Med Chem Res (2012) 21:444–452
DOI 10.1007/s00044-011-9549-9

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and crystal structure of chalcones as well as on cytotoxicity and antibacterial properties

Jianzhang Wu · Cong Wang · Yuepiao Cai · Jing Peng · Donglou Liang · Yunjie Zhao · Shulin Yang · Xiaokun Li · Xiaoping Wu · Guang Liang

Received: 21 August 2010 / Accepted: 4 January 2011 / Published online: 18 January 2011

Abstract A series of chalcones derivatives were synthesized and evaluated for cytotoxic and antibacterial activities in vitro. These modifications changed their bioactivity profile and indicated a combination of SAR analysis toward the substituents in rings A and B of chalcones. Compounds **2, 6–8, 14–17**, and **32** exhibited good cytotoxic properties against two human cancer cell lines HT29 and SGC7901. Compounds **16** and **17** showed high antibacterial activity toward 14 clinically isolated multidrug-resistant strains. Subsequently, the structure of bi-bioactive compound **16** was determined using single-crystal X-ray diffraction. This study presents a few novel leading compounds for the development of potential antitumor and antibacterial agents.

Keywords Chalcones · Synthesis · Cytotoxicity · Antibacterial activity · Crystal structure

Jianzhang Wu, Cong Wang contribute equally to this work.

J. Wu · C. Wang · Y. Cai · J. Peng · D. Liang · Y. Zhao · X. Li · X. Wu · G. Liang (✉)
Bioorganic & Medicinal Chemistry Research Center, School of Pharmacy, Wenzhou Medicinal College, Wenzhou, Zhejiang 325035, People's Republic of China
e-mail: cuiliang1234@gmail.com

J. Wu · S. Yang · X. Li · G. Liang
Institute of Biotechnology, Nanjing University of Science and Technology, Nanjing 210094, People's Republic of China

C. Wang · X. Wu
Institute of Tissue Transplantation and Immunology, Jinan University, Guangzhou 510632, People's Republic of China

Introduction

Chalcones, with the common skeleton of 1,3-diaryl-2-propen-1-ones, are essential intermediate compounds in flavonoid biosynthesis in plants (Nowakowska, 2007). Chemically, they are open-chained molecules consisting of two aromatic rings linked by a three-carbon α,β-unsaturated carbonyl system. Extensive investigations have demonstrated active biological properties of natural and synthetic chalcones, including antiinflammatory (Tanaka *et al.*, 2009; Park *et al.*, 2009; Heidari *et al.*, 2009; Ko *et al.*, 2003), antioxidant (Padhye *et al.*, 2009; Ghosh *et al.*, 2009), antimalarial (Bhattacharya *et al.*, 2009; Tomar *et al.*, 2010; Dominguez *et al.*, 2005), antitumoral (Ratkovic *et al.*, 2010; Hua *et al.*, 2009; Henmi *et al.*, 2009; Tang *et al.*, 2008; Kamal *et al.*, 2008; Boumendjel *et al.*, 2008; Rao *et al.*, 2004), and antibacterial (Batovska *et al.*, 2009; Nowakowska *et al.*, 2008; Liu *et al.*, 2008; Avila *et al.*, 2008; Nielsen *et al.*, 2005) properties in vitro and in vivo. Among currently identified anti-tumor agents, chalcones represent an important class of naturally small molecules useful in cancer chemotherapy (Ratkovic *et al.*, 2010; Boumendjel *et al.*, 2008; Kumar *et al.*, 2003). It is demonstrated that chalcones exhibit antimitotic properties caused by inhibition of tubulin polymerization by attaching to the colchicine-binding site (Boumendjel *et al.*, 2008).

Cancer and infective disease are two of the leading causes of human death in developing as well as advanced countries. Although many therapeutic strategies including chemotherapy and radiotherapy are available, high systemic toxicity and drug resistance limit the successful outcomes in many cases. Therefore, novel treatment approaches and therapeutic agents are urgently needed for the cancer therapy and multi-drug-resistant infective disease. Among chalcones and their synthetic derivatives,

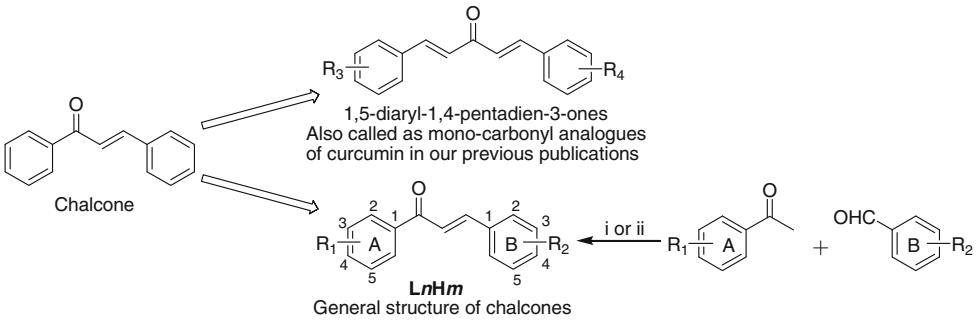
several compounds displayed cytotoxic activity toward cultured tumor cells (Ratkovic *et al.*, 2010; Hua *et al.*, 2009; Henmi *et al.*, 2009; Tang *et al.*, 2008; Kamal *et al.*, 2008; Boumendjel *et al.*, 2008; Rao *et al.*, 2004) and inhibitory properties against pathogenic bacteria (Batovska *et al.*, 2009; Nowakowska *et al.*, 2008; Liu *et al.*, 2008; Avila *et al.*, 2008; Nielsen *et al.*, 2005). They were also effective in vivo as anti-tumor, chemopreventing, antibacterial, and anti-inflammatory agents.

To find the broad spectrum biologically active chalcones, we have previously described the anti-inflammatory, antimicrobial, and antitumor activities of chalcone analogs containing 5-C central linker (Liang *et al.* 2009). As part of our ongoing research for potential anticancer drug candidates, in the present study, a series of chalcones derivatives with 3-C central linker were synthesized and tested for cytotoxicity and antibacterial activity in vitro. Most compounds exhibited good cytotoxic properties against human cancer cell lines HT29 and SGC7901. Several compounds displayed good antibacterial activity, especially against multidrug-resistant gram-positive bacteria. Subsequently, a structure–activity relationship analysis was also carried out to understand the structural requirements for optimum activity and the most active compound **16** was determined through single-crystal X-ray diffraction.

Results and discussion

Chemistry

An amount of 40 chalcones whose structures were shown in Scheme 1 were synthesized by Claisene-Schmidt condensation between acetophenones (acetophenone, 4′-aminoacetophenone, 3′-aminoacetophenone, 4′-methoxyacetophenone, 2′-hydroxyacetophenone, and 3′,5′-difluoroacetophenone) and aryl aldehydes. As shown in Scheme 1, compounds **3**, **12** and **16** were synthesized by reflux in acidic media using HCl as catalyst, while other chalcones were synthesized at 5°C with a catalytic amount of NaOH. All reactions were monitored on the silica gel thin layer chromatography. The yields of products were in the range of 10 and 95% after purification. In general, the yields of reactions using NaOH as catalyst were greater than that of reactions under HCl circumstance. Details of yields, melting points, ^{1}H-NMR, IR, and ESI–MS analysis are described in experimental section. The ^{1}H-NMR spectral evidence that the coupling constants of double bonding hydrogens reached 15–16 Hz confirmed the trans-conformation in the structures of all chalcones. Before used to the biological experiments, compounds were purified using re-crystallization or column chromatography and were determined by HPLC method to meet purity more than 98%.



Scheme 1 Design, chemical structures, and general synthesis of chalcones. *Reagents and conditions* (i) HCl and reflux for **3**, **12**, and **16**; (ii) 5°C and NaOH for other compounds

Comp.	R_1 in ring A	R_2 in ring B	Comp.	R_1 in ring A	R_2 in ring B
1	4-NH_2	2-Cl,4-Cl	**21**	3-F, 5-F	2-OCH_3, 4-OCH_3
2	4-OCH_3	2-Cl,4-Cl	**22**	4-NH_2	2-OCH_3
3	2-OH	2-Cl,4-Cl	**23**	4-OCH_3	2-OCH_3
4	3-NH_2	4-$N(CH_3)_2$	**24**	3-F, 5-F	2-OCH_3
5	3-F,5-F	4-$N(CH_3)_2$	**25**	4-NH_2	2-OCH_3, 3-OCH_3
6	4-NH_2	2-Cl	**26**	3-NH_2	2-OCH_3, 3-OCH_3
7	4-NH_2	2-Cl	**27**	4-OCH_3	2-OCH_3, 3-OCH_3
8	H	4-OCH_3	**28**	H	2-CF_3
9	4-NH_2	4-OCH_3	**29**	4-NH_2	2-CF_3
10	3-NH_2	4-OCH_3	**30**	4-OCH_3	2-CF_3
11	4-OCH_3	4-OCH_3	**31**	4-NH_2	2-Br
12	2-OH	4-OCH_3	**32**	4-OCH_3	2-Br
13	3-F,5-F	4-OCH_3	**33**	4-OCH_3	2-F, 4-F
14	4-NH_2	2-F	**34**	3-OCH_3, 4-OCH_3	2-Cl,4-Cl
15	4-OCH_3	2-F	**35**	3-OCH_3, 4-OCH_3	4-$N(CH_3)_2$
16	H	4-OH	**36**	3-OCH_3, 4-OCH_3	2-Cl
17	4-OCH_3	4-OH	**37**	3-OCH_3, 4-OCH_3	4-OCH_3
18	H	2-OCH_3, 4-OCH_3	**38**	3-OCH_3, 4-OCH_3	2-OCH_3, 4-OCH_3
19	4-NH_2	2-OCH_3, 4-OCH_3	**39**	3-OCH_3, 4-OCH_3	2-OCH_3, 3-OCH_3
20	2-OH	2-OCH_3, 4-OCH_3	**40**	3-OCH_3, 4-OCH_3	2-Br

Pharmacology

Cytotoxic properties

It has been reported that chalcones possess a wide-spectrum of anti-tumor properties, especially against digestive cancers. The in vitro cytotoxicity against HT29 (human colorectal cancer) and SGC7901 (human gastric cancer cell) were evaluated using the MTT method. Table 1 lists the IC_{50} values of present chalcones against two cell lines. 5-Fluorouracil (5-Fu) was used as a positive control. Most of the chalcones exhibited antiproliferative activities against selected tumor cells. Arbitrary thresholds of IC_{50}<30 μM were used to identify promising compounds. Among tested chalcones, 19 ones were found to have IC_{50} values that were lower than 30 μM against different cell lines, and several compounds demonstrated higher cytotoxicity than the positive 5-Fu, especially against SGC7901 cells.

As an excellent leading compound, chalcones have been extensively investigated in medicinal chemistry. The simple structure and the ease of preparation render chalcones an attractive scaffold for structure–activity relationship (SAR) studies, and a wide number of substituted chalcones have been synthesized to evaluate effects of various functional groups on biological activities (Ratkovic *et al.*, 2010; Hua *et al.*, 2009; Henmi *et al.*, 2009; Tang *et al.*, 2008; Kamal *et al.*, 2008; Boumendjel *et al.*, 2008; Rao *et al.*, 2004). Present compounds were designed to evaluate the SAR on different substitutions (both electron-donating and electron-withdrawing groups), as well as different positions on two phenyl rings (A and B).

The data given in Table 1 indicate that the change in the substituent of the phenyl B ring affects the cytotoxic activity of chalcones. When the B ring is substituted for strong electron-donating 4-$N(CH_3)_2$ or 2,4-dimethoxy groups, the compounds generally display little bioactivity, such as **4, 5**, **18–21, 35**, and **38**. The introduction of either electron-withdrawing or slightly weak electron-donating substituents (derivatives **1–3**, **6–7**, **8–13**, **14–15**, **16–17**, **25–27**, **31–34**, **36**, and **40**) enhanced antiproliferative activity. No significant difference was observed between chalcones substituted by 2-halogen in ring B. The slightly weak electron-donating substituents including 4-methoxy and 2,3-dimethoxy in ring B also increased the inhibitory activity against tumor cells. For example, the anti-SGC7901 effects of **8** and **26** were highest among all tested compounds and positive control, with IC_{50} of 7.25 and 6.21 μM, respectively.

Several studies have demonstrated the positive influence of a polymethoxylated ring A on cytotoxicity against tumor cell lines. For example, Ducki and colleagues reported the importance of methoxylation on the A-ring (Ducki, 2009).

Table 1 Cytotoxicity of present compounds against three tumor cells

Compound	IC_{50} (μM)	
	HT29	SGC7901
1	39.98 ± 1.37	87.71 ± 23.26
2	18.87 ± 2.12	12.58 ± 0.77
3	42.36 ± 0.83	ND
4	NA	N.D.
5	NA	NA
6	29.44 ± 3.02	11.64 ± 2.01
7	26.25 ± 1.45	11.15 ± 1.37
8	24.19 ± 1.54	7.25 ± 1.87
9	55.68 ± 5.6	20.17 ± 3.19
10	42.78 ± 2.86	53.76 ± 0.89
11	49.06 ± 9.04	19.21 ± 7.4
12	59.54 ± 24.36	62.10 ± 16.63
13	101.04 ± 23.62	NA
14	47.82 ± 5.43	7.44 ± 0.88
15	18.87 ± 4.55	21.24 ± 0.9
16	24.60 ± 2.94	13.74 ± 0.16
17	18.56 ± 4.66	14.69 ± 3.17
18	130.65 ± 52.38	ND
19	NA	NA
20	NA	NA
21	NA	55.54 ± 0.83
22	ND	7.87 ± 6.25
23	ND	NA
24	53.20 ± 9.12	75.41 ± 15.13
25	69.58 ± 34.85	61.24 ± 1.36
26	43.74 ± 3.66	6.21 ± 0.98
27	41.74 ± 4.13	13.08 ± 2.18
28	42.86 ± 0.54	40.34 ± 10.55
29	ND	15.72 ± 12.05
30	36.43 ± 15.34	ND
31	35.40 ± 10.32	39.61 ± 4.74
32	25.74 ± 5.82	19.97 ± 5.26
33	11.13 ± 4.48	48.41 ± 7.60
34	18.69 ± 3.23	60.5 ± 2.68
35	49.83 ± 8.48	NA
36	15.45 ± 1.35	24.71 ± 1.78
37	54.62 ± 9.75	101.72 ± 19.84
38	65.50 ± 4.44	NA
39	35.86 ± 7.88	70.67 ± 0.50
40	13.95 ± 2.38	21.43 ± 3.53
5-Fu	9.72 ± 1.21	29.19 ± 4.72

ND not determined; *NA* no activity

Recently, Boumendjel *et al.* demonstrated that dimethoxylation or trimethoxylation at 2,4,6-carbons was highly beneficial to cell cycle arrest at G2/M, while hydroxylation at 2-position was generally detrimental (Boumendjel *et al.*,

2008). However, the results from Tzeng group contrasted with previous results, showing high cytotoxicity of 2-hydroxylated chalcones against Jurkat and U937 cancer cells (Rao *et al.*, 2004). Thus, a clear correlation between the substituting pattern of chalcones on the ring A and their cytotoxicity appears to be difficult to establish. As depicted in Table 1, all the chalcones with electron-donating substituents including -NH_2, -OCH_3 and -OH on the ring A displayed a strong capacity to inhibit cell proliferation (compounds **4**–**5** and **18**–**21** are still inactive due to electron-donating-substituted B ring), while the weak activities were observed on 3,5-difluoride chalcones **13**, **21**, and **24**. We also analyzed the effects of different position (2′, 3′, or 4′) in ring A where an electron-donating moiety occupied. Both 2-substituted and 3-substituted chalcones (**3** and **10**) with electron-donating groups exhibited cytotoxicity, but with relatively high IC_{50} values. Taking together, the analysis of substituents in ring A showed that the cytotoxic activity of chalcones could be increased by placing an electron-donating substituent, especially at 4-position, and could be reduced through the induction of an electron-withdrawing substituent. Without the electron-effective moiety in ring A, the compounds also exhibited excellent cytotoxic properties. For example, the IC_{50} values of **8** and **16** (against SGC7901) were all below 15 μM.

Digestive cancers are most sensitive to chalcones. Here, we observed that a majority of compounds exerted high cytotoxicity against two digestive cancer cell lines HT29 and SGC7901. The underlying molecular mechanism and the development of new anti-cancer agents from chalcones are the focus of our continuing research.

Antibacterial activity

A number of bacteria with multiple drug resistance pose a serious threat to human health. Many clinically efficacious antibiotics have become less effective due to the development of drug resistance (Rodriguez *et al.*, 2010; Lee *et al.*, 2009). Natural products are a vital source of structurally diverse compounds to spur the development of new antibacterial agents. Among chalcone derivatives, licochalcone A, naturally isolated from the roots and rhizomes of *Glycyrrhiza inflata*, is an active leading compound against a wide range of Gram-positive organisms (Tsukiyama *et al.*, 2002). Recently, the antibacterial activity of chalcones has been increasingly documented. Many research groups synthesized novel chalcones that possess antibacterial activity (Batovska *et al.*, 2009; Nowakowska *et al.*, 2008; Liu *et al.*, 2008; Avila *et al.*, 2008; Nielsen *et al.*, 2005; Rizvi *et al.*, 2010).

One of our long-term goals is to find novel antibiotic agents that can reverse multidrug resistance. Here, 14 multidrug-resistant bacteria strains were used for these anti-bacterial activity studies. All strains were clinically isolated from local hospitalized patients in the First Affiliated Hospital of Wenzhou Medical College and characterized by the Microbial Department in this hospital. *Staphylococcus aureus* ATCC 25923 (*S. aureus* 25923) purchased from ATCC was used as a non-resistant control. Details of the 14 strains are described in the reference. The antibacterial activity of present chalcones was evaluated by the minimum inhibitory concentration (MIC) method.

Table 2 listed the MIC values of six active chalcones against seven Grams-positive strains and eight Gram-negative strains. Other chalcones synthesized in this study exhibited no activity against these strains. The quinolones antibiotic levofloxacin served as a positive control. As expected, some quinolones-resistant strains were not sensitive to levofloxacin. In general, these compounds possessed higher activity against gram-positive bacteria than gram-negative bacteria. Among these drug-resistant bacteria, *Enterococcus faecalis* 609230, *S. aureus* 25923, and *S. aureus* 701230 were more sensitive to these compounds. The 4-(dimethylamino) substituent in ring B of chalcones may be of importance, for example, **16**, **17**, **25,** and **26** inhibited Gram-positive bacteria growth in a broad spectrum. Batovska *et al.* (2009) reported that **16** had strong anti-staphylococcal activity with an MIC of 125 μg/ml. Here, our data further supported earlier result, showing that **16** and **17**, especially **17**, have strong broad spectrum antibacterial activity. Thus, the electron-donating substituents at 4-position of ring B may contribute to the antibacterial activity of chalcones. Although the antibacterial activity of chalcones is not very strong, these stains are all multidrug-resistant and several are insensitive to levofloxacin. Inhibition against them suggests more significant meanings in clinical therapy. The antibacterial mechanism of the active chalcones may differ from the current drugs. Compound **17** showed highest in vitro activity (MIC $<$80 μg/ml) and it is currently under further investigation as a potential anti-bacterial agent.

The bacterial burden on human health is quickly outweighing available therapeutics due to the multidrug resistance. Combined with a diminishing number of new agents entering clinical practice, such resistance is widely recognized as a major threat to public health. Our results may lead to development of new lead compounds as antibacterial agents against multidrug-resistant clinical strains.

X-ray crystallographic analysis of 16

Finally, compound **16**, which displayed duplicate activity, was re-crystallized from CH_3OH/CH_2Cl_2 (1:2) and its single-crystal structure were determined by X-ray diffraction. The structural analysis revealed that two **16** molecules combined by a hydrogen bonding in one unit cell. All H

Table 2 In vitro antibacterial activity of chalcones against bacteria strains

Compound	Minimum inhibitory concentration (MIC, μg/ml) against gram-positive bacteria strains						
	S.a 25923	S.a 701230	S.a 701219	S.e 620206	S.e 619083	E.f 609230	E.fm 609205
8	279	>1000	>1000	>1000	>1000	279	>1000
9	537	537	>1000	>1000	>1000	537	>1000
16	158	158	158	158	158	158	315
17	40	81	81	81	20	81	>1000
25	325	325	325	325	325	325	325
26	81	161	161	>1000	81	>1000	323
Levofloxacin	12.5	12.5	100	>1000	125	>1000	>1000
Compound	Minimum inhibitory concentration (MIC, μg/ml) against gram-negative bacteria strains						
	E.c 629119	E.c 629247	K.p 626511	K.p 626238	B-5 302234	B-8 303211	P.a 630266	P.a 701218
8	>1000	>1000	279	279	>1000	>1000	279	279
16	158	158	>1000	>1000	158	158	>1000	158
17	548	548	>1000	>1000	548	548	>1000	>1000
25	325	325	325	325	325	325	325	325
Levofloxacin	>1000	25	125	>1000	>1000	12.5	325	>1000

Information of clinically isolated multidrug-resistant strains: S. a 25923 = *Staphylococcus aureus* ATCC 25923; S.a 701230 = *Staphylococcus aureus* (gentamicin resistant); S.a 701219 = *Staphylococcus aureus* (penicillin G resistant); S. e 620206 = *Staphylococcus epidermidis* (ampicillin, penicillin G, bactrim, erythromycin, gentamicin and oxacillin resistant); S. e 619083 = *Staphylococcus epidermidis* (bactrim, clindamycin and amoxicillin resistant); E. f 609230 = *Enterococcus faecalis* (moxifloxacin, levofloxacin, rifampicin and tetracycline resistant); E. fm 609205 = *Enterococcus faecium* (levofloxacin, penicillin G, rifampicin and tetracycline resistant); E. c 629119 = *Escherichia Coli* (Ciprofloxacin, Tetracycline, erythromycin, ampicillin, gentamicinampicillin and gentamicin resistant); E. c 629247 = *Escherichia Coli* (ampicillin, aztreonam, ceftriaxone, cefepime, bactrim, sulbactam, cefazolin and ceftazidime resistant); K. p 626511 = *klebsiella pneumonia* (ampicillin resistant); K. p 626238 = *Klebsiella pneumonia* (ampicillin, ciprofloxacin, nitrofurantoin and levofloxacin resistant); B5 302234 = baumanii (ampicillin, sulbactam, aztreonam, ciprofloxacin, ceftriaxone, cefazolin, nitrofurantoin, cefepime, gentamicin, tienam, levofloxacin, bactrim, ceftazidime, Tobramycin, Piperacillin, Rifampicin and Tetracycline resistant); B8 303211 = baumanii (ampicillin, nitrofurantoin and cefazolin resistant); P. a 630266 = *Pseudomonas aeruginosa* (ampicillin, sulbactam, ceftriaxone, cefazolin, nitrofurantoin and bactrim resistant); P. a 701218 = *Pseudomonas aeruginosa* (ampicillin, sulbactam, ceftriaxone, cefazolin, nitrofurantoin, bactrim, tienam, ciprofloxacin and levofloxacin resistant))

atoms were located in different Fourier maps and were refined freely in idealized positions, with C–H = 0.93 Å for aromatic H and vinyl H or 0.96 Å for methylene H. The crystal structure of the unit cell of **16** is shown in Fig. 1. The details of the crystal data, data collection, and refinement are listed in Table 3. The geometry lengths of the intermolecular hydrogen bond are given in Table 4. The results showed that the crystal of **16** belongs to the orthorhombic system, space group $P2(1)2(1)2(1)$ with $a = 5.3796$ (6) Å, $b = 12.9543$ (14) Å, $c = 17.1522$ (18) Å, $\alpha = \beta = \gamma = 90°$, $V = 1195.2$ (2) Å^3, $Z = 4$, $F_{000} = 472$, $D_x = 1.246$ Mg m^{-3}, and $\mu = 0.08$ mm^{-1}. A dihedral angle between the two phenyl rings C4/C5/C6/C7/C8/C9 and C10/C11/C12/C13/C14/C15 is 25.39°.

Conclusion

In summary, a series of chalcones were synthesized as potent cytotoxic agents against two human digestive tumor cell lines and antimicrobial agents against 14 clinically isolated multidrug-resistant strains. Our structural design altered bioactivity profile of the chalcones and indicated a combination of SAR analysis toward the substituents in rings A and B of chalcones. The electron-donating modification in ring A may be pharmacologically favorable for chalcones-based anti-tumor drug development. Our data also yielded a few novel leading compounds for the development of potential antibacterial candidates with the ability of reversal of multidrug resistance. In addition, the crystal structure of compound **16** with strong cytotoxicity and anti-bacterial activities was reported.

Experimental section

General procedure for the synthesis of chalcones

^{1}H-NMR spectra were recorded on a Bruker AVANCE 600 MHz spectrometer. Electron-spray ionization mass spectra in positive mode (ESI–MS) data were recorded on Bruker Esquire HCT ion-trap mass spectrometer equipped

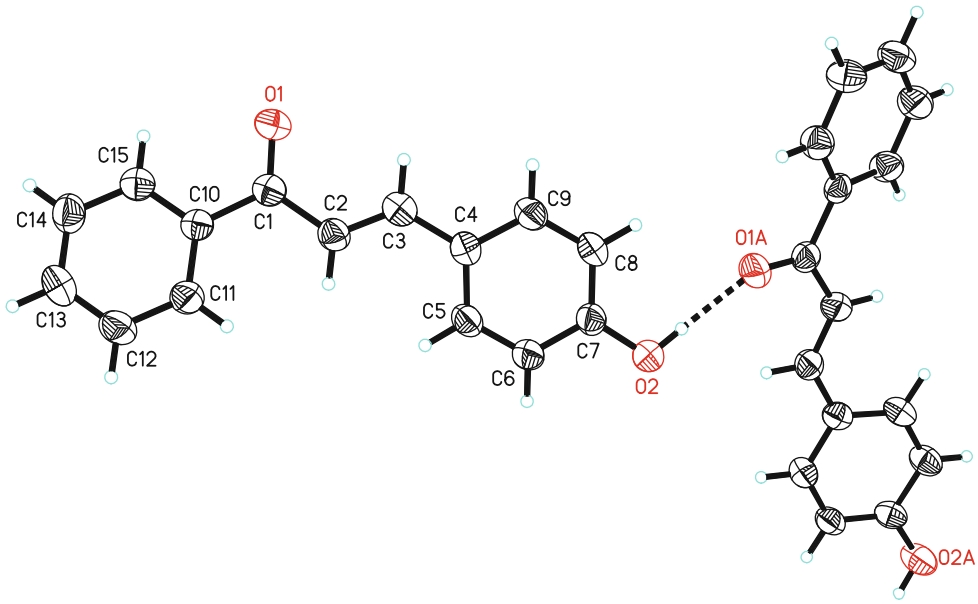


Fig. 1 The crystal structure of **16** in a unit cell with labeled non-hydrogen atom, showing displacement ellipsoids at the 50% probability level. *Dashed lines* indicate intramolecular hydrogen bonding

Table 3 Crystal data of **16**

Crystal data		
$C_{15}H_{12}O_2$	$\alpha = \beta = \gamma = 90°$	$\lambda = 0.71073$ Å
$FW = 224.25$	Cell parameters from 1584 reflections	
Orthorhombic, $P2(1)2(1)2(1)$		$V = 1195.2$ (2) Å^3
Prismatic, green	$Z = 4$	$\theta = 4.8$–$45.2°$
$a = 5.3796$ (6) Å	$F_{000} = 472$	$\mu = 0.08$ mm^{-1}
$b = 12.9543$ (14) Å	$D_x = 1.246$ Mg m^{-3}	$T = 293$ (2) K
$c = 17.1522$ (18) Å	Mo Kα radiation	0.40 × 0.38 × 0.27 mm
Data collection		
$T_{min} = 0.592$	6525 independent reflections,	$h = -6 \rightarrow 6$; $k = -15 \rightarrow 15$; $l = -21 \rightarrow 9$
$T_{mix} = 1.000$	1167 reflections with $I > 2\sigma$ (I)	
$\theta_{max} = 26.0°$	$\theta_{min} = 2.0°$	$R_{int} = 0.056$
Refinement		
$R[F^2 > 2\sigma(F^2)] = 0.039$	1396 reflections	$(\Delta/\sigma)_{max} < 0.001$
$wR(F^2) = 0.095$	159 parameters	$\Delta\rho_{max} = 0.09$ e Å^{-3}
$S = 1.04$	$w = 1/[\sigma^2(F_o^2) + (0.0537P)^2]$	$\Delta\rho_{min} = -0.14$ e Å^{-3}

Table 4 Hydrogen-bond geometry for compound **16**

D–H···*A*	*D*–H (Å)	H···*A* (Å)	*D*···*A* (Å)	*D*–H···*A* (°)
O2–H2A···O1^{i}	0.85 (3)	1.84 (3)	2.691 (2)	175 (3)

Symmetry codes: (i) $-x+3/2$, $-y+2$, $z - 1/2$

with an electrospray ion source. Melting points were determined on a Fisher-Johns melting apparatus and are corrected. Column chromatography purifications were carried out on Silica Gel 60 (E. Merck, 70-230 mesh).

General procedure for synthesis of chalcones: To a solution of 10 mmol acetophenone in EtOH (10 ml) was added 10 mmol aryl aldehydes. The solution was stirred at 5–10°C, and 40%(w/v) NaOH in H_2O (10 mmol) was added dropwise. The mixture was stirred at room temperature for 4–8 h. The resulting precipitate was filtered and washed with water and cold ethanol, and then dried in vacuum. For **3**, **12** and **16**: To a solution of 10 mmol acetophenones in dried EtOH (10 ml) was added 10 mmol aryl aldehydes. The mixture was heated until a clear solution was obtained. Then a dried HCl gas producted by the reaction of H_2SO_4 and NaCl was inleted to the solution until saturated. The mixture was stirred and refluxed for 24 h. The resulting precipitate was filtered and washed with water and cold ethanol, and then dried in vacuum.

Compounds **7, 11, 15–17, 28, 29**, **31**, **32** were purified by recrystallization using alcohol and CH_2Cl_2 (1:2–2:1). Other compounds were purified by silica gel column

chromatography using CH_2Cl_2/CH_3OH as the eluent. The structural data in ^{1}HNMR and ESI–MS of newly described compounds were listed as following.

4.1.1 (E)-2,4-dichloro-4′-aminochalcone (1): orange yellow power, 22.1% yield, mp 189.0–190.5°C. ^{1}H-NMR (DMSO), δ: 8.215 (d, $J = 8.4$ Hz, 2H, H-2′, H-6′), 7.947 (d, $J = 15.6$ Hz, 1H, H-β), 7.935 (d, $J = 8.4$ Hz, 1H, H-6), 7.854 (d, $J = 15.6$ Hz, 1H, H-α), 7.727 (s, 1H, H-3), 7.520 (d, $J = 8.7$ Hz, 1H, H-5), 6.621 (d, $J = 8.4$ Hz, 2H, H-3′, H-5′), 4.180 (brs, 2H, NH_2-4′). ESI–MS m/z: 290.3$(M-1)^-$, calcd for $C_{15}H_{11}Cl_2NO$: 292.16.

4.1.2 (E)-4-dimethylamino-3′,5′-difluorochalcone (5): orange yellow power, 5.43% yield, mp 125.0–129.7°C. ^{1}H-NMR ($CDCl_3$), δ: 7.821 (d, $J = 15.6$ Hz, 1H, H-β), 7.552 (d, $J = 9.0$ Hz, 2H, H-2, H-6), 7.500 (dd, $J = 1.8$ Hz, 7.8 Hz, 2H, H-2′, H-6′), 7.200 (d, $J = 15.0$ Hz, 1H, H-α), 6.987 (tt, $J = 2.4$ Hz, 8.4 Hz, 1H, H-4′), 6.697 (d, $J = 9.0$ Hz, 2H, H-3, H-5), 3.062 (s, 6H, OCH_3-3′, OCH_3-4′). ESI–MS m/z: 288.3$(M + 1)^+$, calcd for $C_{17}H_{15}F_2NO$: 287.3.

4.1.3 (E)-4-methoxy-3′,5′-difluorochalcone (13): light yellow power, 67.7% yield, mp 101.2–102.1°C. ^{1}H-NMR ($CDCl_3$), δ: 7.820 (d, $J = 15.6$ Hz, 1H, H-β), 7.615 (dd, $J = 1.8$ Hz, 8.4 Hz, 2H, H-2, H-6), 7.511 (m, $J = 2.4$ Hz, 7.8 Hz, 2H, H-2′, H-6′), 7.285 (d, $J = 15.6$ Hz, 1H, H-α), 7.019 (tt, $J = 1.8$ Hz, 1H, H-4), 6.951 (dd, $J = 1.8$ Hz, 8.4 Hz, 2H, H-3, H-5), 3.869 (s, 3H, OCH_3-4). ESI–MS m/z: 275.1$(M + 1)^+$, calcd for $C_{16}H_{12}F_2O_2$: 274.26.

4.1.4 (E)-2-fluoro-4′-aminochalcone (14): yellow power, 91.6% yield, mp 138.7–140.4°C. ^{1}H-NMR ($CDCl_3$), δ: 7.936 (d, $J = 7.8$ Hz, 2H, H-2′ H-6′), 7.863 (d, $J = 15.6$ Hz, 1H, H-β), 7.653 (d, $J = 15.6$ Hz, 1H, H- α), 7.619–7.647 (m, 1H, H-6), 7.345–7.362 (m, 1H, H-4), 7.170–7.196 (m, 1H, H-5), 7.105–7.138 (m, 1H, H-3), 6.704 (d, $J = 7.8$ Hz, 2H, H-3′, H-5′), 4.185 (brs, 2H, NH_2-4′). ESI–MS m/z: 240.2$(M-1)^-$, calcd for $C_{15}H_{12}FNO$: 241.26.

4.1.5 (E)-2,4-dimethoxy-3′,5′-difluorochalcone (21): yellow power, 69.4% yield, mp 144.4–149.3°C. ^{1}H-NMR ($CDCl_3$), δ: 8.069 (d, $J = 16.2$ Hz, 1H, H-β), 7.564 (d, $J = 8.4$ Hz, 1H, H-6), 7.500 (dd, $J = 1.8$ Hz, 7.2 Hz, 2H, H-2′, H-6′), 7.429 (d, $J = 15.6$, 1H, H-α), 6.997 (tt, $J = 1.8$ Hz, 8.4 Hz, 1H, H-4′), 6.547 (dd, $J = 1.8$ Hz, 8.4 Hz, 1H, H-5), 6.483 (d, $J = 1.8$ Hz, 1H, H-3), 3.916 (d, 3H, OCH_3-2), 3.867 (d, 3H, OCH_3-4). ESI–MS m/z: 305.3$(M + 1)^+$, calcd for$C_{17}H_{14}F_2O_3$: 304.29.

4.1.6 (E)-2-trifluoromethyl-4′-aminochalcone (29): yellow crystal, 71.7% yield, mp 179.8–181.3°C. ^{1}H-NMR ($CDCl_3$), δ: 8.196 (d, $J = 7.8$ Hz, 1H, H-3), 8.067 (d, $J = 15.6$ Hz, 1H, H-β), 7.988 (d, $J = 8.4$ Hz, 2H, H-2′, H-6′), 7.867(d, $J = 15.6$ Hz, 1H, H-α), 7.819(d, $J = 7.8$ Hz, 1H, H-6), 7.750 (t, 1H, H-5), 7.635 (t, 1H, H-4), 6.760 (d, $J = 8.4$ Hz, 2H, H-3′, H-5′). ESI–MS m/z: 292.5$(M + 1)^+$, calcd for $C_{16}H_{12}F_3NO$: 291.27.

4.1.7 (E)-2-bromo-4′-aminochalcone (31): yellow power, 38% yield, mp 143.2–147.9°C. ^{1}H-NMR ($CDCl_3$), δ: 8.083(d, $J = 15.6$ Hz, 1H, H-β), 7.930 (d, $J = 8.4$ Hz, 2H, H-2′, H-6′), 7.719 (d, $J = 7.8$ Hz, 1H, H-3), 7.630 (d, $J = 7.8$ Hz, 1H, H-6), 7.432 (d, $J = 15.6$ Hz, 1H, H-α), 7.347 (t, 1H, H-5), 7.229 (t, 1H, H-4), 6.705(d, $J = 8.4$ Hz, 2H, H-3′, H-5′), 4.150 (brs, 2H, NH_2-4′). ESI–MS m/z: 302.0 $(M + 1)^+$, calcd for $C_{15}H_{12}BrNO$: 302.17.

4.1.8 (E)-2,6-difluoro-4′-methoxychalcone (33): white power colorless crystal, 55% yield, mp 88.4–90.5°C. ^{1}H-NMR ($CDCl_3$), δ: 8.037 (d, $J = 8.4$ Hz, 2H, H-2′, H-6′), 7.823 (d, $J = 15.6$ Hz, 1H, H-β), 7.618 (d, $J = 16.2$ Hz, 1H, H-α), 7.322–7.3369 (m, 1H, H-4), 7.061–7.088 (m, 2H, H-3, H-5), 6.992 (d, $J = 8.4$ Hz, H-3′, H-5′), 43.900 (s, 3H, -OCH_3-4′).IR (cm^{-1}): 1655 (C = O), 1491, 1572, 1598 (Ar), 1600 (C = C), 3410 (NH_2), 1187 (C–O).. ESI–MS m/z: 274.1$(M + 1)^+$, calcd for $C_{16}H_{12}F_2O_2$: 274.26.

4.1.9 (E)-2-chloro -3′,4′-dimethoxychalcone (36): white power, 93.7% yield, mp 70.2–72.8°C. ^{1}H-NMR ($CDCl_3$), δ: 8.186 (d, $J = 15.6$ Hz, 1H, H-β), 7.770 (d, $J = 7.8$ Hz, 1H, H-6′), 7.704 (d, $J = 8.4$ Hz, 1H, H-6), 7.651 (s, 1H, H-2′), 7.522 (d, $J = 15.6$ Hz, 1H, H-α), 7.475 (d, $J = 7.8$ Hz, 1H, H-3), 7.345–7.358 (m, 2H, H-4, H-5), 6.964 (d, $J = 8.4$ Hz, 1H, H-5′), 3.985 (s, 6H, OCH_3-3′, OCH_3-4′). ESI–MS m/z: 303.6$(M + 1)^+$, 305.6$(M + 1)^+$, calcd for $C_{17}H_{15}ClO_3$: 302.75.

4.1.10 (E)-2,3-dimethoxy -3′,4′-dimethoxychalcone (39): white power, 69.1% yield, mp 82.5–84.2°C. ^{1}H-NMR ($CDCl_3$), δ: 8.072 (d, $J = 15.6$ Hz, 1H, H-β), 7.782 (dd, $J = 1.8$ Hz, 8.4 Hz, 1H, H-6′), 7.628 (d, $J = 15.6$ Hz, 1H, H-α), 7.629 (d, $J = 1.8$ Hz, 1H, H-2′), 7.275 (dd, $J = 1.2$ Hz, 7.8 Hz, 1H, H-6), 7.098 (t, $J = 8.4$ Hz, 1H, H-5), 6.967 (dd, $J = 1.2$ Hz, 7.8 Hz, 1H, H-4), 6.932 (d, $J = 8.4$ Hz, 1H, H-5′), 3.973 (s, 3H, OCH_3-2), 3.970 (s, 3H, OCH_3-3′), 3.897 (s, 3H, OCH_3-3), 3.890 (s, 3H, OCH_3-4′). ESI–MS m/z: 329.0$(M + 1)^+$, calcd for $C_{19}H_{20}O_5$: 328.36.

4.1.11 (E)-2-bromo-3′,4′-dimethoxychalcone (40): white power, 75.3% yield, mp 99.2–103.5°C. ^{1}H-NMR ($CDCl_3$), δ: 8.107 (d, $J = 15.6$ Hz, 1H, H-β), 7.230 (dd, $J = 1.2$ Hz, 7.8 Hz, 1H, H-3), 7.675 (dd, $J = 1.8$ Hz, 8.4 Hz, 1H, H-3′), 7.642 (dd, $J = 1.2$ Hz, 8.4 Hz, 1H, H-6), 7.622 (d, $J = 1.8$ Hz, 1H, H-2′), 7.432 (d, $J = 15.6$ Hz, 1H, H-α), 7.364 (t, $J = 7.8$ Hz, 1H, H-5), 7.243 (dd, $J = 1.2$ Hz, 7.2 Hz, 1H, H-4), 6.935 (d, $J = 8.4$ Hz, 1H, H-5′), 3.974 (s, 6H, OCH_3-3′, OCH_3-4′). ESI–MS m/z: 349.6$(M + 1)^+$, 347.7$(M + 1)^+$, calcd for $C_{17}H_{15}BrO_3$: 347.2.

Methyl thiazolyl tetrazolium (MTT) assay

Cells were cultured in RMPI-1640 containing 10% FBS and seeded in 96-well plates with 5000 cells per well. After incubated in a CO_2 incubator for 24 h, compounds in DMSO at the concentrations of 100 μM, 33 μM, 11 μM and 3.7 μM and vehicle control were added to each plate, respectively. After 72 h treatment, a fresh solution of MTT (5 mg/ml) prepared in NaCl solution (0.9%) was added to each single well of the 96-well plate. The plate were then incubated for 3 h, cells dissolved with 100 μl DMSO, and then analyzed in a multi-well-plate reader at 570 nM. Each compound was tested at least three runs for each concentration. IC_{50} values were determined using Prism GraphPad software.

MIC Test

MICs were determined by preparing doubling dilutions of each test sample in Mueller–Hinton broth. Each dilution was then inoculated with a standard inoculum of an overnight culture of bacteria adjusted so that each test received 3×10^5–5×10^5 CFU (colony-forming units)/ml. All tests were performed in triplicate with negative growth controls (DMSO plus inoculum). The MIC was defined as the lowest drug concentration that prevented visible growth.

Determination of crystal structure

The crystal and refinement data of **16** are presented in Table 3. A yellow rhombus crystal of **16** (E)-3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one, from CH_3OH/CH_2Cl_2 (1:2) having approximate dimensions of $0.40 \times 0.38 \times 0.27$ mm was mounted on a glass fiber. All measurements were made on a Bruker APEX II CCD area-detector diffractometer with graphite monochromated Mo Kα radiation ($\lambda = 0.71073$ Å). Of the 6525 reflections that were collected in the range $2.51 < \theta < 24.26°$, 1167 were unique ($R_{int} = 0.056$). Data were collected and integrated using the Bruker SAINT software package (Bruker AXS Inc, 1999). All non-hydrogen atoms were refined anisotropically. All other hydrogen atoms were included in calculated positions but not refined. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.09 and −0.14 eÅ-3, respectively. All refinements were performed using the SHELXTL-97 crystallographic software package (Bruker AXS Inc, 1997).

Supplementary data

Details of the compounds which have been published previously, including yields, melting points, NMR, and ESI–MS analysis are described in Online Supplemental Data. Supplementary crystallographic data for **16** (CCDC No. 717888) can be obtained free of charge at CCDC database.

Acknowledgments This study was partially supported by the National Natural Science Funding of China (20802054), Young Talent Funding of Zhejiang Department of Health (2009QN020), Key Project of Wenzhou Sci & Tech Bureau (Y20090009 & Y20100006), and Zhejiang Natural Science Funding (Y2101108 & Y4090261).

References

Avila HP, Smania Ede F, Monache FD, Smania A (2008) Structure-activity relationship of antibacterial chalcones. Bioorg Med Chem 16:9790–9794

Batovska D, Parushev S, Stamboliyska B, Tsvetkova I, Ninova M, Najdenski H (2009) Examination of growth inhibitory properties of synthetic chalcones for which antibacterial activity was predicted. Eur J Med Chem 44:2211–2218

Bhattacharya A, Mishra LC, Sharma M, Awasthi SK, Bhasin VK (2009) Antimalarial pharmacodynamics of chalcone derivatives in combination with artemisinin against *Plasmodium falciparum* in vitro. Eur J Med Chem 44:3388–3393

Boumendjel A, Boccard J, Carrupt P, Nicolle E, Blanc M, Geze A, Choisnard L, Wouessidjewe D, Matera E, Dumontet C (2008) Antimitotic and antiproliferative activities of chalcones: forward structure–activity relationship. J Med Chem 51:2307–2310

Bruker (1997) SHELXTL Version 5.1, Bruker AXS Inc., Madison, Wisconsin, USA

Bruker (1999) SAINT Version 6.02, Bruker AXS Inc., Madison, Wisconsin, USA

Dominguez JN, Leon C, Rodrigues J, Gamboa de Dominguez N, Gut J, Rosenthal PJ (2005) Synthesis of chlorovinyl sulfones as structural analogs of chalcones and their antiplasmodial activities. J Med Chem 48:3654–3658

Ducki S (2009) Antimitotic chalcones and related compounds as inhibitors of tubulin assembly. Anticancer Agents Med Chem 9:336–347

Ghosh A, Mandal S, Banerji A, Kar M, Banerji J (2009) A new chalcone from Pongamia pinnata and its antioxidant properties. Nat Prod Commun 4:209–210

Heidari MR, Foroumadi A, Noroozi H, Samzadeh-Kermani A, Azimzadeh BS (2009) Study of the anti-inflammatory and analgesic effects of novel rigid benzofuran-3, 4-dihydroxy chalcone by formalin, hot-plate and carrageenan tests in mice. Pak J Pharm Sci 22:395–401

Henmi K, Hiwatashi Y, Hikita E, Toyama N, Hirano T (2009) Methoxy- and fluoro-chalcone derivatives arrest cell cycle progression and induce apoptosis in human melanoma cell A375. Biol Pharm Bull 32:1109–1113

Hua SZ, Luo JK, Wang XB, Wang JS, Kong LY (2009) Two novel monoterpene–chalcone conjugates isolated from the seeds of Alpinia katsumadai. Bioorg Med Chem Lett 19:2728–2730

Kamal A, Shankaraiah N, Prabhakar S, Reddy ChR, Markandeya N, Reddy KL, Devaiah V (2008) Solid-phase synthesis of new pyrrolobenzodiazepine-chalcone conjugates: DNA-binding affinity and anticancer activity. Bioorg Med Chem Lett 18:2434–2439

Ko HH, Tsao LT, Yu KL, Liu CT, Wang JP, Lin CN (2003) Structure-activity relationship of antibacterial chalcones. Bioorg Med Chem 11:105–111

Kumar SK, Hager E, Pettit C, Gurulingappa H, Davidson NE, Khan SR (2003) Design, synthesis and evolution of novel boronic-chalcone derivatives as antitumor agents. J Med Chem 46:2813–2815

Lee CM, Yeh SC, Lim HK, Liu CP, Tseng HK (2009) High prevalence rate of multidrug resistance among nosocomial pathogens in the respiratory care center of a tertiary hospital. J Microbiol Immunol Infect 42:401–404

Liang G, Shao LL, Wang Y, Zhao CG, Chu YH, Xiao J, Zhao Y, Li XK, Yang SL (2009) Exploration and synthesis of curcumin analogues with improved structural stability both in vitro and in vivo as cytotoxic agents. Bioorg Med Chem 17:2623–2631

Liu X, Xu Y, Go M (2008) Functionalized chalcones with basic functionalities have antibacterial activity against drug sensitive Staphylococcus aureus. Eur J Med Chem 43:1681–1687

Nielsen SF, Larsen M, Boesen T, Schonning K, Kromann H (2005) Cationic chalcone antibiotics design, synthesis, and mechanism of action. J Med Chem 48:2667–2677

Nowakowska Z (2007) A review of anti-infective and anti-inflammatory chalcones. Eur J Med Chem 42:125–137

Nowakowska Z, Kdzia B, Schroeder G (2008) Functionalized chalcones with basic functionalities have antibacterial activity against drug sensitive *Staphylococcus aureus*. Eur J Med Chem 43:707–713

Padhye S, Ahmad A, Oswal N, Sarkar FH, Hematol J (2009) Emerging role of Garcinol, the antioxidant chalcone from Garcinia indica Choisy and its synthetic analogs. Oncol 2:38

Park PH, Kim HS, Jin XY, Jin F, Hur J, Ko G, Sohn DH (2009) KB-34, a newly synthesized chalcone derivative, inhibits lipopolysaccharide-stimulated nitric oxide production in RAW 264.7 macrophages via heme oxygenase-1 induction and blockade of activator protein-1. Eur J Pharmacol 606:215–224

Rao Y, Fang S, Tzeng Y (2004) Structure-activity relationship of antibacterial chalcones. Bioorg Med Chem 12:2679–2686

Ratkovic Z, Juranic ZD, Stanojkovic T, Manojlovic D, Vukicevic RD, Radulovic N, Joksovic MD (2010) Synthesis, characterization, electrochemical studies and antitumor activity of some new chalcone analogues containing ferrocenyl pyrazole moiety. Bioorg Chem 38:26–32

Rizvi SU, Siddiqui HL, Parvez M, Ahmad M, Siddiqui WA, Yasinzai MM (2010) Antimicrobial and antileishmanial studies of novel(2E) -3-(2-chloro-6-methyl/methoxyquinolin-3- yl) -1-(aryl)prop-2-en-1-ones. Chem Pharm Bull 58:301–306

Rodriguez CH, De Ambrosio A, Bajuk M, Spinozzi M, Nastro M, Bombicino K, Radice M, Gutkind G, Vay C, Famiglietti A (2010) In vitro antimicrobials activity against endemic *Acinetobacter baumannii* multiresistant clones. J Infect Dev Ctries 4:164–167

Tanaka H, Nakamura S, Onda K, Tazaki T, Hirano T (2009) Sofalcone, an anti-ulcer chalcone derivative, suppresses inflammatory crosstalk between macrophages and adipocytes and adipocyte differentiation: implication of heme-oxygenase-1 induction. Biochem Biophys Res Commun 381:566–570

Tang Y, Simoneau AR, Xie J, Shahandeh B, Zi X (2008) Effects of the kava chalcone flavokawain a differ in bladder cancer cells with wild-type versus mutant p53. Cancer Prev Res 1:439–451

Tomar V, Bhattacharjee G, Rajakumar S, Kamaluddin, Srivastava K, Puri SK (2010) Synthesis of new chalcone derivatives containing acridinyl moiety with potential antimalarial activity. Eur J Med Chem 45:745–751

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