum activity and new mode of action.

Chalcones (1,3-diphenyl-2-propen-1-ones) are open-chained molecules consisting of two aromatic rings linked by a three-carbon α,β -unsaturated carbonyl system. The chalcones are precursors in the biosynthetic pathways of flavonoids, isoflavonoids, and aurone, which reportedly have wide-ranging pharmacological activities, such as antifungal (Zhao et al.,

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2007), antibacterial (Nielsen et al., 2005), antioxidant (Aichaoui et al., 2009), anticancer (Mahmoodi et al., 2014), antiprotozoal (Kiat et al., 2006), anti-inflammatory (Seo et al., 2005), antimalarial (Awasthi et al., 2009), anti-HIV (Cheenpracha et al., 2006), anti-angiogenic (Varinska et al., 2012), cyclooxygenase inhibitory (Sharma, 2014) and tyrosine phosphatase 1B inhibitory activities (Sun et al., 2012). Chalcones and their α,β -dibromo derivatives (2,3-dibromo-1,3-diphenyl-2-propen-1-one) are used as intermediates for the syntheses of various heterocyclic compounds, such as flavones, flavonols, aziridines, coumarones, quinolines, pyrazoles and isooxazoles (Tökés et al., 1992; Wróblewski et al., 2000; Agrawal & Soni, 2007). A survey of the literature revealed that α,β -dibromochalcones and their derivatives also possess a wide range of biological activities, which include antioxidant (Lahsasni et al., 2014), antimicrobial (Rahman et al., 2012), anti-angiogenic (Robinson et al., 2005), quinine reductase-inducing (Dinkova-Kostova et al., 1998), cytotoxic (Modzelewska et al., 2006) and cholesterol-lowering activities (Piantadosi et al., 1973).

The survey of the literature also revealed that chalcone derivatives exhibited excellent bactericidal activity against such resistant bacterial strains as drug-sensitive strains of *S. aureus* (Alcaráz et al., 2000; Liu et al., 2008; Nowakowska, 2007). The antibiotics usually exert their bactericidal activity via different mechanisms: inhibition of cell wall synthesis, inhibition of protein synthesis, inhibition of nucleic acid synthesis and anti-metabolism. However, Nielsen et al. (2005) reported that the chalcone derivatives containing various substituents, e.g. aliphatic amino group, halogens, methoxy and alkyl groups etc., exerted an antibacterial action by the non-selective disruption of cell membranes. Sivakumar et al. (2009) also reported that the antibacterial action of chalcone derivatives bearing methoxy, hydroxyl, thiomethyl, sulphonylmethyl and nitro substituents was revealed by damaging the cell wall of *S. aureus*. Battenberg et al. (2013) reported on 4-hydroxydericin, a chalcone derivative which inhibited protein biosynthesis by forming a covalent bond with seryl-tRNA synthetase (STS) of *S. aureus* and controlled the aminoacylation of tRNAs, an essential enzymatic pathway for bacterial viability. Hence, interest is increasing within the research community to synthesise and screen new analogues of chalcone derivatives.

Due to the observed wide-ranging bioactivities of chalcone derivatives and in line with an ongoing search for novel biologically active molecules, a series of chalcones (1,3-diphenyl-2-propen-1-ones) and their α,β -dibromo derivatives (2,3-dibromo-1,3-diphenyl-2-propen-1-ones) were synthesised and their antimicrobial activities evaluated against 11 pathogenic and food-borne bacterial strains, e.g. *Staphylococcus aureus* (JMC 2151), *Listeria monocytogenes* (ATCC

43256), *Enterococcus faecalis* (CARS 2011-012), *Bacillus subtilis* (IFO 13719), *Klebsiella pneumonia* (JCM 1662), *Citrobacter freundii* (JCM 1657), *Cronobacter sakazakii* (CARS 2012-J-F), *Salmonella enteritidis* (ATCC 13076), *Escherichia coli* (CARS 2011-016), *Yersinia pestis* (CARS 2013-027) and *Pseudomonas aeruginosa* (PA01), and their antioxidant properties examined. In addition, the quantitative structure-activity relationships (Q-SAR) with respect to antibacterial activities were studied using physicochemical parameters. Moreover, to predict the binding mode of these chalcone derivatives as plausible bacterial cell wall biosynthesis inhibitors, molecular docking studies were carried out on the penicillin-binding protein (PBP-1b) of *S. aureus* using AutoDock 4.2, PyRx 0.8 and Discovery studio 4.0 software. In the present study, the chalcone derivatives possessing different types of substituents only in phenyl ring B were synthesised to observe their effect on antibacterial and antioxidant activity. The results obtained here would be of use in a further study synthesising more chalcone derivatives with various types of substituents in both phenyl rings A and B.

Experimental

All reagents and solvents used in the study were of analytical grade purity and procured from Sigma-Aldrich (USA). The melting points of the synthesised compounds were measured using a Stuart SMP3 apparatus (Barloworld Scientific, UK) and the results are uncorrected. The FTIR spectra (KBr discs technique) were obtained using an MB100 spectrophotometer (Bomem, Canada). The NMR spectra were recorded using a Bruker 400 MHz spectrometer in CDCl_3 using TMS as an internal standard; the mass spectra were acquired using a Jeol JMS-700 mass spectrometer. Elemental analyses (C, H, N) were performed on a PerkinElmer 2400 II CHN elemental analyser (PerkinElmer, USA). Absorbance for antioxidant activity was measured on an Optizen 2120 UV-VIS spectrophotometer (Mecasys Co., Korea).

General procedure for preparation of 1,3-diphenyl-2-propen-1-ones (IIa–IIj)

A mixture of acetophenone (240.30 mg, 2 mmol), substituted benzaldehyde (*I*, 2 mmol) and KOH (224 mg, 4 mmol) in ethanol (20 mL) was kept at ambient temperature for 72–96 h followed by dilution with ice-cold water, acidification with cold dilute HCl, and extraction with ether. The solvent was evaporated and the residue was subjected to silica gel column chromatography using *n*-hexane/dichloromethane ($\varphi_{\text{r}} = 10 : 1$) as a solvent to afford the desired compounds (*IIb*, *IIe*, *IIg*, *IIi*, *IIj*) as solids or liquids. Compounds *IIa*, *IIc*, *IId*, *IIf* and *IIh* were synthesised as previously described (Winter et al., 2010). The purity

of the compounds was verified by TLC and elemental analyses. For IR, ^1H and ^{13}C NMR and mass spectrometry (MS) data, see Table S1 in Supplementary data.

General procedure for preparation of 2,3-dibromo-1,3-diphenylpropan-1-ones (IIIa–IIIj)

Bromine (2 mmol) in CHCl_3 (10 mL) was added slowly over 10 min to a stirred solution of the corresponding chalcone (IIa–IIj, 1 mmol) in CHCl_3 (20 mL). The stirring was continued for 3–4 h (monitored by TLC). After completion of the reaction, ethanol/water ($\varphi_{\text{r}} = 1 : 1$) was added and the product was extracted with ether. The organic layer was washed with water (3×50 mL), dried with Na_2SO_4 , filtered and the solvent evaporated to afford the corresponding brominated product as solid or liquid. The purity of the compounds was verified by TLC and elemental analyses. For IR, ^1H and ^{13}C NMR and MS data, see Table S1 in Supplementary data.

Antibacterial screening

A filter paper disc diffusion method described previously (Alam & Lee, 2011) against 11 strains was used to determine the in vitro antibacterial effects of all compounds. Briefly, nutrient agar (NA) media (Difco Laboratories, Lawrence, KS, USA) was used as a basal medium for test bacteria. Agar media were inoculated with 0.2 mL of 24-h liquid cultures containing the microorganisms. Sample discs were placed carefully on pre-inoculated agar plates and incubated aerobically at 37 °C for 24 h. Discs treated with DMSO only were used as controls, and nalidixic acid was used as a positive control. Inhibitory activities were assessed (in mm) by measuring the diameters of the observed inhibition zones. These evaluations were performed in triplicate for each compound of a concentration of 300 μg per disc. The minimum inhibitory concentrations (MIC, in $\mu\text{g mL}^{-1}$) of selected compounds were determined against *S. aureus* (JMC 2151, G⁺), *L. monocytogenes* (ATCC 43256, G⁺), *E. faecalis* (CARS 2011-012, G⁺), *B. subtilis* (IFO 13719, G⁺), *K. pneumonia* (JCM 1662, G⁻), *C. freundii* (JCM 1657, G⁻), *C. sakazakii* (CARS 2012-J-F, G⁻), *S. enteritidis* (ATCC 13076, G⁻), *E. coli* (CARS 2011-016, G⁻), and *Y. pestis* (CARS 2013-027, G⁻) using nutrient broth media (DIFCO) and a serial dilution technique (Nishino et al., 1987). MIC was defined as the lowest concentration of the tested compound (in DMSO) that inhibited bacterial growth.

DPPH radical-scavenging activity

The free radical-scavenging activities (DPPH-RSA) of the synthesised compounds were assayed using the Blois method with some modifications (Blois, 1958)

and using a DPPH-based method. Briefly, the DPPH solution was added to 0.1 mL samples of different concentrations in ethanol (4 mL of 1.5×10^{-5} M), thoroughly mixed, then left to stand at ambient temperature in a dark place for 30 min. Solution absorbances were measured at 520 nm using an Optizen 2110 UV-VIS spectrophotometer. DPPH-RSA was calculated using the following equation: DPPH-RSA (%) = $(A_{\text{C}} - A_{\text{S}})/A_{\text{C}} \times 100$, where A_{C} is the absorbance of the control and A_{S} is the absorbance of the sample.

Computational analysis

The molecular geometries of the synthesised compounds were constructed with standard bond lengths and angles using the ChemBio3D Ultra Version 14 molecular modelling program (CambridgeSoft Corporation, Cambridge, MA, USA) and SMILES were used to calculate the physicochemical properties using Molinspiration Cheminformatics software (Molinspiration Cheminformatics, Slovenský Grob, Slovakia). Maps of the molecular lipophilicity potentials (MLP) and polar surface areas (PSA) were viewed in a Molinspiration Galaxy 3D Structure Generator (v2013.02 beta) using the optimised structures of the synthesised compounds.

Molecular docking studies

The 3D structures of compound IIIf were constructed using the ChemBio3D Ultra Version 14 molecular modelling program (CambridgeSoft Corporation, Cambridge, MA, USA), and energy minimised using the semi-empirical molecular orbital PM3 method (Stewart, 2004) with 439 iterations and minimum RMS gradient of 0.10. The crystal structure of the penicillin-binding protein (PBP-1b) of *S. aureus* was obtained from the Protein Data Bank (PDB code: 2Y2H) in order to prepare the protein for the docking studies. All the bound waters and ligands were eliminated and the polar hydrogens and the Kollman-united charges were added to the molecule, respectively. The pdb and pdbqt files of the ligand and receptor were prepared using the AutoDock 4.2 software. All torsions were allowed to rotate during docking. The docking procedure was followed using the standard protocol implemented in AutoDock Vina interface (Trott & Olson, 2010) in PyRx 0.8 software and the geometry of the resulting complexes was studied using Discovery Studio 4.0 (Accelrys, San Diego, CA, USA).

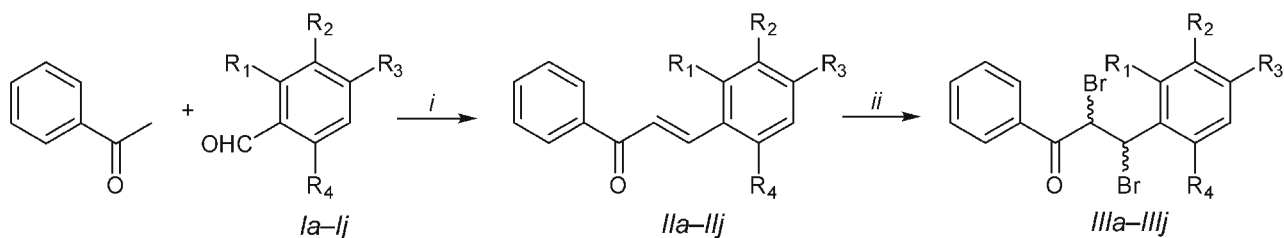
Results and discussion

Spectral characterisation

Starting chalcones IIa–IIj were prepared by the condensation of acetophenone and a substituted ben-

Table 1. Characterisation data of synthesised compounds

Compound	R ₁	R ₂	R ₃	R ₄	Formula	M _r	w ₁ (calc.)/% w ₁ (found)/%			Yield %	M.p. °C
							C	H	N		
<i>IIa</i>	OH	H	OH	H	C ₁₅ H ₁₂ O ₃	240.26	74.99 75.10	5.03 5.09	–	71	168–169
<i>IIb</i>	OCH ₃	H	OCH ₃	H	C ₁₇ H ₁₆ O ₃	268.31	76.10 76.21	6.01 6.07	–	77	69–70
<i>IIc</i>	OH	H	OH	OH	C ₁₅ H ₁₂ O ₄	256.26	70.31 70.47	4.72 4.78	–	82	158–159
<i>IId</i>	H	OH	OH	H	C ₁₅ H ₁₂ O ₃	240.26	74.99 75.11	5.03 5.11	–	85	liquid
<i>IIe</i>	H	OCH ₃	OCH ₃	H	C ₁₇ H ₁₆ O ₃	268.31	76.10 76.19	6.01 6.09	–	74	91–92
<i>IIf</i>	H	H	OH	H	C ₁₅ H ₁₂ O ₂	224.26	80.34 80.43	5.39 5.35	–	79	185–86
<i>IIg</i>	H	OCH ₃	OH	H	C ₁₆ H ₁₄ O ₃	254.29	75.57 75.67	5.55 5.60	–	89	liquid
<i>IIh</i>	OH	H	H	H	C ₁₅ H ₁₂ O ₂	224.26	80.34 80.43	5.39 5.45	–	85	143–144
<i>IIi</i>	H	H	Cl	H	C ₁₅ H ₁₁ ClO	242.70	74.23 74.31	4.57 4.61	–	89	110–111
<i>IIj</i>	H	H	N(CH ₃) ₂	H	C ₁₇ H ₁₇ NO	251.33	81.24 81.32	6.82 6.79	5.57 5.64	86	113–114
<i>IIIa</i>	OH	H	OH	H	C ₁₅ H ₁₂ Br ₂ O ₃	400.07	45.03 45.15	3.02 3.10	–	86	134–135
<i>IIIb</i>	OCH ₃	H	OCH ₃	H	C ₁₇ H ₁₆ Br ₂ O ₃	428.12	47.69 47.78	3.77 3.81	–	79	155–156
<i>IIIc</i>	OH	H	OH	OH	C ₁₅ H ₁₂ Br ₂ O ₄	416.07	43.30 43.42	2.91 2.85	–	83	213–214
<i>IIId</i>	H	OH	OH	H	C ₁₅ H ₁₂ Br ₂ O ₃	400.07	45.03 45.12	3.02 3.10	–	78	115–116
<i>IIIe</i>	H	OCH ₃	OCH ₃	H	C ₁₇ H ₁₆ Br ₂ O ₃	428.12	47.69 47.78	3.77 3.82	–	84	86–87
<i>IIIf</i>	H	H	OH	H	C ₁₅ H ₁₂ Br ₂ O ₂	384.07	46.91 47.02	3.15 3.18	–	81	125–126
<i>IIIg</i>	H	OCH ₃	OH	H	C ₁₆ H ₁₄ Br ₂ O ₃	414.09	46.41 46.52	3.41 3.50	–	85	liquid
<i>IIIh</i>	OH	H	H	H	C ₁₅ H ₁₂ Br ₂ O ₂	384.07	46.91 47.03	3.15 3.21	–	81	liquid
<i>IIIi</i>	H	H	Cl	H	C ₁₅ H ₁₁ Br ₂ ClO	402.51	44.76 44.84	2.75 2.82	–	84	174–175
<i>IIIj</i>	H	H	N(CH ₃) ₂	H	C ₁₇ H ₁₇ Br ₂ NO	411.14	49.66 49.73	4.17 4.22	3.41 3.49	80	liquid

**Fig. 1.** Synthesis of chalcones *IIa–IIj* and 2,3-dibromo derivatives *IIIa–IIIj*. Reaction conditions: *i*) EtOH/KOH, ambient temperature, 72–96 h, yield of 77–96 %; for *IIa*, *IIc*, *IId*, *IIf* and *IIh*: MeOH, piperidine, reflux, yield of 77–96 %; *ii*) CHCl₃/Br₂, ambient temperature, 3–4 h, yield of 78–86 %.

zaldehyde (*I*) (Fig. 1; for substituents R₁, R₂, R₃, R₄, see Table 1) in ethanol/KOH solution or in

MeOH/piperidine solution by heating under reflux (for *IIa*, *IIc*, *IId*, *IIf*, *IIh*) with good yields (71–

Table 2. In vitro bactericidal profiles^a of chalcones *IIa–IIj* and 2,3-dibromo derivatives *IIIa–IIIj*

Compound	Inhibition zone diameter/mm										
	<i>S.</i> <i>aureus</i>	<i>L.</i> <i>monocytogenes</i>	<i>E.</i> <i>faecalis</i>	<i>B.</i> <i>subtilis</i>	<i>K.</i> <i>pneumonia</i>	<i>C.</i> <i>freundii</i>	<i>C.</i> <i>sakazakii</i>	<i>S.</i> <i>enteritidis</i>	<i>E.</i> <i>coli</i>	<i>Y.</i> <i>pestis</i>	<i>P.</i> <i>aeruginosa</i>
<i>IIa</i>	10 ± 0.5	10 ± 0.5	na	15 ± 1.0	18 ± 1.0	12 ± 0.5	9 ± 0.5	12 ± 1.0	17 ± 1.0	13 ± 0.5	13 ± 0.5
<i>IIb</i>	11 ± 0.5	12 ± 0.5	13 ± 1.0	10 ± 0.5	12 ± 0.5	11 ± 1.0	12 ± 1.0	12 ± 0.5	11 ± 0.5	12 ± 0.5	12 ± 1.0
<i>IIc</i>	9 ± 0.5	9 ± 0.5	10 ± 0.5	11 ± 0.5	9 ± 0.5	na	9 ± 0.5	9 ± 0.5	9 ± 0.5	na	13 ± 0.5
<i>IId</i>	10 ± 0.5	na	15 ± 1.0	11 ± 0.5	9 ± 0.5	10 ± 0.5	14 ± 1.0	10 ± 0.5	9 ± 0.5	13 ± 1.0	13 ± 1.0
<i>IIe</i>	10 ± 0.5	11 ± 0.5	12 ± 0.5	10 ± 0.5	11 ± 0.5	11 ± 0.5	12 ± 0.5	12 ± 1.0	10 ± 0.5	10 ± 0.5	11 ± 0.5
<i>IIf</i>	11 ± 0.5	10 ± 0.5	11 ± 0.5	10 ± 0.5	10 ± 0.5	10 ± 0.5	11 ± 0.5	9 ± 0.5	10 ± 0.5	10 ± 0.5	9 ± 0.5
<i>IIg</i>	10 ± 0.5	11 ± 0.5	13 ± 1.0	10 ± 0.5	11 ± 0.5	12 ± 1.0	10 ± 0.5	11 ± 0.5	9 ± 0.5	11 ± 0.5	11 ± 0.5
<i>IIh</i>	10 ± 0.5	11 ± 0.5	11 ± 0.5	18 ± 1.0	10 ± 0.5	10 ± 0.5	11 ± 0.5	na	11 ± 0.5	10 ± 0.5	11 ± 0.5
<i>IIi</i>	12 ± 0.5	13 ± 0.5	9 ± 0.5	13 ± 1.0	18 ± 1.0	10 ± 0.5	12 ± 0.5	10 ± 0.5	13 ± 0.5	9 ± 0.5	9 ± 0.5
<i>IIj</i>	9 ± 0.5	9 ± 0.5	11 ± 0.5	na	12 ± 0.5	10 ± 0.5	na	13 ± 0.5	12 ± 0.5	10 ± 0.5	10 ± 0.5
<i>IIIa</i>	12 ± 0.5	15 ± 1.0	na	10 ± 0.5	12 ± 0.5	23 ± 1.0	10 ± 0.5	9 ± 0.5	12 ± 0.5	14 ± 0.5	14 ± 1.0
<i>IIIb</i>	10 ± 0.5	11 ± 0.5	na	11 ± 0.5	na	12 ± 0.5	12 ± 0.5	11 ± 0.5	10 ± 0.5	12 ± 0.5	11 ± 0.5
<i>IIIc</i>	32 ± 1.5	35 ± 1.5	13 ± 1.0	9 ± 0.5	19 ± 1.0	13 ± 1.0	10 ± 0.5	9 ± 0.5	14 ± 1.0	15 ± 0.5	16 ± 1.0
<i>IIId</i>	10 ± 0.5	20 ± 1.0	na	12 ± 0.5	na	12 ± 0.5	10 ± 0.5	9 ± 0.5	13 ± 0.5	9 ± 0.5	10 ± 0.5
<i>IIIe</i>	9 ± 0.5	9 ± 0.5	na	na	10 ± 0.5	12 ± 0.5	11 ± 0.5	11 ± 0.5	11 ± 0.5	11 ± 0.5	11 ± 0.5
<i>IIIf</i>	42 ± 1.5	9 ± 0.5	33 ± 1.5	33 ± 1.5	15 ± 1.0	22 ± 0.5	18 ± 1.0	14 ± 1.0	20 ± 1.0	13 ± 1.0	23 ± 1.0
<i>IIIg</i>	35 ± 1.5	10 ± 0.5	23 ± 1.0	15 ± 1.0	12 ± 0.5	14 ± 1.0	13 ± 1.0	11 ± 0.5	13 ± 1.0	15 ± 1.0	17 ± 1.0
<i>IIIh</i>	10 ± 0.5	9 ± 0.5	20 ± 1.0	21 ± 1.0	12 ± 0.5	12 ± 0.5	12 ± 1.0	11 ± 0.5	11 ± 0.5	13 ± 1.0	11 ± 0.5
<i>IIIi</i>	20 ± 1.0	10 ± 0.5	9 ± 0.5	na	9 ± 0.5	13 ± 1.0	15 ± 1.0	14 ± 0.5	11 ± 0.5	9 ± 0.5	9 ± 0.5
<i>IIIj</i>	9 ± 0.5	9 ± 0.5	12 ± 1.0	11 ± 0.5	9 ± 0.5	13 ± 1.0	13 ± 1.0	13 ± 0.5	10 ± 0.5	9 ± 0.5	9 ± 0.5
NA	25 ± 1.0	07 ± 0.5	13 ± 1.0	21 ± 1.0	22 ± 1.0	30 ± 1.5	25 ± 1.0	19 ± 1.0	25 ± 1.0	na	24 ± 1.0

a) Results are mean ± SD of at least three experiments; compounds were loaded at 300 µg per disc and positive control (NA, nalidixic acid) was loaded at 50 µg per disc; na: not active.

89 %). The characterisation data and elemental analyses of *IIa–IIj* are presented in Table 1. The spectral data (Table S1, Supplementary data) are in accordance with the structures of the synthesised compounds *IIa–IIj*. The IR spectra of compounds containing a hydroxyl group (*IIa*, *IIc*, *IId*, *IIf*, *IIh*, *IIi*) exhibited an absorption band at ~3342–3549 cm⁻¹. The stretching absorption band of the C=O groups appeared at 1641–1679 cm⁻¹. ¹H NMR spectra of compounds *IIa–IIj* exhibited the characteristic two doublets at δ 7.36–7.96 and 7.54–8.15, which were assigned to H_α and H_β protons, respectively. The coupling constants of olefinic H_α and H_β protons were 15–16 Hz, indicating the *E* configuration around the carbon-carbon double bond of the chalcones *IIa–IIj* (Markham & Geiger, 1994). The methoxyl protons of compounds *IIb*, *IIe* and *IIh* appeared as a singlet at δ 3.84–3.91 and the *N,N*-dimethylamino protons of *IIj* appeared as a singlet at δ 2.96. ¹³C NMR spectra of compounds *IIa–IIj* exhibited two characteristic peaks at δ 119.65–122.80 and δ 141.01–146.84 for α and β carbon, respectively, while these peaks disappeared in compounds *IIIa–IIIj*. The aromatic, carbonyl, methoxyl and *N,N*-dimethylamino functional group carbons were assigned in the usual way. In addition, compounds *IIa–IIj* exhibited molecular ion peaks in the mass spectra.

The electrophilic addition of bromine to the asymmetrical C=C double bond of compounds *IIa–IIj* using the Br₂/CHCl₃ solution afforded the corresponding 2,3-dibromo derivatives (*IIIa–IIIj*) with good

yields (79–86 %) as racemates (Fig. 1). These are new compounds, with the exceptions of *IIf* and *IIj* (Varinska et al., 2012), and *IIIi* (Berthelot et al., 1995). Their physical properties and elemental analyses are presented in Table 1. Spectral data (IR, ¹H and ¹³C NMR and MS) are listed in Table S1 (see Supplementary data). In the IR spectra of compounds *IIIa*, *IIIc*, *IIId*, *IIIf*, *IIIh* and *IIIi*, the characteristic O—H stretching absorption bands appeared at 3326–3462 cm⁻¹. The absorption bands at 1649–1717 cm⁻¹ and 711–736 cm⁻¹ were assigned to C=O stretching and C—Br stretching, respectively. In the ¹H NMR spectra of *IIIa–IIIj*, the characteristic two doublets at δ 5.35–5.83 and δ 5.51–6.01 were assigned to H_α and H_β protons (Lahsasni et al., 2014), respectively. Aromatic and methoxyl protons were observed in the ¹H NMR spectra. In the ¹³C NMR spectra, α carbon appeared downfield (δ 49.12–59.11) in relation to β carbon (δ 42.02–46.90) for compounds *IIIa–IIIj*, while the opposite positions were observed in compounds *IIa–IIj* (Table S1). Aromatic, carbonyl, methoxyl and *N,N*-dimethylamino functional group carbons also appeared in the usual manner. The mass spectra of *IIIa–IIIj* showed molecular ion peaks with appropriate intensities.

Antibacterial activity

The in vitro antibacterial activities of compounds *IIa–IIj* and *IIIa–IIIj* were evaluated against four Gram-positive bacteria and seven Gram-negative bac-

Table 3. MIC values of some selected chalcones *II* and 2,3-dibromo derivatives *III* for selected bacterial strains^a

Com- pound	MIC/($\mu\text{g mL}^{-1}$)									
	<i>S. aureus</i>	<i>L. monocytogenes</i>	<i>E. faecalis</i>	<i>B. subtilis</i>	<i>K. pneumonia</i>	<i>C. freundii</i>	<i>C. sakazakii</i>	<i>S. enteritidis</i>	<i>E. coli</i>	<i>Y. pestis</i>
<i>Ila</i>	nd	nd	nd	100	50	nd	nd	nd	50	100
<i>Ilb</i>	nd	nd	nd	nd	nd	nd	200	nd	nd	nd
<i>Ild</i>	nd	nd	25	nd	nd	nd	150	nd	nd	100
<i>Ile</i>	nd	nd	100	nd	nd	nd	nd	nd	nd	nd
<i>Ilh</i>	nd	nd	nd	50	nd	nd	nd	nd	nd	nd
<i>Ili</i>	nd	nd	150	nd	50	nd	nd	nd	nd	nd
<i>IIIa</i>	nd	25	nd	nd	nd	25	nd	nd	nd	100
<i>IIIc</i>	12.5	12.5	50	nd	50	nd	nd	nd	nd	50
<i>IIIe</i>	nd	25	nd	nd	nd	nd	nd	nd	nd	nd
<i>IIIg</i>	6.25	200	12.5	12.5	25	50	50	50	25	100
<i>IIIh</i>	6.25	nd	50	100	nd	100	nd	nd	nd	50
<i>IIIi</i>	nd	nd	50	25	nd	nd	nd	nd	nd	50
<i>IIIj</i>	75	nd	nd	nd	nd	nd	50	nd	nd	nd
NA	6.25	25	12.5	6.25	12.5	6.25	6.25	12.5	6.25	nd

a) NA: nalidixic acid; nd: not determined.

teria using disc diffusion methods. Table 2 shows that all the compounds tested significantly inhibited the growths of Gram-positive and Gram-negative bacteria at 300 μg per disc, and their activities were comparable with the activity of nalidixic acid (positive control at 50 μg per disc). All the compounds exhibited bactericidal activity against *S. aureus*, *L. monocytogenes* (except for *Ild*), *K. pneumonia* (except for *IIIb* and *IIIe*), *C. freundii* (except for *Ile*), *C. sakazakii* (except for *Ilg*), *S. enteritidis* (except for *Ilh*), *E. coli*, *Y. pestis* (except for *Ile*) and *P. aeruginosa*. In addition, most compounds also exhibited antibacterial activity against *E. faecalis* (except for *Ila*, *IIIa*, *IIIb*, *IIIe*) and *B. subtilis* (except for *Ilg*, *IIIe*, *IIIi*). In detail, compound *IIIg* exhibited the highest activity against *S. aureus* followed by compounds *IIIg*, *IIIc* and *IIIi*. Compound *IIIg* exhibited the highest activity against *E. faecalis* (followed by *IIIg*, *IIIh*, *IIIe*), *B. subtilis* (followed by *IIIh*, *Ilg*, *Ila*, *IIIg*), *C. sakazakii* (followed by *IIIi* and *Ild*), *S. enteritidis* (*IIIi*), *E. coli* (followed by *Ila* and *IIIc*) and *P. aeruginosa* (followed by *IIIg*, *IIIc*, *IIIa*). Compound *IIIc* exhibited the highest activity against *L. monocytogenes* (followed by *IIIe* and *IIIa*) and *K. pneumonia* (followed by *Ila* or *IIIi*), while compound *IIIa* exhibited the highest activity against *C. freundii* followed by *IIIg* and *IIIg*. Compounds *IIIc* and *IIIg* showed the highest activity against *Y. pestis*. It is worth noting that the majority of the compounds synthesised exhibited good activity against *Y. pestis*, whereas nalidixic acid showed no activity at 50 μg per disc. The minimum inhibitory concentrations (MICs) of the most active compounds were determined against some selected bacterial strains, and the results obtained are summarised in Table 3. The MIC values of *IIIg* against *S. aureus* (6.25 $\mu\text{g mL}^{-1}$) and *E. faecalis* (12.5 $\mu\text{g mL}^{-1}$) were similar to the MIC value of the standard antibiotic, nalidixic acid. Compound *IIIg*

exhibited the same MIC value (6.25 $\mu\text{g mL}^{-1}$) against *S. aureus* as *IIIg* and nalidixic acid, whereas compound *IIIc* exhibited lower activity (12.5 $\mu\text{g mL}^{-1}$). The MIC values (25 $\mu\text{g mL}^{-1}$) of compounds *IIIa* and *IIIe* against *L. monocytogenes* were identical with the MIC value of nalidixic acid.

Taking into consideration the basic structures of the favourable compounds, bromination at the α,β -position of compounds *Ile*, *Ilg* and *Ilg* to yield compounds *IIIc*, *IIIg* and *IIIg*, respectively, resulted in significant increases in activity against almost all the bacterial strains. The influences of the types and positions of substituents on the antibacterial abilities of compounds *IIIa–IIIj* may be summarised as follows: compound *IIIg* bearing one OH group ($R_3 = \text{OH}$) exhibited the highest activity against all strains except *L. monocytogenes*, *K. pneumonia*, *C. freundii* and *Y. pestis*. Compound *IIIa* with two OH groups ($R_1 = R_3 = \text{OH}$) exhibited better activity than *IIIg* against *C. freundii*. Compound *IIIc* with three OH groups ($R_1 = R_3 = R_4 = \text{OH}$) exhibited better activities than *IIIg* and *IIIa* against *L. monocytogenes*, *K. pneumonia* and *Y. pestis*. Compound *IIIg* with one OH (R_3) and one OMe (R_2) also displayed good activity against *S. aureus*, *E. faecalis*, *Y. pestis* and *P. aeruginosa* whereas compounds *IIIe* and *IIIh* (R_2 or $R_1 = \text{OH}$, respectively) showed lower activity than *IIIg*. Moreover, replacement of the OH group of *IIIg* ($R_3 = \text{OH}$) with other substituents (cf. *IIIi* or *IIIj*) substantially decreased its potency. From the above structure activity relationships, it may be inferred that a R_3 hydroxyl group and a bromine atom at the 2,3-position in the open-chain are crucial for antibacterial activity.

Quantitative structure–activity relationship (Q-SAR) study

The physicochemical properties of drugs such as

Table 4. Physico-chemical properties of chalcones *IIa–IIj* and 2,3-dibromo derivatives *IIIa–IIIj*

Compound	clog <i>P</i> ^a	TPSA ^b /Å ²	Donor Num ^c	Acceptor Num ^d	RB Num ^e	Volume/Å ³
<i>IIa</i>	3.069	57.527	2	3	3	217.888
<i>IIb</i>	3.673	35.539	0	3	5	252.944
<i>IIc</i>	2.985	77.755	3	4	3	225.906
<i>IId</i>	2.843	57.527	2	3	3	217.888
<i>IIe</i>	3.458	35.539	0	3	5	252.944
<i>IIf</i>	3.332	37.299	1	2	3	209.87
<i>IIg</i>	3.15	46.533	1	3	4	235.416
<i>IIh</i>	3.572	37.299	1	2	3	209.87
<i>IIi</i>	4.489	17.071	0	1	3	215.388
<i>IIj</i>	3.914	20.309	0	2	4	247.758
<i>IIIa</i>	3.94	57.527	2	3	4	259.897
<i>IIIb</i>	4.544	35.539	0	3	6	294.953
<i>IIIc</i>	3.856	77.755	3	4	4	267.915
<i>IIId</i>	3.534	57.527	2	3	4	259.897
<i>IIIe</i>	4.149	35.539	0	3	6	294.953
<i>IIIf</i>	4.023	37.299	1	2	4	251.88
<i>IIIg</i>	3.842	46.533	1	3	5	277.425
<i>IIIh</i>	4.443	37.299	1	2	4	251.88
<i>IhIIi</i>	5.18	17.071	0	1	4	257.398
<i>IIIj</i>	4.605	20.309	0	2	5	289.768

a) Calculated octanol/water partition coefficient; b) molecular polar surface area; c) number of hydrogen bond (OH—NH interactions) donors; d) number of hydrogen bond (O—N interactions) acceptors; e) number of rotatable bonds.

octanol/water partition coefficient (clog*P*) and polar surface area (PSA) play important roles in eliciting biological responses. Accordingly, log*P* and PSA are viewed as meaningful parameters in structure-activity relationship studies (Desai et al., 2014; Alam et al., 2013). To explain the quantitative structure-activity relationships (Q-SARs) of chalcones *IIa–IIj* and the corresponding 2,3-dibromo analogues *IIIa–IIIj*, physicochemical calculations were performed using Molinspiration Cheminformatics software. The physicochemical parameters of *IIa–IIj* and *IIIa–IIIj* are listed in Table 4. As these two series of compounds have different molecular geometries (Fig. S1, see Supplementary data), correlations between their values (clog*P* and PSA) and inhibitory potencies against each bacterial strain were considered individually. Figs. S2 and S3 (see Supplementary data) show correlations of clog*P* and PSA values with the inhibitory potencies of *IIa–IIj*, respectively. The results indicated that antibacterial activity increased with increases in log*P* against *S. aureus*, *L. monocytogenes*, *K. pneumonia*, *C. sakazakii*, and *E. coli*, but decreased with increases in log*P* against *E. faecalis* and *P. aeruginosa* (Fig. S2). Conversely, antibacterial activity increased on decreasing PSA against *S. aureus*, *L. monocytogenes*, *C. sakazakii*, *S. enteritidis* and *E. coli*, and activity increased on increasing PSA against *E. faecalis*, *C. freundii*, *Y. pestis* and *P. aeruginosa* (Fig. S3). No good correlation between the log*P* or PSA values and inhibitory potencies was observed for *IIa–IIj* against *B. subtilis*.

Fig. S4 (see Supplementary data) shows that a good correlation trend was observed between clog*P* values and the inhibitory potencies of compounds

IIIa–IIIj, whereby antibacterial activity increased with decreasing log*P* against *E. faecalis*, *C. freundii* and *P. aeruginosa* and activity decreased with decreasing log*P* against *C. sakazakii*. On the other hand, the active compounds among *IIIa–IIIj* also showed a good correlation between the PSA values and their inhibitory potencies, where opposite trends were observed (Fig. S5, see Supplementary data), e.g. activity increased with increasing PSA against *L. monocytogenes*, *K. pneumonia*, *C. freundii*, *E. coli*, *Y. pestis* and *P. aeruginosa*, but decreased with increasing PSA against *B. subtilis*, *C. sakazakii* and *S. enteritidis*.

It is worth noting that the correlations between PSA values versus inhibitory potencies were indirectly proportional for the two compound series (*IIa–IIj* and *IIIa–IIIj*) against *S. aureus*, *L. monocytogenes* (data not shown), *K. pneumonia* and *E. coli* (Fig. S3 and S5); this suggests that compounds *IIa–IIj* and *IIIa–IIIj* might act differently against these bacterial strains. However, a parallel correlation trend was observed between PSA values versus inhibitory potencies for these two compound series against *C. freundii*, *C. sakazakii*, *S. enteritidis*, *Y. pestis* and *P. aeruginosa*. Fig. 2 shows that activity increased with decreasing PSA against *C. sakazakii* and *S. enteritidis*, whilst activity increased with increasing PSA against *Y. pestis* and *P. aeruginosa*. These results lead to the inference that the mode of action of *IIa–IIIj* and *IIIa–IIIj* might be identical for *C. sakazakii* and *S. enteritidis*, but different for *C. freundii*, *Y. pestis* and *P. aeruginosa*. However, the above correlations should be treated with caution since a few exceptions were observed, e.g. clog*P* of *IIc* (2.985) was almost the same as that of *IIa* (3.069) but it exhibited lower activity against *K. pneu-*

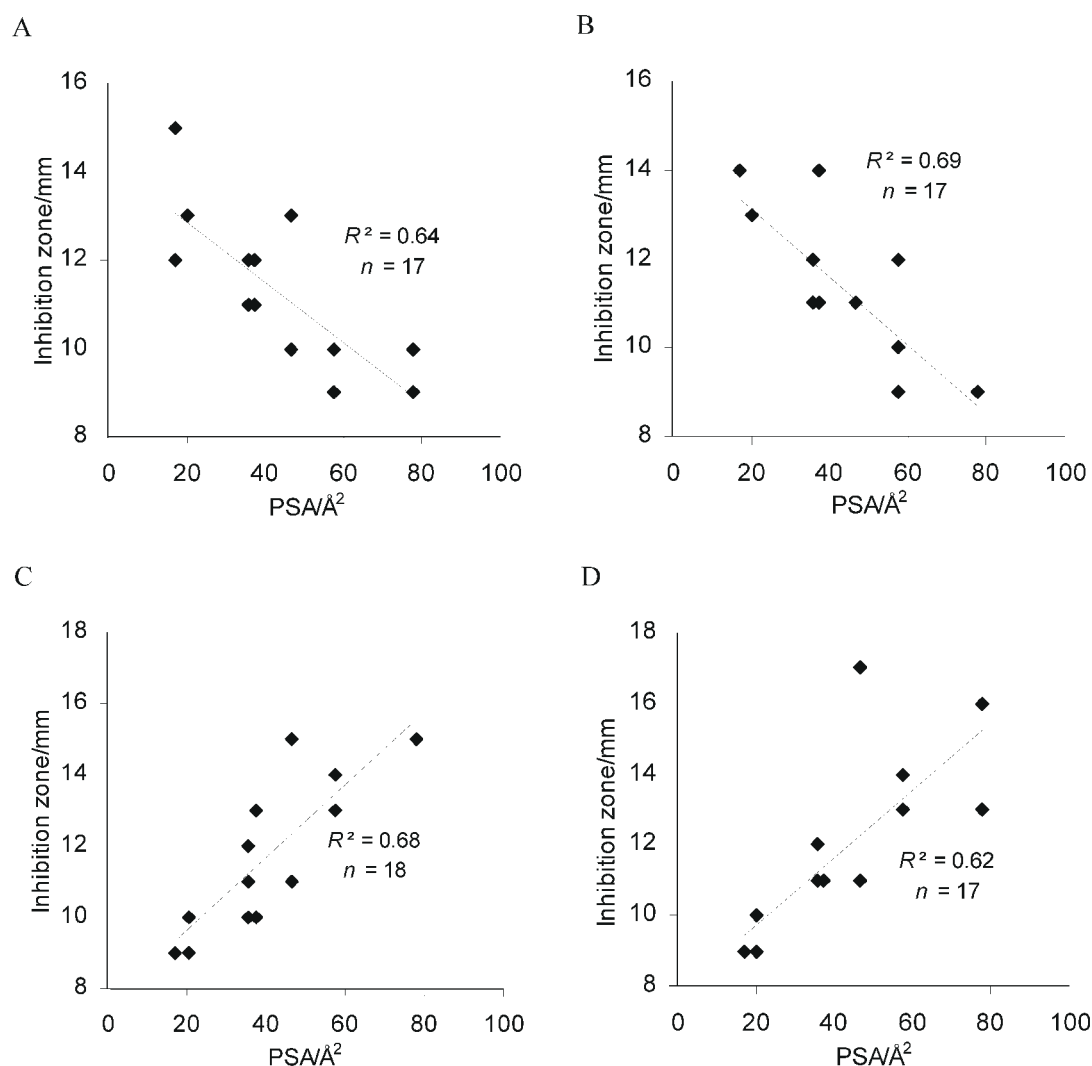


Fig. 2. Correlation between polar surface areas (PSA) and inhibitory potencies for selected chalcones and 2,3-dibromo analogues against *C. sakazakii* (A), *S. enteritidis* (B), *Y. pestis* (C) and *P. aeruginosa* (D).

monia and no activity against *Y. pestis*. Similarly, *IIIc* (3.856) had a similar $\text{clog}P$ to *IIIf* (3.842) but showed lower activity against *E. faecalis*, *B. subtilis*, *C. freundii*, *C. sakazakii* and *S. enteritidis*, and higher activity against *L. monocytogenes*. Moreover, the PSA value of the low activity compound *IIf* (37.299) was the same as that of the moderately active compound *IIIc* (37.299) against *C. sakazakii*. Therefore, the PSA and MLP maps were compared for compounds *IIf*, *IIf*, *IIIb* and *IIIc* (Fig. 3). It was found that the polar surface areas and lipophilicity potentials differed for all molecules, suggesting that the distributions of lipophilic and polar areas at specific locations on the molecular surfaces are also important for biological activity against selected bacteria.

Molecular docking studies

Bacteria possess a variable number (2 to 16) of penicillin-binding proteins (PBPs) and are classified

as A, B and C (Sauvage et al., 2008). The catalytic sites of PBPs are located on the exterior of the bacterial cell wall, allowing the ligand access without the need to cross the lipid bilayer. Hence, PBPs remain excellent targets for antibacterial agents. The PBPs inhibitors exert their bactericidal activity by inhibiting the peptidoglycan biosynthesis leading to the cell lysis (Murray et al., 2006). However, PBP-1a and PBP-1b belong to the class A protein, and are considered to be the primary targets for such structurally diverse antibacterial agents as β -lactams, glycopeptides and other small molecule antibiotics (Yousif et al., 1985; Konaklieva, 2014; Alves et al., 2014). It is considered that PBP-1b acts as a major enzyme for peptidoglycan synthesis (Charpentier et al., 2002) and the bacterial cell lysis capacity of antibiotics is correlated with their affinity for PBP-1b compared with that of PBP-1a (Spratt et al., 1977). Moreover, among the high-molecular mass PBPs, PBP-1b exhibits both transglycosylase and transpeptidase activities (Terrak et al.,

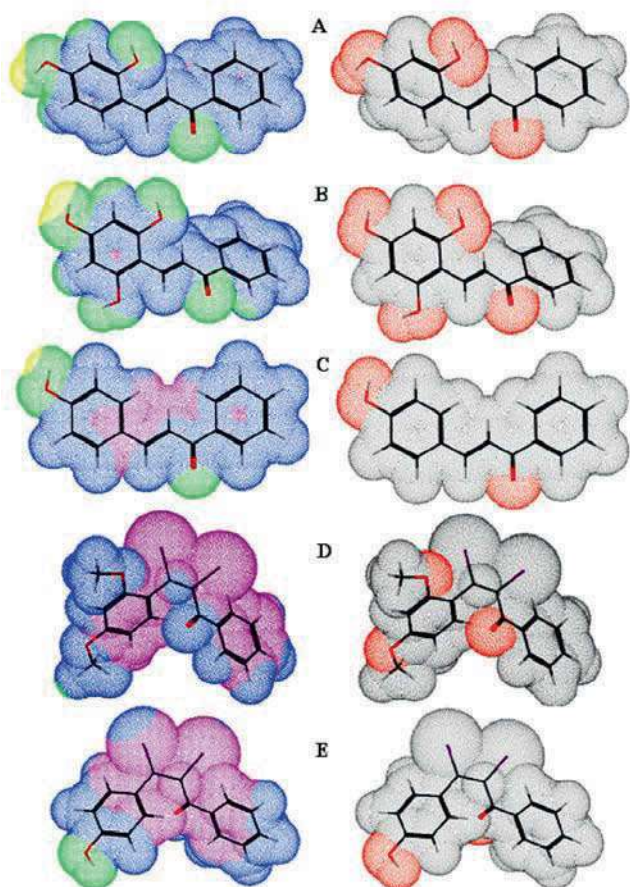


Fig. 3. Molecular lipophilicity potentials (left) and polar surface areas (right) for *Iia* (A), *Iic* (B), *Iif* (C), *Iib* (D) and *Iif* (E) showing areas of lipophilicity (blue), intermediate lipophilicity (pink), greatest hydrophilicity (yellow), and intermediate hydrophilicity (green); non-polar and polar areas are depicted in grey/white and red, respectively.

1999), while other isoforms of PBP behave only as transpeptidases (Adam et al., 1997). However, among the bacterial strains used in the present study, the synthesised compounds showed the highest bactericidal activity against *S. aureus* (cf. *IIIc*, *IIIf*, *IIIg*). Therefore, to predict the possible mechanism by which the chalcone derivatives can induce antibacterial activity, molecular docking of the potent antibacterial compound *IIIf* was performed on the binding model based on PBP-1b of *S. aureus* (2Y2H.pdb) and the docking result of *IIIf* was compared with the interaction of *N*-alkyl boronic acid analogues, the PBP-1b inhibitors of *S. aureus* (Contreras-Martel et al., 2011). The docking results show (Fig. S6, Supplementary data) that compound *IIIf* binds to the same active pocket of the PBP-1b receptor as that of endogenous boronate ligands. The active site of PBP-1b of *S. aureus* consists of two pockets, e.g. pocket 1 surrounded by ALA459, SER457, ASN518 and MET556 amino acid residues and pocket 2 surrounded by ALA499, VAL628, ASP658, and GLN686 amino acid residues. However, only the alkyl boronic acid analogues binding to pocket 2 show antibacterial activity (Contreras-Martel et al., 2011). Fig. 4 shows that compound *IIIf* significantly interacts with ALA499, VAL628, and GLN686 including other amino acid residues in pocket 2 of PBP-1b receptor.

The binding affinity of $-6.30 \text{ kcal mol}^{-1}$ (1 kcal = 4.1868 kJ) was observed with RMSD (root mean square deviation) of 1.41 Å from the endogenous boronate ligand binding pose. It was observed that six amino acid residues (THR654, TYR690, TYR498, GLY689, GLN687 and MET661) are involved in electrostatic and covalent interactions and five other amino acid residues (ALA499, THR629, VAL628, TYR515 and GLN686) are also involved in the van

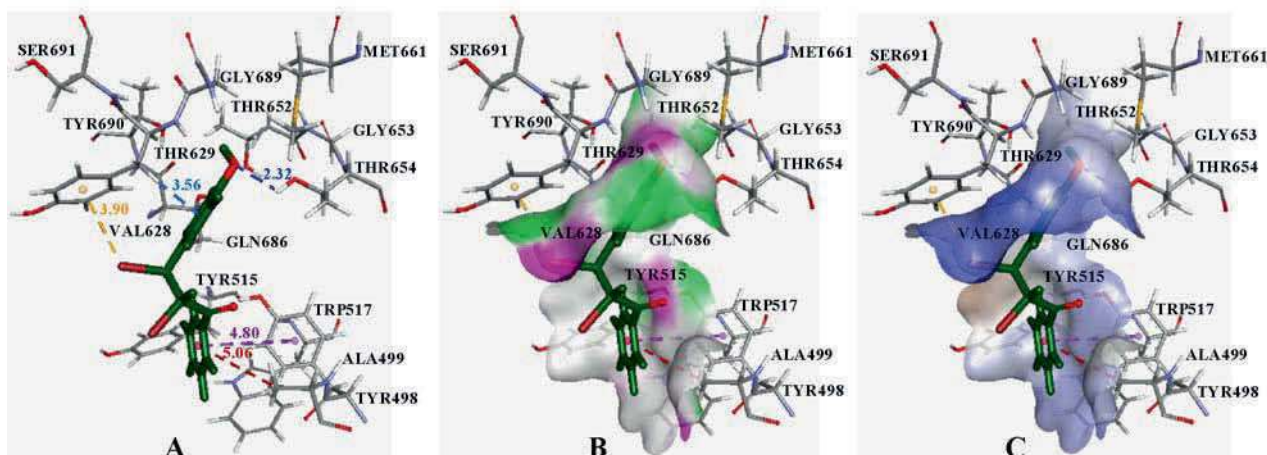


Fig. 4. Molecular docking modelling of compound *IIIf* (dark green) with penicillin-binding protein (PBP-1b: H-bond (Ph—OH and THR654, 2.32 Å), π - σ (Ph—OH and TYR690, 3.56 Å), π - π (Ph and Tyr498, 4.80 Å, and π -alkyl (Ph and ALA499, 5.06 Å; β -Br and TYR690, 3.90 Å) interactions (A); hydrogen bond interaction showing donor (pink) and acceptor surfaces (green) (B); hydrophobic interaction showing hydrophobic surface (purple) (C).

Table 5. DPPH radical-scavenging activities of chalcones *IIa–IIj*, 2,3-dibromo derivatives *IIIa–IIIj* and ascorbic acid (AA)

IC ₅₀ /μM																				
<i>IIa</i>	<i>IIb</i>	<i>IIc</i>	<i>IId</i>	<i>IIe</i>	<i>IIf</i>	<i>IIg</i>	<i>IIh</i>	<i>IIi</i>	<i>IIj</i>	<i>IIIa</i>	<i>IIIb</i>	<i>IIIc</i>	<i>IIId</i>	<i>IIIe</i>	<i>IIIf</i>	<i>IIIg</i>	<i>IIIh</i>	<i>IIIi</i>	<i>IIIj</i>	AA
5.15	29.5	1.68	5.20	27.96	9.36	2.46	4.90	25.20	7.98	8.75	21.56	1.44	5.25	5.84	14.30	12.10	13.0	31.25	12.17	1.03

der Waals interaction with compound *IIIj*, in which one hydrogen bond, one π - σ , one π - π and two π -alkyl interactions were observed between *IIIj* and the protein receptor. In the binding model (Fig. 4A), *IIIj* is bound to the cellular protein receptor via the hydrogen bond between the oxygen atom of the hydroxyl group of *IIIj* and the hydrogen atom of the hydroxyl group of THR654 (Ph—O···H—O, distance: 2.32 Å). The major electrostatic π - σ and π - π interactions were observed between phenyl ring B and TYR690 (distance: 3.56 Å) and phenyl ring A and TYR498 (distance: 4.80 Å), whereas two π -alkyl interactions were observed between phenyl ring A and ALA499 (distance: 5.06 Å) and β -methylene bromine and TYR690 (distance: 3.90 Å). Since *S. aureus* is one of the most hydrophobic organisms (Sivakumar et al., 2009), *IIIj* exhibited a prominent hydrophobic interaction around bromine in the β -methylene position and phenyl ring B with TYR690, THR629 and VAL628 residue of the receptor (Fig. 4C), which is consistent with the result of the structure activity relationship study.

Antioxidant activity

Free radical-scavenging activities using DPPH were evaluated for compounds *IIa–IIj* and *IIIa–IIIj*. Table 5 shows that all compounds exhibited significant DPPH radical-scavenging activity. Of the compounds tested, *IIIc* showed the highest activity followed by *IIc*, *IIg*, *IIh*, *IIa*, *IId*, *IIId*, *IIIe* and *IIj*. Furthermore, the IC₅₀ values of compounds *IIIc* (1.44 μM) and *IIc* (1.68 μM) were comparable to that of the standard antioxidant agent, ascorbic acid (1.03 μM). Polyphenolic compounds containing several hydroxyl groups, such as *IIIc* or *IIc*, are known to exhibit excellent antioxidant activities. In effect, compound *IIc* exhibited the highest antioxidant activity with an IC₅₀ value of 1.68 μM, and bromination at the olefinic double bond of *IIc* afforded *IIIc*, which exhibited slightly more activity with an IC₅₀ value of 1.44 μM. Bromination of *IId* yielded *IIId*, but the activity was unchanged whereas bromination of *IIa*, *IIf* and *IIh*, producing compounds *IIIa*, *IIIf* and *IIIh*, respectively, resulted in 1.53–2.65-fold decreases in radical-scavenging activities. Of the non-hydroxylated analogues, *IIIe* and *IIj* showed significant activity, whereas the activities of *IIb*, *IIe*, *IIi*, *IIIb*, *IIIi* and *IIIj* were relatively weak. In addition, the bromination of *IIb* and *IIe*, affording *IIIb* and *IIIe*, resulted in 1.37-fold and 4.79-fold increases in activity, respectively. Finally, the bromi-

nation of *IIi* and *IIj*, yielding *IIIi* and *IIIj*, resulted in 1.24-fold and 1.52-fold reductions in activity, respectively. The above results are in agreement with the known antioxidant activities of polyhydroxylated chalcone analogues.

Conclusions

The present study details the synthesis of chalcone derivatives and the results of their biological evaluations as antibacterial agents and antioxidants. The inhibitory effects on eleven bacterial strains and DPPH radical-scavenging activities were investigated in twenty chalcones. All the compounds significantly inhibited Gram-positive and Gram-negative bacteria, but compound *IIIj* exhibited the greatest inhibition zones against all bacterial strains except for *K. pneumonia* JCM 1662, *C. freundii* JCM 1657 and *Y. pestis* CARS 2013-027. Furthermore, compound *IIIj* had MIC values of 6.25 μg mL⁻¹ and 12.5 μg mL⁻¹ against *S. aureus* JMC 2151 and *E. faecalis* CARS 2011-012, respectively, and these values were similar to those of the standard antibiotic agent, nalidixic acid. Quantitative structure–activity relationship studies (Q-SAR) using physicochemical parameters indicated that the bactericidal activities of the chalcone derivatives correlated well with the calculated PSA and MLP values. Docking simulation was performed to position compound *IIIj* into the active site of the PBP-1b receptor of *S. aureus* to study the probable binding mode. In addition, most of the compounds synthesised exhibited significant DPPH radical-scavenging activity; *IIc* and *IIIc* had potencies similar to that of the standard antioxidant, ascorbic acid.

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Supplementary data

The supplementary data associated with this article can be found in the online version of this paper (DOI: 10.1515/chempap-2015-0113).

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