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The synthesis and synergistic antifungal effects of chalcones against drug resistant *Candida albicans*

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ABSTRACT

To identify effective and low toxicity synergistic antifungal compounds, 24 derivatives of chalcone were synthesized to restore the effectiveness of fluconazole against fluconazole-resistant *Candida albicans*. The minimal inhibitory concentration (MIC_{80}) and the fractional inhibitory concentration index (FICI) of the antifungal synergist fluconazole were measured against fluconazole-resistant *Candida albicans*. This was done via methods established by the clinical and laboratory standards institute (CLSI). Of the synthesized compounds, 2′-hydroxy-4′-methoxychalcone (**8**) exhibited the most potent in vitro (FICI = 0.007) effects. The structure activity relationship of the compounds are then discussed.

Candida albicans is the most common pathogenic fungus and is frequent in persons with weaker immunity or bacterial flora imbalance including those with cancer, chronic wasting disease, or excessive use of broad-spectrum antibiotics, immunosuppressive agents, and hormones. Fluconazole is the most commonly used drug to treat *C. albicans* in prophylaxis and therapy; however, widespread and repeated use of fluconazole resulted in resistance to or failure of fluconazole therapy.[1]

One promising approach aimed at overcoming azole resistance has been sensitizing *C. albicans* toward fluconazole via small molecules such as berberine,[2,3] caffeic acid amides[4] and minocycline.[5] Recently, natural flavonoids have been reported that have synergistic antifungal activity[6,7] such as baicalein and quercetin—this encouraged us to study new flavonoids with synergistic antifungal activity. Chalcones are naturally occurring flavonoids composed of two aromatic rings connected by a three-carbon unit to form an α,β unsaturated carbonyl group. Some research has demonstrated that chalcones have antitumor, anti-fungal and anti-inflammatory activities,[8–10] but to the best of our knowledge, no study has yet described the synergistic antifungal activity of chalcones against drug-resistant *C. albicans*. This current work is motivated by a recent study describing new active scaffolds containing an α,β unsaturated carbonyl group against drug-resistant *C. albicans*.[4] Inspired by these, we designed and synthesized some chalcones—most of which, as expected, exhibited activity. Herein, we report the results and the SAR (structure activity relationship) is investigated and discussed.

Previous studies have reported that baicalein (BE, Fig. 1) and caffeic acid amides (CAA, Fig. 1) exert synergistic antifungal activity on fluconazole-resistant *C. albicans*. Bitencourt's study reported non-substituted chalcone (NSC, Fig. 1) that was effective against *Trichophyton rubrum*. In the structures of BE, CAA and NSC, all contain the an α,β unsaturated carbonyl and catechol moiety, but the catechol moiety was believed to be a pan assay interference compounds (PAINS)[11] and thus should be avoided in compound design. Thus, we designed the structure of chalcone to exclude the catechol moiety and keep the BE moiety (1-alkoxyl and 5-hydroxyl structure (Compd **1**–**6**, Fig. 1 and Table 1)). We also kept only the 5-hydroxyl and/or introduced other groups (Compd **6**–**24**, Fig. 1 and Table 1) to investigate SAR.

The synthetic route to target compounds is outlined in Scheme 1. The chalcones were synthesized by aldol condensation catalyzed by 30% NaOH. The 2′,6′-dihydroxyacetophenone was used as a starting material for the synthesis of compounds **1**–**6**. First, 2′,6′-dihydroxyacetophenone was treated with CH_3I and K_2CO_3 in dry DMF to give 2′-hydroxy-6′-methoxyacetophenoe, which then reacted with a series of aromatic aldehydes to achieve

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0960-894X/

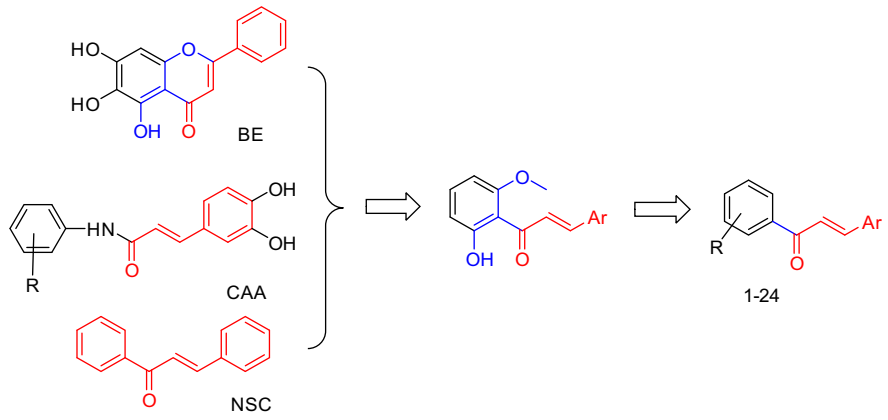


Figure 1. The design of title compounds.

Table 1
Structures and interaction modes of the title compounds and their MIC_{80} and FICI values

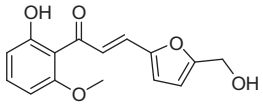
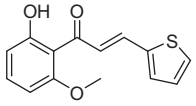
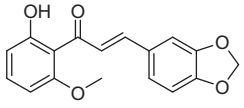
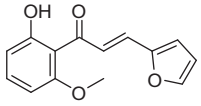
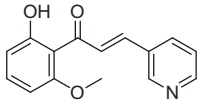
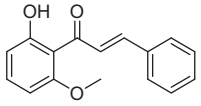
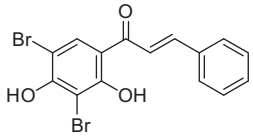
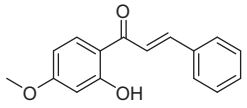
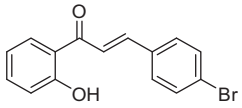
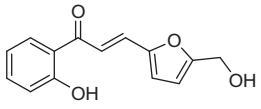
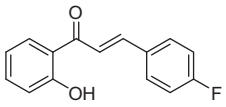
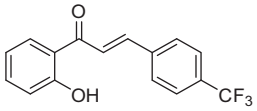
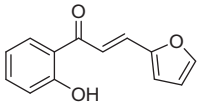
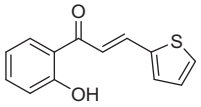
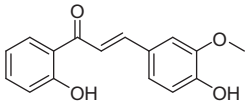
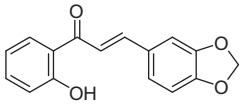
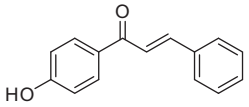
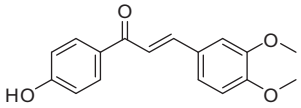
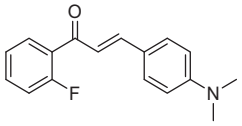
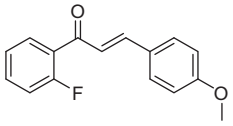
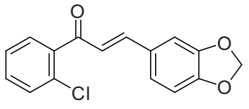
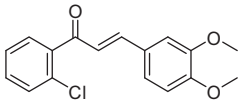
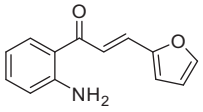
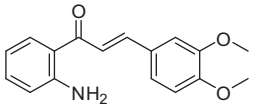
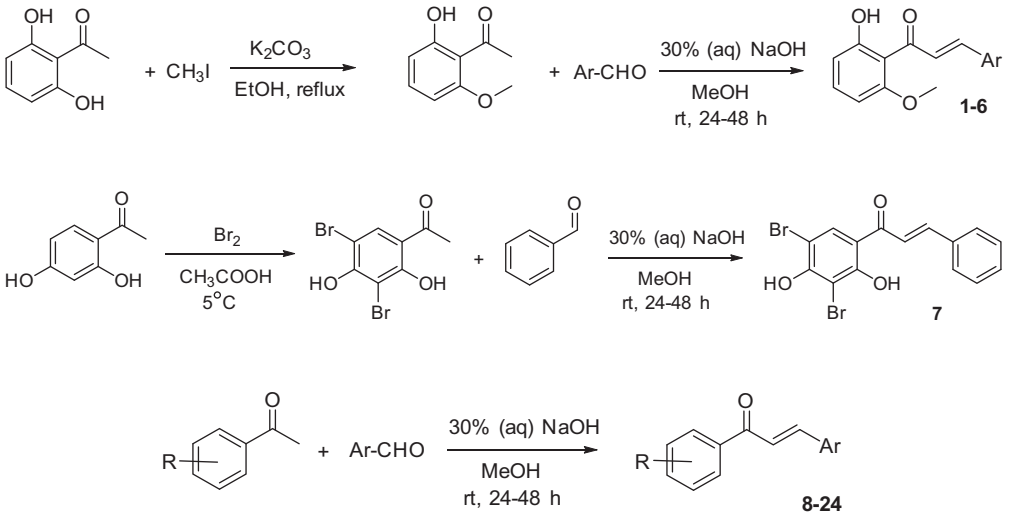
No.	Structure	MIC_{80} (μg mL^{-1})		FICI	Mode of interaction
		Alone	With FLC[a]		
1*		>64	8	0.125	Synergy
2*		>64	4	0.094	Synergy
3		>64	4	0.094	Synergy
4		>64	4	0.094	Synergy
5*		>64	8	0.125	Synergy
6		>64	16	0.188	Synergy
7		64	4	0.125	Synergy
8		>64	1	0.007	Synergy
9		>64	16	0.188	Synergy
10		>64	8	0.125	Synergy

Table 1 (*continued*)

No.	Structure	MIC_{80} (μg mL^{-1})		FICI	Mode of interaction
		Alone	With FLC[a]		
11		>64	32	0.315	Synergy
12		>64	64	0.506	Indifferent
13		>64	64	0.506	Indifferent
14		>64	>64	>1.06	Indifferent
15		>64	>64	>1.06	Indifferent
16		>64	32	0.315	Synergy
17		>64	>64	>1.06	Indifferent
18		>64	>64	>1.06	Indifferent
19		>64	8	0.125	Synergy
20		>64	64	0.506	Indifferent
21		>64	32	0.315	Synergy
22		>64	>64	>1.06	Indifferent
23		>64	>64	>1.06	Indifferent
24		>64	32	0.315	Synergy
BE	–	32	4	0.188	Synergy

[a] MIC_{80} value [μg/mL] of compound in column 1 in combination with 8.0 μg/mL fluconazole.



Scheme 1. The synthetic route for the title compounds.

compounds **1**–**6**. The 3′,5′-dibromo-2′,4′-dihydroxyacetophenone—an intermediate for the synthesis of compound **7**—was obtained by 2′,4′-dihydroxyacetophenone react with Br_2/AcOH at 5 °C. Compounds **1**, **2** and **5** are reported for the first time. The structures of all compounds were confirmed by ^1H NMR, ESI-MS.

The in vitro synergistic antifungal activities of the title compounds were tested using the microbroth dilution method according to the standards of the Clinical and Laboratory Standards Institute, USA.[12] The MIC_{80} of fluconazole (FLC) against the fluconazole-resistant *C. albicans* (clinical isolate 103) was determined to be 128.0 μg/ml. The MIC_{80} of each title compound when used alone and when combined with fluconazole (8.0 μg/ml) are described in Table 1. Furthermore, the fractional inhibitory concentration index (FICI) of each agent was calculated by adding the ratios of the MIC_{80} (with FLC)/MIC_{80} (used alone). The interaction modes—synergistic or indifferent—were defined according to FICI values of $\leqslant$0.5 or >0.5, respectively.[4]

Several chalcones displayed synergistic antifungal activity in the range of FICI from 0.007 to 0.315 as we expected (Table 1). This indicated that they have synergistic antifungal activity with FLC. However, the MIC_{80} (used alone) values of title compounds are all $\geqslant$64—this suggested that they have no antifungal activity themselves, but that they can restore the sensitivity of the fluconazole-resistant *C. albicans* to fluconazole. In a series of 2′-hydroxyl and 6′-menthoxyl substituted chalcones, all compounds (**1**–**6**) showed synergistic antifungal activity. Moreover, when the B ring was a heterocyclic ring (**1**–**5**), the synergistic effect was better than phenyl ring (**6**).

Of the series of 2′-hydoxychalcons (**7**–**16**) and 4′-hydroxychalcones (**17**, **18**), only compounds **7**, **8**, **9**, **10**, **11** showed synergistic effects—of these compound **8** was the most potent with a FICI of 0.0781 in all synthetic compounds. Moreover, compounds substituted by fluoro (**19**, **20**), chloro (**21**, **22**) or amino (**23**, **24**) at the 2′-position (except for compound **19**) showed synergistic effects. All others had no effect. These results show that the chalcones with only one substituent at the 2′-position or the 4′-position were not conducive to antifungal or synergistic effects. The 2′-hydroxyl-6′-menthoxyl and 2′-hydroxy-4′-methoxyl chalcones had good effects. Our results are mainly consistent with Dai's[4] in terms of structure and FICI. This indicates that the α,β unsaturated carbonyl moiety is critical pharmacophores of chalcone for synergistic function with fluconazole against fluconazole-resistant *C. albicans*.

Natural flavonoids have been reported for their antibacterial, antifungal, and synergistic antifungal effects including baicalein,[6,13,14] quercetin, and catechin.[7] Cao and Dai et al.[6,14] reported that baicalein could dramatically decrease biofilm production by *C. albicans* and induce apoptosis in *C. albicans* cells. The apoptosis was associated with a breakdown of mitochondrial membrane potential. However, we have been investigating their antifungal mechanism and target based on our studies on biofilms and apoptosis in *C. albicans*, but have not achieved any clear results thus far. Bitencourt et al.[15] reported that the trans-chalcone showed antifungal activity against *T. rubrum* and reduced ergosterol levels and modulated the expression of FAS1 and ERG6. Lacka et al.[16] reported that the oxathiolone-fused chalcone derivative AMG-148 showed antifungal activity against several strains of *C. albicans* with MIC values from 1 to 16 μg mL^{-1} in vitro. This was an inhibitor of beta (1→3)glucan synthase of fungi, which may be helpful for us to investigate their mechanism against drug-resistant fungi. Thus, these findings suggest that chalcone is a new synergist of fluconazole against fluconazole-resistant *C. albicans*.

In this study, 24 chalcones have been synthesized, and the bioassay results showed most of the compounds displayed synergistic antifungal effects. Compound **8** was the most potent (FICI = 0.0781). The SAR study indicates that the 2′-hydroxyl-6′-menthoxyl or 2′-hydroxyl-4′-menthoxyl are important groups on chalcone because of their synergistic activity with fluconazole against fluconazole-resistant *C. albicans*. These results are important for the discovery of fluconazole synergists against fluconazole-resistant *C. albicans*. Further studies on structural optimization are in progress in our laboratory.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.05.013.

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