Original article

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SYNTHESIS AND SCREENING OF ANTIMICROBIAL ACTIVITY OF TWO BROMO-3',4'-DIMETHOXYCHALCONE DERIVATIVES

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Bromochalcone derivatives were synthesized, structurally characterized and screened for their *in vitro* antibacterial activity against a panel of two Gram-positive (*Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538) and two Gram-negative (*Escherichia coli* ATCC 8739 and *Salmonella typhimurium* ATCC 14028) laboratory control strains in a disc diffusion assay. The antimicrobial test revealed that 4-bromo-3',4'-dimethoxysubstituted chalcone was active against two involved Gram-negative strains, exhibiting stronger bactericidal effect on *E. coli* (11 ± 0.3 mm) than on *S. typhimurium* (15 ± 0.7 mm). Such observed difference in activity highlighted A-ring position 4- as potentially favourable for creating effective Gram-negative antibacterial agents.

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Key words: *chalcone derivatives, synthesis, antibacterial activity*

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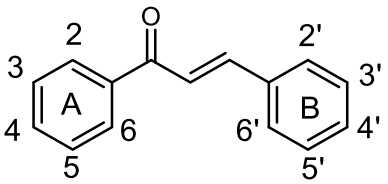


Figure 1. Structural representation of (*E*)-chalcone scaffold

Introduction

Chalcones represent a class of phenolic compounds widely distributed in the kingdom Plantae. Being both intermediates and end products in flavonoid biosynthesis, chalcones act as defensive compounds preventing molecular damage caused by pathogens, pests and herbivores, contributing to the medicinal value of herbs. Structurally this diverse class of plant secondary metabolites can be considered as open-chain flavonoids, representing a precursor unit in flavonoid and isoflavonoid biosynthesis (1). Using chemistry language, chalcones are 1,3-diaryl-2-propen-1-ones in which two benzene rings are joined by an α,β-unsaturated carbonyl system (2) as is shown in Figure 1.

Naturally occurring chalcones and their synthetic derivatives were involved in numerous pharmacological studies, showing a wide range of biological activities. It has been shown that this group of compounds expresses antiarrhythmic, antitrombic, antineoplastic, antiangiogenic, antiinflammatory, antihistaminic, antioxidant, antidiabetic, hypolipidemic, antihypertensive, antimicrobial, antiprotozoal, antiulcer, antigout, immunosuppressant, sedative, hypnotic, antispasmodic, analgesic, estrogenic, vasorelaxant and other diverse activities (3, 4). Taking into account the wide spectrum of their biological activities and the fact that they cannot be isolated from natural sources in large quantities, great efforts have been made towards bioinspired synthesis of chalcone derivatives. Chemical modulations of chalcone scaffold mainly involve the modification of substitution pattern on aromatic rings, but also the replacement of phenyl rings with heteroaryls and formation of hybrid molecules through conjugation with other pharmacologically active compounds (4, 5); this achieves enhanced activity and reduction of toxicity of synthetic analogs in comparison to their

natural counterparts (6). A small number of chalcone derivatives have reached the point of inclusion in clinical studies, some of which are clinically approved for the treatment of several conditions [for example metochalcone used as a choleretic agent, sofalcone as a muciprotective agent and hesperidin methyl chalcone as a vascular protective agent (7)] or registered as ingredients in cosmetic preparations (8, 9).

Chalcones reportedly have exhibited strong inhibitory activities against bacteria that are pathogenic to humans (10). The antibacterial effects are due to reactions between these compounds and the cell membrane of the target microorganism, their ability to attach with outer cell, absorbable proteins and the cell walls (11). Like other biological properties, microbiological activity is generally attributed to the α,β-unsaturated keto moiety and is also found to be dependent on the presence, the number and the position of functional groups such as methoxy, glycosides, hydroxyl, halogens in both phenyl rings (12, 13). Chemical substitutions of the phenyls are also the subject of interest, and useful conclusions about the structure-activity relationship facilitate the synthesis of pharmacologically active chalcones. An excellent review exploring recent developments of chalcones as potential antibacterial agents in medicinal chemistry, summarising the structure-activity relationships and mechanisms of antibacterial action has provided some important guidance for design and synthesis in future (14). Prompted by all these observations, studying the effect of bromine substitution on antimicrobial potential of diverse 1-(bromophenyl)-3-(3′,4′-dimethoxyphenyl) prop-2-en-1-ones, we report herein the synthesis and preliminary antibacterial evaluation of two chalcone derivatives, the A-ring 3- and 4-positional isomers:
(*E*)-1-(3-bromophenyl)-3-(3′,4′-dimethoxyphenyl) prop-2-en-1-one (**4**) and (*E*)-1-(4-bromophenyl)-3-(3′,4′-dimethoxyphenyl) prop-2-en-1-one (**5**).

Experimental

Chemicals

All chemicals used were of analytical reagent grade. Unless specified otherwise, all reagents and standards were purchased from Merck (Darmstadt, Germany).

Chemical synthesis procedure

A procedure for the preparation of (*E*)-1-(3-bromophenyl)-3-(3′,4′-dimethoxyphenyl) prop-2-en-1-one (**4**) and (*E*)-1-(4-bromophenyl)-3-(3′,4′-dimethoxyphenyl) prop-2-en-1-one (**5**).

A mixture of 3- and 4-bromoacetophenones (compounds **1** and **2**, 3 mmol) and 3,4-dimethoxyphenylaldehyde (compound **3**, 3 mmol) was stirred in ethanol (12 mL) and then 60% aqueous solution of sodium hydroxide (3 mL) was added dropwise with continuous stirring at 0 ^{O}C (Scheme 1). The mixture was stirred for 2-3 hours in the ice-bath. The reaction progress was monitored by thin layer chromatography (TLC) on silica gel 60 pre-coated F_{264} plates (Merck), (hexane/ethyl acetate, 4:1). Developed plates were examined with UV lamps (254 nm).

The chalcone derivatives **4** and **5** precipitate out as solids. The mixture was diluted with ice-cold water, filtered under reduced pressure and washed with cold water until neutral pH. The obtained crude products were recrystallized from 96% ethanol. Purity of obtained chalcones was above 95.0% (95.1% for compound **4**, and 99.0% for compound **5**), checked by high-performance liquid chromatography (HPLC), Agilent Technologies 1200 (Wilmington, DE, USA) equipped with photo diode array detector.

NMR analysis

^{1}H and ^{13}C NMR spectra of compounds **4** and **5** were measured at Bruker Avance III – 300 spectrometer. All NMR spectra were recorded at 298 K in $CDCl_3$ (isotopic enrichment 99.95%) solution. Chemical shifts are reported on the δ (ppm) scale and are relative to residual $CHCl_3$ signals (7.24 for ^{1}H and 77.0 ppm, central line, for ^{13}C spectra, respectively) and are given as: s (singlet), d (doublet), t (triplet) or m (multiplet). Scalar coupling constants are reported in hertz (Hz). The experi-mental error in the measured ^{1}H-^{1}H coupling con-stants was ± 0.5 Hz.

Antibacterial activity

Bacterial strains

Antibacterial activity of the synthesized compounds was tested *in vitro* against a panel of laboratory control strains belonging to the American Type Culture Collections, Maryland, USA: two Gram-positive (*Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538) and two Gram-negative (*Escherichia coli* ATCC 8739 and *Salmonella typhimurium* ATCC 14028) laboratory control strains were obtained from the National Collection of Type Cultures. All microorganisms were maintained at -20 ^{O}C under appropriate conditions and regenerated twice before use in the manipulations.

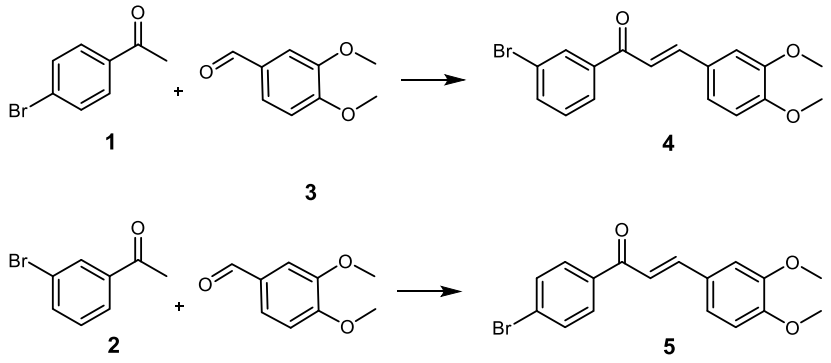
Screening of antibacterial activity

The *in vitro* antibacterial activity of the synthesised compounds **4** and **5** was determined using the disc diffusion assay recommended by NCCLS (15), described in detail in Lazarević et al. (16). For growing the Gram-positive and Gram-negative bacteria, Antibiotic Medium 1 (Difco Laboratories, Detroit, MI, USA) was used. The nutrition medium was prepared according to the instructions of the manufacturers. All agar plates were prepared in 90 mm Petri dishes with 22 mL of agar, giving the final depth of 4 mm. A suspension of the tested microorganisms (0.1 mL, 10^{8} cells per mL) was spread on the solid media plates. Dimethyl sulph-oxide (10%

aqueous solution) was used to dissolve and to dilute samples to the highest concentration to be tested (stock concentrations 1 mg/mL). Sterile filter paper disks ("Antibiotica Test Blättchen", Macherey-Nagel, Düren, Germany, 9 mm in diameter) were impregnated with 10 µL of the tested sample solution and placed on the inoculated plates. These plates, after standing at 4 ^{O}C for 2 h, were incubated at 37 ^{O}C for 24 h. Standard disks of tetracycline and gentamicin (Institute of Immunology and Virology "Torlak", 30 µg of the active component, diameter 6 mm) were used as the positive controls, while disks imbued with 10 µL of 10% dimethyl sulphoxide were used as the negative controls. The diameters of the inhibition zones were measured in millimetres using a "Fisher-Lilly Antibiotic Zone Reader" (Fisher Scientific Co., USA). Each experiment was replicated three times. Mean values are presented.

Results and discussion

Chemistry and spectral data on synthetized compounds: Reaction of 3- (**1**) and 4-bromoacetophenone (**2**) with 3,4-dimethoxybenzaldehyde (**3**) in basic medium, formed chalcone derivatives **4** and **5** (Scheme 1) which were characterized using ^{1}H and ^{13}CNMR experiments (Supplemental data). The reaction led to the expected products in high yield and in pure form (purity confirmed by HPLC).



Scheme 1. Synthesis of chalcone derivatives **4** and **5**

(*E*)-1-(3-Bromophenyl)-3-(3',4'-dimethoxyphenyl) prop-2-en-1-one (4)

Yield 79.7%; ^{1}HNMR (300 MHz, $CDCl_3$) δ (ppm): 8.13 (s, ^{1}H), 7.93 (d, J = 7.6 Hz, ^{1}H), 7.77 (d, J = 15.6 Hz, ^{1}H), 7.71–7.69 (m, ^{1}H), 7.38 (t, J = 8.0 Hz, ^{1}H), 7.32 (d, J = 15.6 Hz, ^{1}H), 7.26–7.23 (m, ^{1}H), 7.16 (s, ^{1}H), 6.91 (d, J = 8.0 Hz, ^{1}H), 3.95 (d, J = 9.2 Hz, ^{6}H).

^{13}CNMR (300 MHz, $CDCl_3$), δ (ppm): 187.7, 151.5, 149.0, 145.4, 139.9, 135.5, 130.9, 130.8, 127.5, 127.3, 124.3, 122.3, 119.1, 111.5, 111.1, 55.8.

(*E*)-1-(4-bromophenyl)-3-(3',4'-dimethoxyphenyl) prop-2-en-1-one (5)

Yield 89.3%; ^{1}HNMR (300 MHz, $CDCl_3$): δ (ppm): 7.88 (d, J = 8.4 Hz, ^{2}H), 7.77 (d, J = 15.6 Hz, ^{1}H), 7.64 (d, J = 8.4 Hz, ^{2}H), 7.33 (d, J = 15.6 Hz, ^{1}H), 7.25–7.23 (m, ^{1}H), 7.16 (s, ^{1}H), 6.91 (d, J = 8.4 Hz, ^{1}H), 3.95 (d, J = 6.8 Hz, ^{6}H).

^{13}CNMR (300 MHz, $CDCl_3$), δ (ppm): 188.4, 151.4, 149.0, 145.1, 136.8, 2x131.7, 2x130.4, 127.4, 127.0, 124.2, 119.2, 111.5, 110.8, 55.7, 55.6.

In vitro antibacterial activities. The antimicrobial activity was evaluated in a disc diffusion method, measuring inhibition zones of bacterial growth. The results of preliminary antibacterial testing are presented in Table 1 from which can be seen that not all of the tested compounds were equally effective against the selected bacterial strains. While compound **5** was active, exhibiting stronger bactericidal activity on *E. coli* (11 ± 0.3 mm) than on *S. typhimurium* (15 ± 0.7 mm), a complete absence of activity was observed for compound **4**. Effecting the growth of only Gram-negative strains, compound **5** exhibited also selectivity. The assayed samples were less effective than antibiotics used as reference standards.

To the best of our knowledge, our work is the first study that tested **4** and **5** on *B. subtilis*, *S. aureus*, *E. coli* and *S. typhimurium*. Compound **5** was previously involved in only one antimicrobial study, evaluating growth of avirulent and virulent mycobacteria, however no marked effect on inhibition of mycobacterial growth was observed (17).

Results for the tested compounds from our (antibacterial) activity study together with large number of published papers (14, 18-20) clearly

show that the structure-activity relationship for the A-ring substituted chalcones is strongly conditioned by the position of the halogen. Seems that A-ring bromo-substitution in position 4- for 3′,4′-dimethoxysubstituted chalcones is more favourable for creating active Gram-negative antibacterial agents than the position 3- (Table 1, compounds **4** and **5**).

Table 1. The antimicrobial activity (diameters of growth inhibition zones measured in mm) of 3- and 4-bromo-3′,4′-dimethoxysubstituted chalcones (compounds **4** and **5**, respectively) and of positive (antibiotics tetracycline and gentamicine)/negative (DMSO 10% aqueous solution) control.

	Microorganisms			
	Gram-positive		Gram-negative	
Cpd. entry	*B. subtilis*[a]	*S. aureus*	*E. coli*	*S. typhimurium*
4	na[b]	na	na	na
5	na	na	11 ± 0.3	15 ± 0.7
Tetracycline[c]	32.1 ± 0.5	30.7 ± 0.5	30.5 ± 0.5	31.2 ± 0.7
Gentamicine	22.1 ± 0.3	19.2 ± 0.7	24.2 ± 0.9	22.2 ± 0.3
DMSO 10% aqueous solution[d]	na	na	na	na

[a]Mean value ± SD (in mm) of five experiments, including disc diameter, 9 mm (10 µl per disc).
Values representing bactericidal zones in which the growth of bacteria was not observed.
[b]Not active.
[c]Positive control bactericidal activity (30 µg per disc).
[d]Negative control (10 µl per disc)

Conclusion

In this paper, we have described synthesis and antibacterial evaluation of two chalcone derivatives. To synthesize the compounds, eco-friendly and easy method has been used, including mild reaction conditions, use of recyclable solvent and easy work-up procedures. The products were obtained in high yield and in pure form. Compounds were evaluated for their *in vitro* antimicrobial activities in disc diffusion assay. Based on the results of two synthetized and tested samples as well as on the basis of numerous papers published, most likely the structure-activity relationship for 3- and 4-bromo-substituted chalcones is strongly conditioned by the position of the halogen. For 3- and 4-bromo 3′,4′-dimethoxysubstituted chalcones a difference in activity highlights position 4- as potentially favourable for creating effective Gram-negative antibacterial agents.

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References

1. Mathew B, Suresh J, Anbazghagan S, Paulraj J, Krishnan GK. Heteroaryl chalcones: Mini review about their therapeutic voyage. Biomed Prev Nutr 2014;4: 451-8. [CrossRef]
2. Nowakowska Z. A review of anti-infective and anti-inflammatory chalcones. Eur J Med Chem 2007;42(2): 125-37. [CrossRef] [PubMed]
3. Rudrapal M, Khan J, Dukhyil AAB, Alarousy RMII, Attah EI, Sharma T, et al. Chalcone scaffolds, bio-precursors of flavonoids: Chemistry, bioactivities, and pharmacokinetics. Molecules 2021;26(23):7177. [CrossRef] [PubMed]
4. Mahapatra DK, Bharti SK. Therapeutic potential of chalcones as cardiovascular agents. Life Sci 2016;148: 154-72. [CrossRef] [PubMed]
5. Thapa P, Upadhyay SP, Suo WZ, Singh V, Gurung P, Lee ES, et al. Chalcone and its analogs: Therapeutic and diagnostic applications in Alzheimer's disease. Bioorg Chem 2021;108:104681. [CrossRef] [PubMed]
6. Jasim HA, Nahar L, Jasim MA, Moore SA, Ritchie KJ, Sarker SD. Chalcones: Synthetic chemistry follows where nature leads. Biomolecules 2021;11(8):1203. [CrossRef] [PubMed]
7. Gomes MN, Muratov EN, Pereira M, Peixoto JC, Rosseto LP, Cravo PVL, et al. Chalcone derivatives: promising starting points for drug design. Molecules 2017;22(8):1210. [CrossRef] [PubMed]
8. Jumina J, Harizal H, Kurniawan YS. Chalcones in dermatology. In: Levine MP, Santos JS, editors. Beauty - Cosmetic science, cultural issues and creative developments [Internet]. London: IntechOpen; 2020 [cited 2022 May 17]. Available from: URL: https://www.intechopen.com/chapters/71036 [CrossRef]
9. Ni L, Meng CQ, Sikorski JA. Recent advances in therapeutic chalcones. Expert Opin Ther Pat 2004;14(12): 1669-91. [CrossRef]
10. Kunthalert D, Baothong S, Khetkam P, Chokchaisiri S, Suksamrarn A. A chalcone with potent inhibiting activity against biofilm formation by nontypeable Haemophilus influenzae. Microbiol Immunol 2014;58(10): 581-9. [CrossRef] [PubMed]
11. Okolo EN, Ugwu DI, Ezema BE, Ndefo JC, Eze FU, Ezema C et al. New chalcone derivatives as potential antimicrobial and antioxidant agent. Sci Rep 2021; 11(1):21781. [CrossRef] [PubMed]
12. Singh P, Anand A, Kumar V. Recent developments in biological activities of chalcones: a mini review. Eur J Med Chem 2014;85:758-77. [CrossRef] [PubMed]
13. Gutierrez RMP, Muniz-Ramirez A, Sauceda JV. Review: The potential of chalcones as source of drugs. Afr J Pharm Pharmacol 2015;9(8):237-57. [CrossRef] [PubMed]
14. Dan W, Dai J. Recent developments of chalcones as potential antibacterial agents in medicinal chemistry. Eur J Med Chem 2020;187:111980. [CrossRef] [PubMed]
15. NCCLS Performance Standards for Antimicrobial Disk Susceptibility Test, 6th ed., National Committee for Clinical Laboratory Standards, approved standard: P. A. Wayne, M100- S9, 1997.
16. Lazarević J, Ralić R, Radulović N, Ristić N, Stojanović G. Chemical composition and screening of the antimicrobial and antioxidative activity of extracts of *Stachys* species. J Serb Chem Soc 2010;75(10): 1347-59. [CrossRef]
17. Ventura TL, Calixto SD, de Azevedo Abrahim-Vieira B, de Souza AM, Mello MV, Rodrigues CR et al. Anti-mycobacterial and anti-inflammatory activities of substituted chalcones focusing on an anti-tuberculosis dual treatment approach. Molecules 2015;20(5): 8072-93. [CrossRef] [PubMed]
18. Chen ZH, Zheng CJ, Sun LP, Piao HR. Synthesis of new chalcone derivatives containing a rhodanine-3-acetic acid moiety with potential anti-bacterial activity. Eur J Med Chem 2010;45(12):5739-43. [CrossRef] [PubMed]
19. Tran TD, Do TH, Tran NC, Ngo TD, Huynh TN, Tran CD, et al. Synthesis and anti Methicillin resistant Staphylococcus aureus activity of substituted chalcones alone and in combination with non-beta-lactam antibiotics. Bioorg Med Chem Lett 2012;22(14):4555-60. [CrossRef] [PubMed]
20. Xu M, Wu P, Shen F, Ji J, Rakesh KP. Chalcone derivatives and their antibacterial activities: Current development. Bioorg Chem 2019;91:103133. [CrossRef] [PubMed]

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SINTEZA I PROCENA ANTIMIKROBNE AKTIVNOSTI MONOSUPSTITUISANIH DERIVATA 3',4'-DIMETOKSIHALKONA

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Monosupstituisani 3',4'-dimetoksihalkonski derivati sintetisani su bazno katalizovanom Klajzen-Šmitovom kondenzacijom, strukturno okarakterisani i podvrgnuti *in vitro* ispitivanju antibakterijske aktivnosti na laboratorijske sojeve Gram-pozitivnih (*Bacillus subtilis* ATCC 6633 i *Staphylococcus aureus* ATCC 6538) i Gram-negativnih (*Escherichia coli* ATCC 8739 i *Salmonela typhimurium* ATCC 14028) bakterija. 4-Brom-3',4'-dimetoksihalkon deluje baktericidno na Gram-negativne sojeve, ispoljavajući snažniji antimikrobni efekat prema *E. coli* (11 mm ± 0,3 mm), u odnosu na *S. typhimurium* (15 mm ± 0,7 mm). Rezultati antimikrobnog testa ukazuju na potencijalni značaj pozicije 4-A-prstena halkona u kreiranju antibakterijskih agenasa selektivnog dejstva.

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Ključne reči: *halkonski derivati, sinteza, antibakterijska aktivnost*