Bioorganic & Medicinal Chemistry 16 (2008) 9790–9794

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Structure–activity relationship of antibacterial chalcones

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ARTICLE INFO

Article history:
Received 10 July 2008
Revised 24 September 2008
Accepted 26 September 2008
Available online 30 September 2008

Keywords:
Chalcone
4-Hydroxy-derricin
Antibacterial activity
Bacteria
Minimal inhibitory concentration
Minimal bactericidal concentration

ABSTRACT

The antibacterial activity of 31 chalcones was tested against bacterial strains, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923. Some of the tested chalcones showed fair to significant activity against Gram-positive bacteria. By comparison of the results obtained, the antibacterial activity can be related to features such as the presence of a C-4 hydroxyl group, a C-4′ oxygenated substituent or a C-3′ isoprenoid side chain, while the C-2′ hydroxyl group might have importance for the stability of the molecule. The inhibitory effect of chalcones on human pathogenic microorganisms can be correlated with the substitution patterns of the aromatics rings.

1. Introduction

Flavonoids represent an outstanding class of naturally occurring compounds with a 1,3-diarylpropane skeleton, which may assume different cyclic or alicyclic arrangements, according to varying levels of oxidation. Chalcones are open chain flavonoids in which two aromatic rings are joined by a three carbon α,β-unsaturated carbonyl system, that is, 1,3-diphenyl-2-propen-1-one derivatives. The equilibrium between the open chalcone form and the cyclic flavanone isomer is the key step at the origin of the skeletal modifications of the biosynthetic pathway. Chalcones have shown a wide variety of anticancer,[1] antiinflammatory,[2] antiinvasive,[3] and antifungal activities.[4] In addition to the properties listed above, the antibacterial activity of chalcones is being increasingly documented. Depending on the substitution of the two aromatic rings the chalcones can display different spectra of activity. For instance, (*E*)-chalcones containing 4-alkylthio- or 4-alkoxy side chains and 4′-*N*-piperidine or 4′-*N*-methylpiperidine groups, as *para* substituents, exhibited a narrow spectrum of antibacterial activity, being affective against Gram-positive bacteria.[5] Conversely, broad-spectrum compounds, effective against Gram-positive and Gram-negative bacteria, were obtained by introduction of piperazine or 2,5-dichlorothiophene on the basic skeleton of the chalcones.[6] Finally, 5-[3-(4′-dimethylaminophenyl)acryolyl]-6-methoxybenzo-[1,3]-oxathiol-2-one was shown to display tuberculostatic activity against *Mycobacterium tuberculosis*.[7] In earlier studies, concerning their antimicrobial effect, the activity of chalcones was mainly attributed to the presence of phenolic hydroxyl groups, which have high affinity for proteins and thus may inhibit microbial enzymes.[8] It was generally agreed that at least one phenolic hydroxyl group and a certain degree of lipophilicity were required.[9] The nature of the flavonoid was considered priority, e.g., the flavanone isoxanthoumol is 60 times less active than the corresponding chalcone,[10] but the influence of the substitution pattern on the structure–activity relationship (SAR) was not further investigated. By contrast, a SAR study on the effect of 12 different chalcones, on both established and primary ovarian cancer cells, revealed some important remarks on the influence that structural changes may have on both antiproliferative and binding activity.[11] This paper deals with an investigation on the influence of the substitution pattern, involving hydroxyl, methoxyl, acetoxyl, and methyledioxy groups as well as isoprenoid substituents, of both A and B rings of 31 chalcones on their antibacterial activity against human pathogenic microorganisms.

2. Results and discussion

Table 1 summarizes the results obtained for the MICs and MBCs of the 31 chalcones (Figs. 1 and 2) against the four bacterial species.

Although no definite structure–activity relationship could be determined, some conclusions on the structural changes that may influence the antimicrobial activity can be drawn by the

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doi:10.1016/j.bmc.2008.09.064

Table 1
Antibacterial activity of the 31 chalcones

Chalcone order	*B. cereus*		*E. coli*		*P. aeruginosa*		*S. aureus*	
	MIC[a]	MBC[b]	MIC	MBC	MIC	MBC	MIC	MBC
1	>2000[c]	>2000	>2000	>2000	2000	2000	>2000	>2000
2	1000	>2000	2000	>2000	1000	>2000	2000	>2000
3	2000	>2000	2000	2000	2000	2000	>2000	>2000
4	>2000	>2000	>2000	>2000	2000	2000	>2000	>2000
5	1000	1000	2000	1000	1000	2000	1000	2000
6	1000	1000	1000	2000	1000	>2000	1000	2000
7	2000	>2000	1000	>2000	1000	2000	2000	>2000
8	2000	>2000	1000	>2000	2000	>2000	2000	>2000
9	250	250	2000	2000	2000	2000	1000	1000
10	2000	2000	2000	>2000	2000	>2000	2000	>2000
11	2000	2000	2000	>2000	2000	2000	2000	>2000
12	1000	1000	2000	>2000	2000	2000	2000	2000
13	2000	2000	2000	2000	2000	2000	2000	2000
14	31.2	31.2	1000	1000	1000	1000	31.2	31.2
15	2000	2000	2000	2000	2000	2000	2000	>2000
16	2000	2000	2000	2000	2000	2000	2000	>2000
17	1000	1000	2000	2000	2000	2000	2000	2000
18	3.9	3.9	2000	2000	2000	2000	7.8	7.8
19	2000	2000	2000	>2000	2000	2000	2000	2000
20	62.5	62.5	2000	2000	2000	2000	62.5	62.5
21	15.6	15.6	1000	2000	1000	2000	31.2	31.2
22	15.6	15.6	1000	2000	1000	2000	31.2	31.2
23	1000	>2000	2000	>2000	2000	>2000	1000	>2000
24	2000	2000	1000	1000	2000	2000	2000	2000
25	2000	2000	2000	2000	2000	2000	2000	>2000
26	1000	>2000	2000	>2000	2000	>2000	2000	>2000
27	2000	2000	1000	2000	1000	2000	2000	2000
28	500	500	2000	2000	1000	2000	500	500
29	2000	2000	2000	2000	2000	2000	2000	2000
30	2000	2000	2000	2000	2000	2000	2000	>2000
31	250	250	>2000	1000	2000	2000	250	250
Tetracycline	0.25		2.0		32.0		1.0	

[a,b] Minimal inhibitory concentration and minimal bactericidal concentration, respectively.
[c] Values expressed in μg/mL.

comparison among the structures of compounds with different activities:

- 4-Hydroxyderricin (**18**) was the most active compound, followed by 2′,4,4′-trihydroxy-3-prenyl-3′-geranylchalcone (**21**) and 2′,4,4′-trihydroxy-3′-geranylchalcone (**22**).
- The 2′-hydroxyl group, a very common feature of natural chalcones, is always present in the active compounds[1]; very likely, it participates to stabilize by a hydrogen bond the predominant structure of the chalcone. On the other hand, it is also the key element in the equilibrium chalcone–flavanone. For both these reasons, the 2′-hydroxy substituent may be considered a crucial group for the structure stability, but should not contribute significantly to the activity. Acetylation (as in **7**) or methylation (as for **1**, **3**, **29**, and **30**) of the 2′-OH group seems to lead to less active skeletons, but no comparison was possible with active counterpart with a free 2′-phenol group.
- A free hydroxyl group in position 4 (B ring) appears to be a very important requirement (**18** vs **15** and **14** vs **13**). When the hydroxyl group is methylated (**19** vs **18**), or in other positions (**16** or **17** vs **18**), the chalcone is not more active. Methylenation, as in **3** and **29**, would be expected to have the same negative effect, but in the two structures the absence of a 4′-methoxyl group (in **3**) or the saturated double bond (in **29**) might also be responsible for the decrease in the activity.
- A 4′-methoxyl group seems to be a second requirement, because, when the 4′-hydroxyl group is free (**14** vs **18**) there is a decrease in the activity, whereas, when the 4′-OH bears a more complex substituent (**12** or **25** vs **18**) there is a complete loss of activity. However, a slight activity was shown by **31** (with hydroxyl substituent in both 4- and 4′-positions) and **9** (with a 4′-OMe and no 4-substituent): we may conclude that the two proposed requirements must not be considered in a drastic sense.
- A hydrophobic group (prenyl or geranyl) in 3′ (ring A) seems to be a third condition for the activity. A geranyl group in 3′ is compatible with the presence a 4′-hydroxyl group, because probably brings to a degree of lipophilicity similar to that of a 3′-prenyl-4-methoxyl substitution pattern (**21** or **22** vs **18**). For the same reason, probably, the similarity of 4-hydroxylonchocarpin (**25**) and 4-hydroxyderricin (**18**), both having an isoprenoid substituent in 3′ and an alkylated hydroxyl group, is not enough to give comparable activities.
- An isoprenyl group on the B ring, however, does not improve the activity (**21** vs **22**).
- The negative effect of the reduction of the double bond can be evidenced by the comparison between **20** and **31**, but it should be confirmed by other tests.

In summary, these findings are in agreement with the main points of the report by Barron and Ibrahim[9] about the requirements for the antimicrobial activity of flavonoids, such as the presence of at least one phenolic hydroxyl group, and a certain degree of lipophilicity. On the other hand, our studies seem to confirm that for increasing the antimicrobial potency simple modifications (methylation and acetylation) of the substituents are not sufficient and more drastic interventions on the chalcone scaffold, such as the introduction of cationic aliphatic amino groups[12] or the syn-

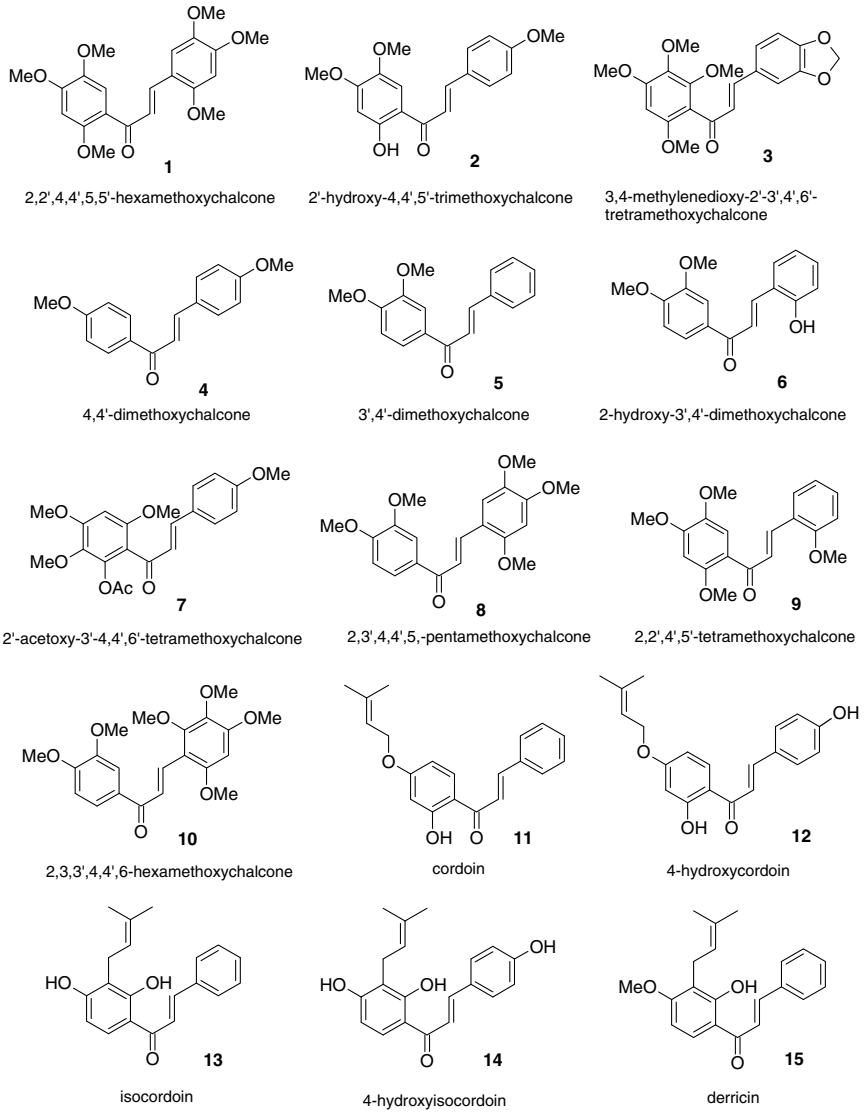


Figure 1. Structures of the tested chalcones.

thesis of hybrid compounds with an A ring containing an oxazolidinone moiety and a heterocyclic B ring,[13] are needed.

3. Conclusion

These studies, focused on the potentially active skeleton of chalcones, pointed out the importance of the positions of the phenolic hydroxyl groups and/or the isoprenyl side chain in the substitution pattern. The importance of prenyl and/or geranyl groups, which confer to the molecule a strong affinity to biological membranes[14] must be once again noticed.[15]

4. Experimental

4.1. Chalcones

Thirty-one natural and/or synthetic chalcones, available in our laboratories, have been assayed in this study. The chalcones (compounds **1**–**10** and **27**) and dihydrochalcones (compounds **28**–**31**) were earlier synthesized by Professor G. Bargellini (1879–1963) and co-worker at beginning of 20th century.[16–19] Afterwards, the compounds were inherited by Professor G.B. Marini Bettolo (1915–1996) and finally exhumed by one of us (F. Delle Monache). Their structures, initially suggested by the information of handwritten labels such as hexamethoxy chalcones from veratraldeyde or tetramethoxy chalcone from anisaldehyde were confirmed by their NMR spectra. The other compounds (**11**–**26**) are naturally occurring chalcones.[20–23]

Figure 1 shows the structures of the 31 chalcones and Figure 2 shows a summary of their substitution patterns.

4.2. Bacterial strains

The assay was carried out using the following bacterial strains: *Escherichia coli* ATCC 25922 (American Type Culture Collection), *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, and *Bacillus cereus* ATCC 11778. Prior to investigation, stock cultures of bacteria were maintained on brain heart infusion—BHI at −20 °C in our laboratory. The inoculum was an overnight culture of each bacterial species in Mueller–Hinton broth diluted in the same media to a final concentration of approximately 10^8 CFU/mL.[24]

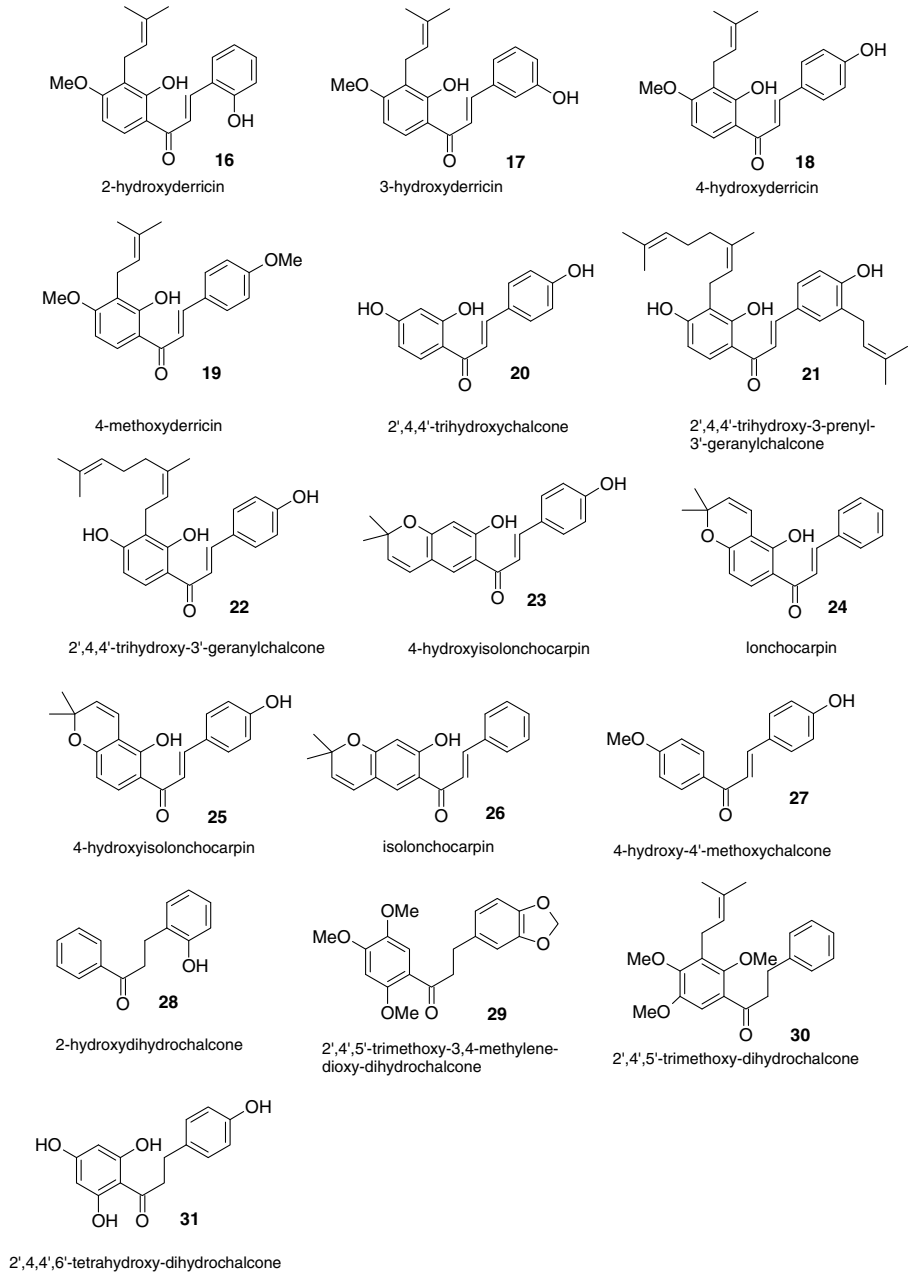


Figure. 1 (*continued*)

4.3. Determination of minimal inhibitory concentration

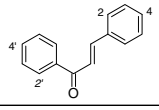
The antibacterial activity was evaluated by determining the minimum inhibitory concentration (MIC) using the microdilution broth method. The compounds were dissolved in 200 μL of dimethyl sulfoxide (DMSO), previously sterilized by autoclavation, and added to 800 μL Mueller–Hinton broth. Further serial dilutions were performed to reach a final concentration range between 2000 and 1.95 μg/mL. One sample (100 μL) of each diluted solution and the samples for both growth and sterility controls (containing sterile culture medium and DMSO, and no antimicrobial agents) were distributed in a 96-well plate. Each well for test and growth control was inoculated with 5 μL of bacterial suspension. Tetracycline was used to assess the MIC values of the reference strains. All experiments were performed in duplicate and the microdilution trays were incubated at 36 °C for 24 h. Bacterial growth was detected by the addition of a solution (0.2 mg/mL) of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltrazolium-chloride (INT) in 70% EtOH. MIC values (μg/mL) of tested substances, that is, the lowest concentration at which no growth occurred, were determined by the INT colorimetric reaction from yellow (absence of growth) to purple.[24]

4.4. Determination of minimal bactericidal concentration

For the minimal bactericidal concentration assay (MBC) assay, aliquots (10 μL) of each culture without visible growth were transferred to 90 μL of Mueller–Hinton broth. Incubation, reading and interpretation were performed according to MIC specifications.[24]

Acknowledgments

We thank Giuliano Delle Monache for a discussion of the manuscript. This work was supported by Fundação de Apoio à Pesquisa



Chalcone	Substituent on the basic skeleton									
	C-2'	C-3'	C-4'	C-5'	C-6'	C-2	C-3	C-4	C-5	C-6
1	OMe		OMe	OMe		OMe		OMe	OMe	
2	OH		OMe	OMe				OMe		
3	OMe	OMe		OMe			OCH_2O			
4			OMe					OMe		
5		OMe	OMe							
6		OMe	OMe			OH				
7	OAc	OMe	OMe					OMe		OMe
8		OMe	OMe			OMe		OMe	OMe	
9	OMe		OMe	OMe		OMe				
10		OMe	OMe			OMe	OMe	OMe		OMe
11	OH		OPr							
12	OH		OPr					OH		
13	OH	Pr	OH							
14	OH	Pr	OH					OH		
15	OH	Pr	OMe							
16	OH	Pr	OMe			OH				
17	OH	Pr	OMe				OH			
18	OH	Pr	OMe					OH		
19	OH	Pr	OMe					OMe		
20	OH		OH					OH		
21	OH	Ge	OH				Pr	OH		
22	OH	Ge	OH					OH		
23	OH		O	Py				OH		
24	OH	Py	O							
25	OH	Py	O					OH		
26	OH		O	Py						
27			OMe					OH		
28						OH				
29	OMe		OMe	OMe			OCH_2O			
30	OMe		OMe	OMe						
31	OH		OH		OH			OH		

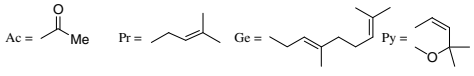


Figure 2. Substitution patterns of the 31 chalcones.

Científica e Tecnológica do Estado de Santa Catarina (FAPESC) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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