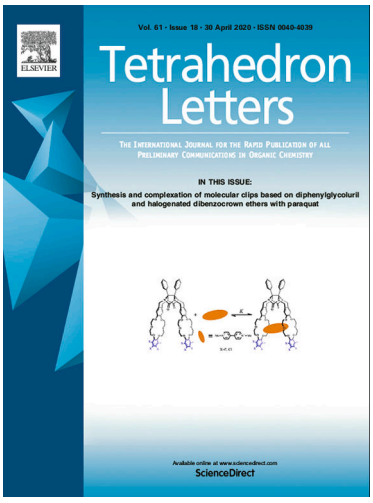



# Synthesis and pharmacological evaluation of childinin E and several derivatives as *anti*-hyphal formation inhibitors against *Candida albicans*

Hitoshi Kamauchi, Momoka Hirata, Koichi Takao, Yoshiaki Sugita

PII: S0040-4039(20)31087-X
DOI: https://doi.org/10.1016/j.tetlet.2020.152588
Reference: TETL 152588

To appear in: *Tetrahedron Letters*

Received Date: 27 August 2020
Revised Date: 14 October 2020
Accepted Date: 19 October 2020

Please cite this article as: Kamauchi, H., Hirata, M., Takao, K., Sugita, Y., Synthesis and pharmacological evaluation of childinin E and several derivatives as *anti*-hyphal formation inhibitors against *Candida albicans*, *Tetrahedron Letters* (2020), doi: https://doi.org/10.1016/j.tetlet.2020.152588

ELSEVIER

Tetrahedron Letters

journal homepage: www.elsevier.com

# Synthesis and pharmacological evaluation of childinin E and several derivatives as *anti*-hyphal formation inhibitors against *Candida albicans*

Hitoshi Kamauchi*, Momoka Hirata, Koichi Takao, Yoshiaki Sugita

*Laboratory of Bioorganic Chemistry, Department of Pharmaceutical Sciences, Faculty of Pharmacy and Pharmaceutical Sciences, Josai University, 1-1 Keyaki-dai, Sakado, Saitama 350-0295, Japan*

ARTICLE INFO

*Article history:*
Received
Received in revised form
Accepted
Available online

*Keywords:*
hyphal formation
*Candida albicans*
benzophenone
*Daldinia* spp.

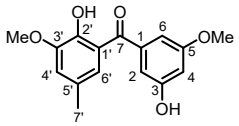
* Corresponding author. Tel./fax: +81-49-271-7256; e-mail: kamauchi@josai.ac.jp

ABSTRACT

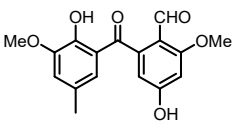
The natural highly substituted benzophenone childinin E (**1**) was previously isolated from the fungus *Daldinia childiae*. Here we describe the total synthesis of childinin E and several derivatives using a linear seven-step sequence. The antifungal properties of the synthesized compounds against *Candida albicans* were evaluated by an *anti*-hyphal formation test. Childnin E and two derivatives exhibited *anti*-hyphal formation activity.



The fungus *Daldinia* is a rich source of polyketides with unique structures. The highly functionalized benzophenones daldinals A and B and childinins D and E (**1-4**) have been isolated from *D. concentrica* and *D. childiae* (Fig. 1) [1, 2] but their bioactivities are poorly investigated. For example, only the nitric oxide production inhibitory activities of daldinals have been evaluated.[3] Natural highly functionalized benzophenones show various bioactivities. Pestalone, isolated from a marine derived fungus, shows *in vitro* cytotoxicity

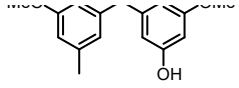



**1** childinin E

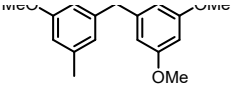



**2** daldinal A

Fig. 1 Structures of benzophenones isolated from *Daldinia* spp.




**3** daldinal B



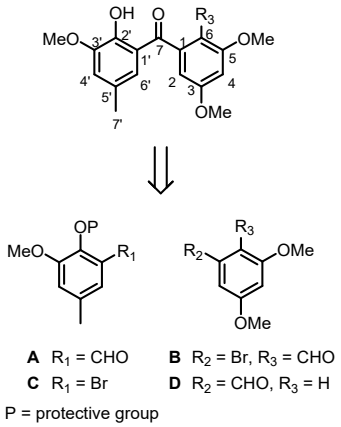

**4** childinin D

against tumor cell lines and antibiotic activity against drug-resistant *Staphylococcus aureus* and *Enterococcus faecium*, [4] and xanthochymol isolated from *Garcinia xanthochymus* fruit shows antifungal activity against *Candida albicans*. [5] Natural highly functionalized benzophenones thus exhibit promising antibiotic properties.

The hyphal-yeast transition of *C. albicans* is an important antifungal target and represents the first step in the development of spatially highly structured and dynamic organized mature biofilms. Biofilms are virulence factors that aid in the adhesion to epithelial and endothelial cell types that resist to current antifungal therapeutics. [6] Farnesol, an autoregulatory molecule produced by *C. albicans*, inhibits the induction of hyphal growth by inhibiting adenylate cyclase (Cyr1). [7] However, few compounds have been identified with *anti*-hyphal formation activity compared to typically used antifungal agents (e.g., azole and polyene type antibiotics).

Here we describe the synthesis of natural benzophenones isolated from *Daldinia* spp. and several derivatives, and their *in vitro* antifungal activities against *C. albicans* determined using an *anti*-hyphal formation assay.

To synthesize the target compounds, we first elaborated the synthesis of 3-methoxy benzophenone derivatives using a multi-step reaction to probe the general feasibility of the synthetic approach. The strategy behind our synthesis is summarized in Scheme 1. The benzophenone scaffold is intended to be assembled using two building blocks. Building block A is a 2,3,5-substituted benzaldehyde and building block B is a 2,3,5-substituted bromobenzene. The two blocks are linked by reaction with a lithiated arene of building block B and subsequent oxidation. The introduction of formyl group at C-6 was placed at a faster stage in the synthesis.

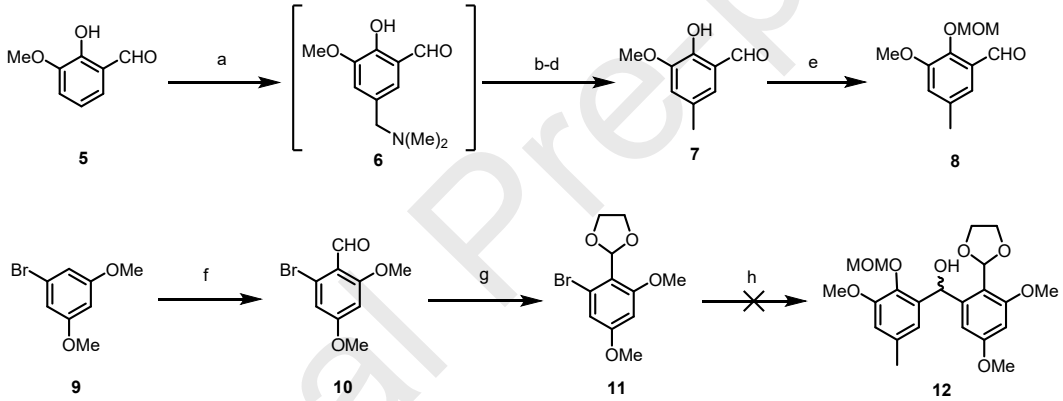



Scheme 1. Retrosynthetic analysis of benzophenone derivatives.

Starting from commercial *o*-vanillin (**5**), benzaldehyde (**7**) was prepared by selective methylation at C-5 *via* intermediate **6**, and the hydroxy group was protected with MOM-Cl, yielding **8** as building block A.[8] Compound 11 as building block B was prepared by Vilsmeier formylation of 1-bromo-3,5-dimethoxybenzene (**9**) followed by and acetal protection (Scheme 2).[9]

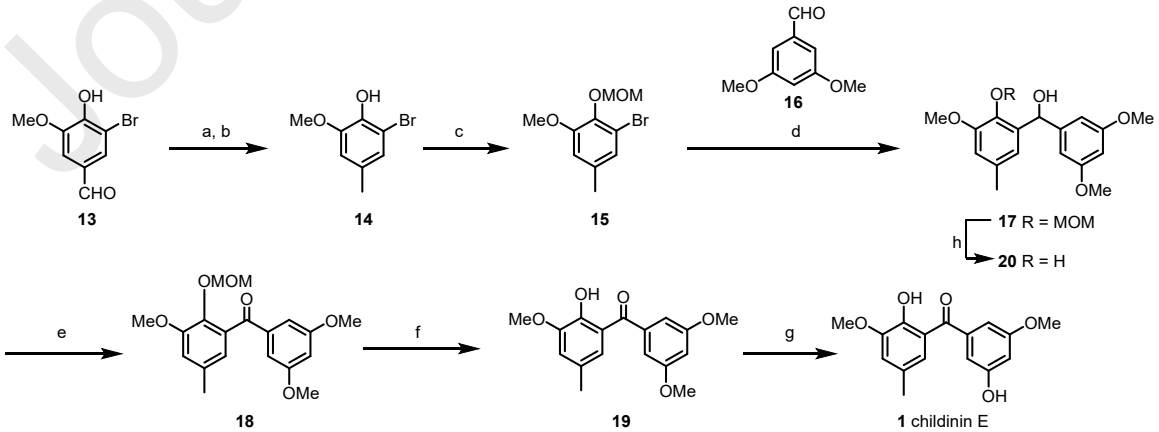
Bromine–lithium exchange of **11** employing *n*-BuLi, followed by reaction with **8**, did not provide the expected benzhydrol **12**. The same reaction using **9** in place of **11** also resulted in no reaction.

We therefore changed our strategy and exchanged the substituted groups on bromobenzene and benzaldehyde (Scheme 1). We



Scheme 2. Synthesis of benzophenone derivatives: a) HCHO, $NH(Me)_2$, reflux, 4 h; b) $Ac_2O$, reflux, overnight; c) HCl, rt, 1.5 h; d) $SnCl_2$, reflux, 0.5 h, 53% over four steps; e) MOMCl, DIPEA, rt, 3 h, 85%; f) DMF, $POCl_3$, 0°C to 100°C, overnight, 61%; g) ethylene glycol, *p*TsOH, reflux, 3 h, 71%. h) *n*-BuLi, **8**, −78°C to rt.

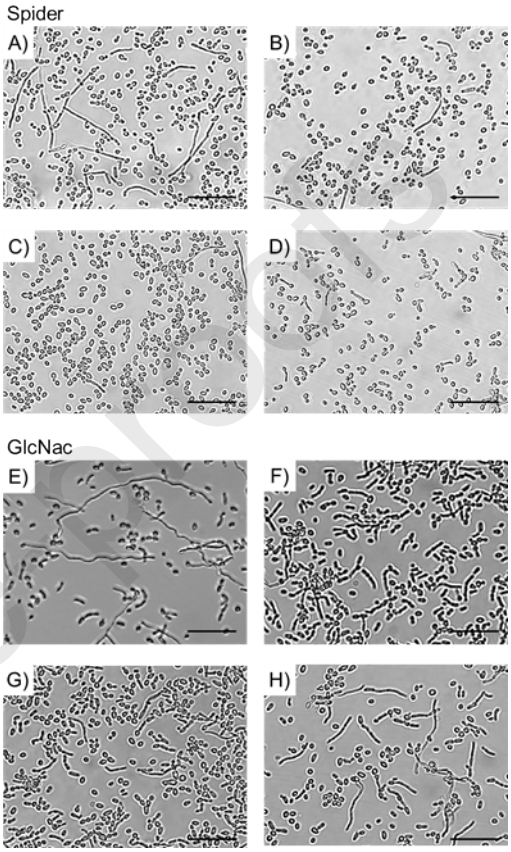
prepared a 2,3,5-substituted bromobenzene as building block C and a 3,5-substituted benzaldehyde as building block D. The benzophenone scaffold was synthesized as described above. The target compound was non-substituted at C-6 due to the complicated process required for formylation at C-6.



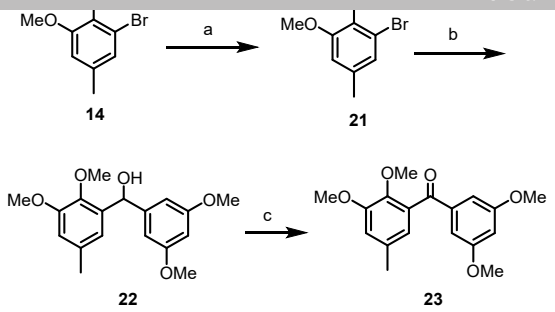
Scheme 3. Synthesis of childinin E: a) hydrazine monohydrate, diethylene glycol, 110°C, 15 min; b) KOH, 150°C, 2 h, 99% over two steps; c) MOMCl, DIPEA, rt, 3 h, 77%; d) *n*-BuLi, **16**, −78°C to rt, 15 min, 41%; e) $MnO_2$, rt, overnight, 41%; f) $BCl_3$, 0°C to rt, overnight, 74%; g) $BBr_3$, −78°C to reflux, 1 h, 25%; h) HCl, rt, 1 h, 67%.

E (**13**) (Scheme 3).[10] The phenolic hydroxy group at C-2 was protected by MOM. Bromine–lithium exchange of **15** employing *n*-BuLi, followed by reaction with 3,5-dimethoxybenzaldehyde (**16**) at −78°C to rt, gave benzhydrol **17** in 41% yield. Oxidation of benzylic position of **17** with $MnO_2$ provided benzophenone derivative **18**. The MOM protecting group was cleaved using a Lewis acid in good yield to give 3-*O*-methyl-childinin E (**19**).

Finally, the methoxy groups in **19** were removed using $BBr_3$. There are three methoxy groups in **19**: one each at C-3, C-5 and C-3'. Demethylation proceeded selectively at C-3 or C-5 and yielded childinin E (**1**) as a white solid. The $^{1}H$, $^{13}C$ and MS spectroscopic properties of synthetic **1** matched those reported for the natural product.

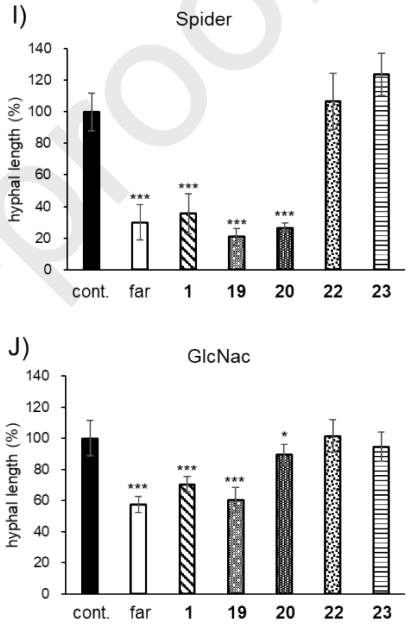



In contrast, the formylation of **18** or **19** by classical Vilsmeier formylation, or dichloro-methylation using $TiCl_4$ or $SnCl_4/CH_2Cl_2$ were unsuccessful. To investigate the structure-activity relationship of these benzophenone derivatives, we focused on synthesizing several derivatives by introducing a number of hydroxy groups using the above synthetic route. The MOM group in benzhydrol **17** was removed using 10% aqueous HCl in dioxane to yield 2'-hydroxy benzhydrol **20** (Scheme 3). The 2'-methoxy derivatives were synthesized from 2,3-dimethoxy-5-methylbromobenzene **21** as the building block (Scheme 4), prepared by methylating **14** with iodomethane.[10] 2'-*O*-Methyl-benzhydrol **22** and 2'-*O*-methyl-benzophenone **23** were obtained by bromine–lithium exchange of **21** followed by reaction with **16** and oxidation of benzylic position of **22** with $MnO_2$.




Scheme 4. Synthesis of 2'-methoxy benzophenone derivatives: a) MeI, $K_2CO_3$, 80°C, overnight, quant.; b) *n*-BuLi, **16**, −78°C to rt, 15 min, 20%; c) $MnO_2$, rt, overnight, 55%.

The *anti*-hyphal formation activities of the synthesized benzophenone and its derivatives (**1**, **19**, **20**, **22** and **23**) were tested against *C. albicans* (SC-5314) using spider medium and *N*-acetylglucosamine (GlcNac) medium. Hyphal formation of *C. albicans* is triggered by several external signals and can be controlled by the choice of medium. GlcNac medium activates the MAPK signaling pathway,



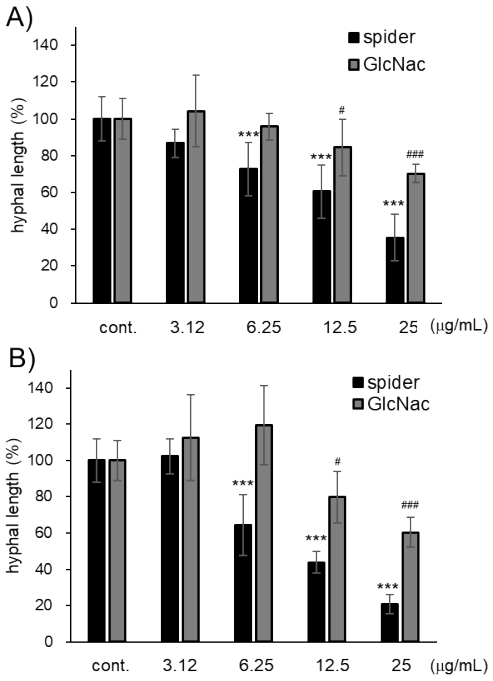

suppressing the Cph1 gene,[11] whereas the cAMP-PKA signaling pathway is activating by spider medium, suppressing the Efg1 transcription factor gene.[12] The induced hyphal cells were picked and measured their length from photo. Evaluation was performed by using the average of hyphal length for each compound.

The results of the *anti*-hyphal formation test are shown in Fig. 2 (all photo was represented in supplementary data). In spider medium, **1**, **19** and **20** showed *anti*-hyphal formation activity at 25 μg/mL, and **1** and **19** also showed inhibitory activity in GlcNac medium, but this activity was weaker than in spider medium. Compound **20** show inhibitory activity only in spider medium. Compounds **22** and **23** showed no inhibitory activity.

We investigated the dose-dependencies of **1** and **19** and found that both compounds showed inhibitory activities of over 25% at 6.25 μg/mL in spider medium whereas activity was observed at 12.5 μg/mL in GlcNac medium (Fig. 3). Toxicity was checked using minimum inhibitory concentration (MIC) values by performing susceptibility testing according to the CLSI M27-A2 microdilution method using spider and GlcNac media. The MIC values of **1** and **19** were 100 μg/mL, whereas **20** showed no toxicity under both conditions.

Fig. 2 Benzophenone derivative inhibition of hyphal formation by *C. albicans*. Hyphal growth of *C. albicans* in spider and GlcNac media in the presence of DMSO (A and E), **1** (B and F), **19** (C and G) and **20** (D and H) after 24 h incubation at 37°C. Measured hyphal length of *C. albicans* in spider (I) and GlcNac (J) media in the presence of the synthesized compounds. Compounds were tested at 25 μg/mL, except for farnesol (far) in GlcNac medium (50 μg/mL), used as a positive control. *, ***: $P < 0.05$, 0.001 vs. control. Scale bars represent 50 μm.

T…nds active in spider medium had a hydroxy group at C-2' whereas compounds active in GlcNac medium also required a carbonyl group at C-7, perhaps because a different signaling pathway is activated in spider and GlcNac media.




Thus, we completed the total synthesis of childinin E and some structural analogues and evaluated their antifungal properties by inhibiting hyphal formation of *C. albicans*. The important substituted groups in benzophenone with *anti*-hyphal formation activity (hydroxy group at C-2' and carbonyl group at C-7) were discovered. The further extension of this study could develop new class of antifungal compounds.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

This work was supported by JSPS KAKENHI Grant Number JP20K16029.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at

## References and notes

1. Hashimoto T, Tahara S, Takaoka S, Tori M, Asakawa Y, *Chem. Pharm. Bull*. 1994; 42: 1528–1530.
2. Zhao ZZ, Chen HP, Huang Y, Zhang SB, Li ZH, Feng T, Liu JK. *Phytochemistry*. 2017; 142: 68–75.
3. Quang DN, Harinantenaina L, Nishizawa T, Hashimoto T, Kohchi C, Soma GI, Asakawa Y. *J. Nat. Med*. 2006; 60: 303–307.
4. Cueto M, Jensen PR, Kauffman C, Fenical W, Lobkovsky E, Clardy J. *J. Nat. Prod*. 2001; 64: 1444–1446.
5. Jackson DN, Yang L, Wu SB, Kennelly EJ, Lipke PN. *Antimicrob. Agents Chemother*. 2015; 59: 6032–6038.
6. Toenjes KA, Munsee SM, Ibrahim AS, Jeffrey R, Edwards JE, Johnson DI. *Antimicrob. Agents Chemother*. 2005; 49: 963–972.
7. Lindsay AK, Deveau A, Piispanen AE, Hogan DA. *Eukaryot Cell*. 2012; 11: 1219–1225.
8. Sinhababu AK, Borchardt RT. *Synth. Commun*. 1983; 13: 677–683.
9. Wang Z, Wang Y, Wang B, Li W, Huang L, Li X. *J. Med. Chem*. 2015; 58: 8616–8637.
10. Nazih A, Benezra C, Lepoittevin JP. *Chem. Res. Toxicol*. 1993; 6: 215–222.
11. Naseem S, Gunasekera A, Araya E, Konopka JB. *J. Biol. Chem*. 2011; 286: 28671–28680.
12. Braun BR, Johnson AD. *Genetics*. 2000; 155: 57–67.

Fig. 3 Inhibition of hyphal formation by *C. albicans* using **1** (A) and **19** (B). ***: $P< 0.001$ vs. control in spider medium, #, ###: $P< 0.05$, 0.001 vs. control in GlcNac medium.

## Highlights

- The first total synthesis of childinin E was achieved from the commercial benzaldehyde.
- Five benzophenone derivatives were obtained by using the synthetic route of childinin E.
- The antifungal properties of the synthesized compounds against *Candida albicans* were evaluated by an *anti*-hyphal formation test.
- Childnin E and two derivatives exhibited *anti*-hyphal formation activity.

## Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

**Synthesis and pharmacological evaluation of childinin E and several derivatives as *anti*-hyphal formation inhibitors against *Candida albicans***

Leave this area blank for abstract info.

Hitoshi Kamauchi*, Momoka Hirata, Koichi Takao, Yoshiaki Sugita
*Laboratory of Bioorganic Chemistry, Department of Pharmaceutical Sciences, Faculty of Pharmacy and Pharmaceutical Sciences, Josai University, 1-1 Keyaki-dai, Sakado, Saitama 350-0295, Japan*