Bioorganic & Medicinal Chemistry Letters 22 (2012) 6343–6346

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis of β-ionone derived chalcones as potent antimicrobial agents

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ARTICLE INFO

Article history:
Received 29 May 2012
Revised 25 July 2012
Accepted 22 August 2012
Available online 30 August 2012

Keywords:
β-Ionone
β-Ionone derived chalcones
Antibacterial activity
Antifungal activity

ABSTRACT

A series of chalcones (**3a**–**v**) have been synthesized by condensation of β-ionone (**1**) with a variety of aldehydes (**2a**–**v**). The synthesized compounds have been screened for their in vitro antimicrobial activity against five bacterial and five fungal strains, using disc diffusion assay. The evaluated compounds display a wide range of activities, from completely inactive to the highly active compounds. Some of the compounds are also active against methicillin resistant *staphylococcus aureus* (MRSA).

Life threatening infections caused by pathogenic fungi and bacteria are becoming increasingly common and widespread epidemics in the world, especially in individuals with suppressed immune system that is, cancer, AIDS patients etc. Antimicrobial treatment used for these infections is also a serious public health threat because of rapid development of resistance by increasing number of pathogenic microorganisms against multiple drugs.[1–3] Therefore, efforts are directed towards the design, synthesis and evaluation of the new chemical therapeutics as alternative to existing antimicrobial therapy. Chalcones represent an important group of natural products. Both naturally occurring and synthetic chalcones (Fig. 1) exhibit remarkable pharmacological activities such as antioxidant,[4] tyrosinase inhibition,[5] antileishmanial,[6] anticancer,[7] antiangiogenic,[8] anti-inflammatory,[9] nitric oxide inhibition,[10] antifungal,[11] antibacterial[12–15] etc. Recently, Liaras et al. have reported some thiazole based chalcones displaying greater activity than reference drugs.[12b] Chalcones such as 4-hydroxychalcone and 2,4-dihydroxychalcone have been found to exhibit moderate activity against MRSA when used alone and showed significant synergistic effect in combination with non beta-lactam antibiotics.[12c]

On other hand, β-ionone is an important phytochemical present in many fruits, vegetables and grains and known to exert diverse biological activities including antibacterial and antifungal activity.[16–18] Taking cognizance of wide range of biological activities of both β-ionone and chalcones, particularly antibacterial and antifungal, it was decided to synthesize some β-ionone derived chalcones so that synergistic effect of both valuable moieties in single scaffold may result in formation of some potential antimicrobial molecules. Recently, some ionones based chalcones have been reported as potential anticancer agents against prostate cancer cells.[7b] β-ionones derived chalcones (**3a**–**v**) were prepared by condensing freshly distilled β-ionone (**1**, Scheme 1, Table 1) with distilled/crystallized subsituted benzaldehydes (**2a**–**v**) in the presence of 10% NaOH water-ethanol solution.[19] The products were purified through chromatography over silica gel (hexane, hexane-benzene gradient) and/or by crystal1ization with diethylether and characterized through spectral data (IR, ^{1}H NMR, ^{13}C NMR, mass) and elemental analysis.

The assigned structures of chalcones are based on detailed spectroscopic analysis. IR spectrum of compound **3i** revealed a strong band at 1650 cm^{-1}, along with resonance in its ^{13}C NMR at δ 188.4 indicating the presence of a keto carbonyl function. Its ^{1}H NMR revealed, besides aromatic proton resonances in the region δ 7.27–7.15, two β-protons of α,β-unsturated carbonyl moiety showed up as doublets at δ 7.97 and 7.43 with *trans* olefinic-H coupling constant J = 15.9 Hz and two α-protons appeared as doublets at δ 6.87 and 6.64 (J = 15.9 Hz).

Antibacterial activity

In vitro antibacterial studies of various compounds (**3a**–**v**) were carried out against Gram negative and gram positive bacterial strains by disk-diffusion assay.[20] The Gram positive strains used were *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 2451) and gram negative bacteria were *Escherichia coli* (MTCC 82), *Pseudomonas aeruginosa* (MTCC 2642), *Salmonella typhimurium* (MTCC 1251). The activity of compounds was determined in com-

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http://dx.doi.org/10.1016/j.bmcl.2012.08.084

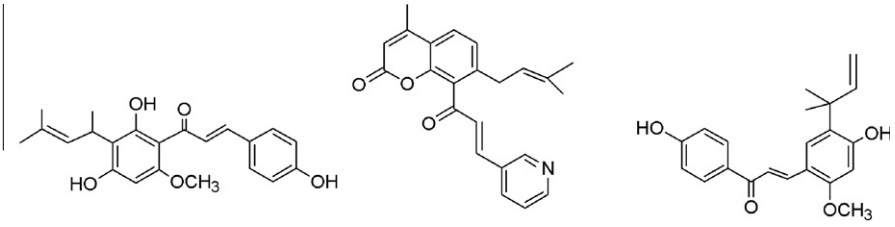
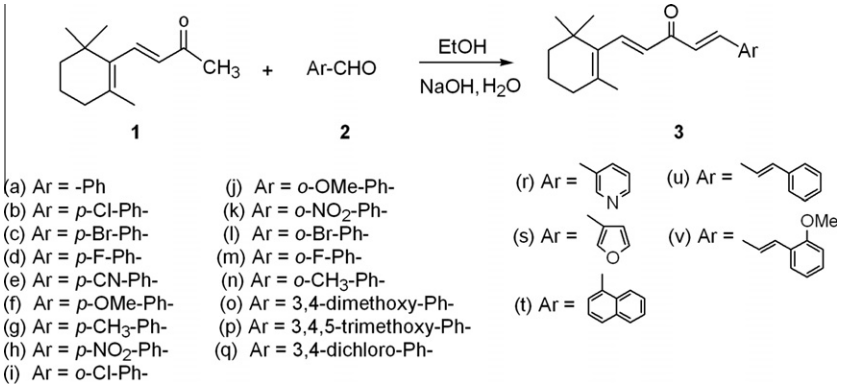


Figure 1. Some biologically active chalcones.



Scheme 1. Synthesis of β-ionones derived chalcones (**3a**–**v**).

Table 1
Time (min) and yield (%) of various β-ionones derived chalcones (**3a**–**v**).

Comp. No.	Time (min)	(%) Yield	Comp. No.	Time (min)	(%) Yield
3a	45	90	**3l**	40	90
3b	40	90	**3m**	40	85
3c	40	85	**3n**	55	80
3d	40	90	**3o**	60	75
3e	40	90	**3p**	60	75
3f	50	85	**3q**	45	90
3g	55	80	**3r**	50	75
3h	40	80	**3s**	50	80
3i	40	90	**3t**	55	85
3j	50	90	**3u**	55	80
3k	40	80	**3v**	60	80

parison to standard antibiotic discs of Amoxicillin (5 μg) and Ciprofloxacin (10 μg). Pre-warmed Mueller–Hinton agar plates were inoculated with 10^6 CFU/mL of test bacteria. Each compound was dissolved in DMSO (1 mg/ml) and then 30 μL of each was pipetted onto sterile paper discs (6 mm diameter) placed on the surface of inoculated agar plates. Plates were incubated at 37 °C for 24 h. Activity was expressed as the diameter of the inhibition zone (mm) produced by the compounds. DMSO was used as negative control. Minimum inhibitory concenteration (MIC) of compounds exhibiting considerable activity (Table 2 and Fig. 2) was determined by using serial tube dilution method.[21] The initial optical density (OD) of the medium was measured by spectrophotometer at 600 nm. The test strains were incubated in nutrient broth until the OD reached 0.4–0.6. Different concentrations of compounds (0.78, 1.56, 3.12, 6.25, 12.5, 25, 50 μg/ml) were tested for the inhibition of growth of these microbes in separate tubes. The 10 ml tubes each containing 5 ml nutrient broth and 1 ml of different concentrations of compounds were incubated for 24 h with shaking at 180 rpm using a rotary shaker. Each tube corresponding to different concentration was observed and concentration showing apparently no turbidity was considered to be the MIC of respective compound.

Table 2
MIC (μg/ml) of compounds **3a**–**v** against various bacterial strains.

Comp. No.	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*S. typhi*
3a	**3.12**	**3.12**	**1.56**	**12.5**	**12.5**
3b	**6.25**	**25**	**3.12**	**12.5**	>100
3c	—	—	>100	>100	>100
3d	**25**	**25.5**	>100	—	—
3e	>100	42	37	—	>100
3f	—	**25**	>100	—	>100
3g	**25**	>100	—	65	>100
3h	**3.12**	**25**	**6.25**	**12.5**	>100
3i	**0.78**	**1.56**	**1.56**	**12.5**	>100
3j	**25**	**22.2**	**31.5**	>100	>100
3k	**3.12**	**6.25**	**12.5**	**3.12**	**25**
3l	40.2	**25**	—	>100	—
3m	—	>100	>100	—	>100
3n	**25**	>100	**30.5**	—	55.5
3o	**22.5**	—	**42**	**55.5**	>100
3p	>100	**19.5**	—	**22.5**	45
3q	**25**	**32.5**	**22**	—	>100
3r	**6.25**	**6.25**	**12.5**	**12.5**	>100
3s	**22.5**	—	>100	>100	74
3t	**25**	**25**	**55.5**	—	—
3u	**3.12**	**6.25**	**6.25**	>100	50
3v	25	>100	>100	32.5	>100
Amoxicillin	**0.5**	**0.5**	**0.12**	**1.0**	**0.9**
Ciprofloxacin	**0.75**	**1.2**	**0.9**	**1.8**	**—**

Compound **3i** showed highest antibacterial potential against both Gram positive and Gram negative bacteria with MIC 0.78 against *B. subtilis*, followed by MIC 1.56 against both *S. aureus* and *E. coli* and MIC 12.5 against *P. aeruginosa*. Compound **3a** displayed valuable antibacterial activity with MIC 1.56 against *E. coli*, followed by MIC 3.12 against both *B. subtilis* and *S. aureus*,

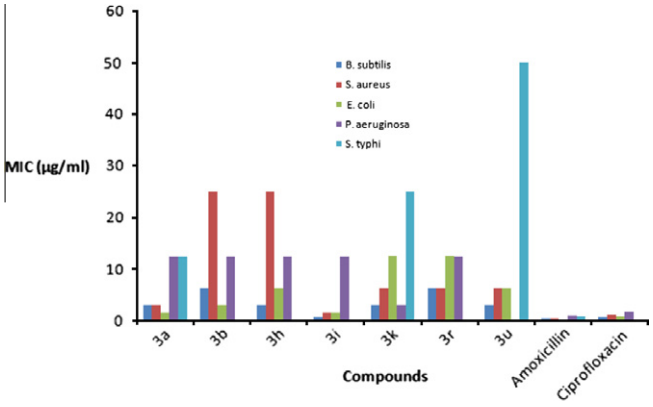


Figure 2. Graphical representation of the antibacterial activity.

Table 3
MIC (mg/ml) of compounds against methecillin resistant *staphylococcus aureus* (MRSA).

Comp. No.	**3a**	**3b**	**3k**	**3n**	**3p**	**3u**	**3r**
MRSA	9	10	9	5	20	4	5

MIC 12.5 against *P. aeruginosa* and *S. typhimurium* Compound **3b** exhibited significant activity with MIC 3.12 against *E. coli*, followed by MIC 6.25 against *B. subtilis* and MIC 12.5 against *P. aeruginosa*. Compound **3h** showed good antibacterial activity with MIC 3.12 against *B. subtilis*, followed by MIC 6.25 against *E. coli* and MIC 12.5 against *S. typhimurium* Compound **3k** showed promising activity with MIC 3.12 against both *B. subtilis* and *P. aeruginosa*, followed by MIC 6.25 against *S. aureus* and MIC 12.5 against *E. coli*. Compound **3u** possesses potential antibacterial activity with MIC 3.12 against *B. subtilis*, followed by MIC 6.25 against both *S. aureus* and *E. coli*. Compound **3r** was also endowed activity with MIC 6.25 against both *B. subtilis* and *S. aureus*, followed by MIC 12.5 against *E. coli* and *P. aeruginosa*. Active compounds **3a**, **3b**, **3k**, **3n**, **3p**, **3u**, **3r** were further evaluated against bacterial resistant strains such as methecillin resistant *S. aureus* (MRSA), a clinically isolate obtained from PGIEMR-Chandigarh and *Klebsiella pneumoniae* (MTCC 530) by using disk-diffusion assay.[20] Compounds were found to be active against MRSA and completely inactive against *K. pneumoniae*. Minimum inhibitory concenteration (MIC) in mg/ml of compounds exhibiting activity (Table 3) was determined by using serial tube dilution method.[21]

Structure–activity relationship can be established on the basis of observed antibacterial activities. Compounds bearing electron widhrawing lipophilic groups such as –Cl and $-NO_2$ subsituents on aromatic ring were endowed with valuable antibacterial activity as compared to compounds bearing electron releasing groups at aromatic ring. However, replacement of aryl moiety with pyridyl (**3r**) leads to enhancement of antibacterial activity.

Antifungal activity

All synthesized compounds **3a**–**v** were tested against five reference fungal strains such as *Aspergillus niger* (MTCC 1344), *Saccharomyces cerevisiae* (MTCC 172), *Candida albicans* (MTCC 3018), *Cryptococcus gastricus* (MTCC 1715) and *Microsporum gypseum* (MTCC 4490). by using disc diffusion method.[20] The antifungal activity of synthesized compounds was determined by observing the zone of inhibition in comparison to the standard antifungal discs (Fluconazole and Griseofulvin). Test compounds were dissolved in DMSO to make a stock solution of 1 mg/ml. The fresh sub culture of strains in normal saline was added to the sterile assay medium (Saboraud Dextrose agar with Chloramphenicol) at 40–45 °C and mixed well. The medium was poured into each of the petridishes. Sterile discs of diameter 6 mm were placed on the medium and 20 µl of each test solution was added to the previously marked discs and the media was allowed to stand for 5 min. The petridishes were kept aside for 1 h and then incubated at 28 °C for 48 h. Zone of inhibition was measured and the average of the three readings was calculated. DMSO was kept as negative control. Minimum inhibitory concentration (MIC) of compounds was determined by serial tube dilution method.[21] Different dilutions of test compounds (1.9 µg/ml–500 µg/ml) were made from stock solution and 1 ml nutrient broth was taken in each test tube and 20 µl of standard strains were added to previously marked test tubes.

Table 4
MIC (µg/ml) of active compounds against various fungal strains.

Comp. No.	*A. niger*	*S. cerevisiae*	*C. albicans*	*C. gastricus*	*M. gypseum*
3a	>100	61.5	>100	78	>100
3b	>100	**12.5**	>100	>100	>100
3d	**15.6**	**15.6**	**12.5**	>100	>100
3e	52.4	>100	80	>100	90.5
3g	>100	40.5	46	>100	>100
3h	**9.6**	**19.5**	**11.2**	90.4	82
3i	33.5	**12.5**	**11.5**	>100	94
3j	92.5	61.4	>100	>100	88.5
3k	32.4	42.5	>100	58.4	>100
3l	**7.6**	**3.9**	31.2	>100	>100
3m	**9.9**	**11.4**	44.5	>100	>100
3n	>100	96	72	>100	>100
3r	**3.9**	**10.5**	35	>100	>100
3s	61.4	>100	74	72.5	>100
3t	55	>100	71.5	>100	98
3u	25	80	>100	55.5	>100
3v	80	34	>100	19.5	71.5
Fluconazole	**1.9**	**1.9**	**3.9**	**31.2**	**1.9**
Griseofulvin	**1.9**	**1.9**	**1.9**	**1.9**	**1.9**

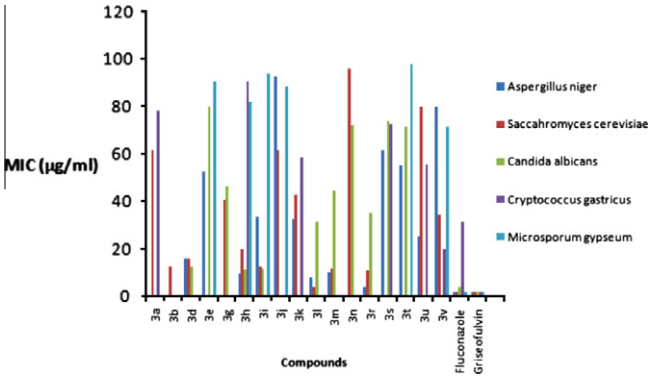


Figure 3. Graphical representation of the antifungal activity.

The compounds evaluated were found to exert prominent antifungal activity against various fungal strains, specially, against *A. niger*, *S. cerevisiae* and *C.albicans* (Table 4, Fig. 3). Compounds **3h**, **3l**, **3m**, and **3r** showed significant inhibitory activity against *A. niger* with MIC < 10, whereas, compounds **3d** showed inhibitory activity with MIC 15.6. Compound **3l** posses maximum inhibitory potential against *S. cerevisiae* with MIC 3.9 whereas, compounds **3b**, **3d**, **3h**, **3i**, **3m** and **3r** displayed good inhibitory potential with MIC < 20. Compounds **3d**, **3h** and **3i** exhibit promising activity against *C. albicans* with MIC < 15.

In conclusion, a series of β-ionone derived chalcones (**3a**–**v**) were synthesize through condensation of β-ionone (**1**) with subsituted benzaldehydes (**2a**–**v**) in the presence of 10% NaOH water-

ethanol solution. The synthesized compounds were evaluated for in vitro antimicrobial activity against various pathogenic bacterial and fungal strains. Chalcone **3i** showed valuable inhibitory activity against all bacterial strains, specially, against *B. subtilis*, whereas the chalcone **3l** displayed the highest inhibitory potential against *A. niger* and *S. cerevisiae*. Antimicrobial activities also revealed that compounds bearing electron withdrawing groups are more potent than compounds bearing electron releasing groups and activities were found to be enhanced on replacement of -aryl with -pyridyl moiety. Some of the compounds were also found to be active against MRSA. These 'lead' compounds can be considered for investigation of their mode of action and for further development.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.08.084.

Reference and notes

1. Dessen, A.; Di Guilmi, A. M.; Vernet, T.; Dideberg, O. *Curr. Drug Targets: Infect. Disord.* **2001**, *1*, 63.
2. Pfeltz, R. F.; Wilkinson, B. J. *Curr. Drug Targets: Infect. Disord.* **2004**, *4*, 273.
3. Roberts, M. C. *Curr. Drug Targets: Infect. Disord.* **2004**, *4*, 207.
4. Vogel, S.; Ohmayer, S.; Heilmann, G. *Bioorg. Med. Chem.* **2008**, *16*, 4286.
5. Nerya, O.; Musa, R.; Khatib, S.; Tamir, S.; Vaya, J. *Phytochemistry* **2004**, *65*, 1389.
6. Suryawanshi, S. N.; Chandra, N.; Kumar, P.; Porwal, J.; Gupta, S. *Eur. J. Med. Chem.* **2008**, *43*, 2473.
7. (a) Lawrence, N. J.; Patterson, R. P.; Ooi, L. L.; Cook, D.; Ducki, S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5844; (b) Zhou, j.; Geng, G.; Batist, G.; Jian, W. H. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1183.
8. Mojzis, J.; Varinska, L.; Mojzisova, G.; Kostova, I.; Mirossay, L. *Pharmacol. Res.* **2008**, *57*, 259.
9. Cheng, J. H.; Hung, C. F.; Yang, S. C.; Wang, J. P.; Won, S. J.; Lin, C. N. *Bioorg. Med. Chem.* **2008**, *16*, 7270.
10. Rojas, J.; Paya, M.; Dominguez, J. N.; Ferrandiz, M. L. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1951.
11. Lahtchev, K. L.; Batovska, D. I.; Parushev, S. P.; Ubiyvovk, V. M.; Sibirny, A. A. *Eur. J. Med. Chem.* **2008**, *43*, 2220.
12. (a) Avila, H. P.; Smania, E.; Monache, F. D.; Smania, A. *Bioorg. Med. Chem.* **2008**, *16*, 9790; (b) Liaras, K.; Geronikaki, A.; Glamoclija, J.; Iric, A. C.; Sokovic, M. *Bioorg. Med. Chem.* **2011**, *19*, 3135; (c) Tran, T. D.; Do, T. H.; Tran, N. C.; Ngo, T. D.; Huynh, T. N. P.; Tran, C. D.; Thai, K. M. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4555.
13. Gerhauser, C.; Alt, A.; Heiss, E.; Gamal-Eldeen, A.; Klimo, K.; Knauft, J.; Neumann, I.; Scherf, H. R.; Frank, N.; Bartsch, H.; Becker, H. *Mol Cancer Ther.* **2002**, *1*, 959.
14. Koneni, V.; Sashidhara; Kumar, A.; Kumar, M.; Sarkar, J.; Sinha, S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7205.
15. Tsukiyama, R. I.; Katsura, H.; Tokuriki, N.; Kobayashi, M. *Antimicrob. Agents Chemother.* **2002**, *46*, 1226.
16. Zhu, J.; Zhang, L.; Jin, X.; Han, X.; Sun, C.; Yan, J.; Mol. Biol. Rep. DOI 10.1007/s11033-009-9793-y.
17. Mikhlin, E. D.; Radina, V. P.; Dmitrovskii, A. A.; Blinkova, L. P.; Butova, L. G. *Priki. Biokhim. Mikrobiol.* **1983**, *19*, 795.
18. Mandiola, J. A.; Santoyo, S.; Cifuentes, A.; Reglero, G.; Ibanez, E.; Senorans, F. J. J. *Food. Prot.* **2008**, *71*, 2138.
19. Gandhi, R. P.; Kumar, S.; Aryan, R. C.; Ishar, M. P. S. *Syn. commun.* **1989**, *19*, 1759.
20. Mitic-Ćulafic, D.; Vukovic-Gacic, B.; Knezevic-Vukcevic, J.; Stankovic, S.; Simic, D. *Arch. Biol. Sci.* **2005**, *57*, 173.
21. Hindler, J.; Mahon, C.C.; Manuselis, G. (Eds.), Textbook of Diagnostic Microbiology Special Antimicrobial Susceptibility Tests **1995**, *89*.