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Diversity-oriented synthesis of pyrazoles derivatives from flavones and isoflavones leads to the discovery of promising reversal agents of fluconazole resistance in *Candida albicans*

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ABSTRACT

Diversity-oriented synthesis of derivatives of natural products is an important approach for the discovery of novel drugs. In this paper, a series of novel 3,4-diaryl-1*H*-pyrazoles and 3,5-diaryl-1*H*-pyrazoles derivatives were synthesized through the one-pot reaction of flavones and isoflavones with the hydrazine hydrate and substituted hydrazine hydrate. Some of these novel compounds exhibited antifungal effects against *Candida albicans* SC5314, and displayed more potent inhibitory activities against the efflux-pump-deficient strain DSY654. In addition, compounds **25**, **28** and **32a** displayed outstanding reversal activity of azole resistance against clinical azole-resistant *Candida albicans* in combination with fluconazole (FLC), with FICI values ranging from 0.012 to 0.141. The preliminary structure-activity relationship (SAR) of these compounds was also discussed. In conclusion, this study provides several novel agents that displayed potent antifungal activities alone or together with fluconazole, which makes progress for development of antifungal drugs.

Candida albicans, isolated from human oral, gastrointestinal, vaginal, cutaneous and mucosal surfaces, is one of the most common human opportunistic fungal pathogen, causing high mortality in nosocomial bloodstream infections. Currently, azole antifungal agents are widely used as first-line antifungal therapy by inhibiting fungal lanosterol 14α-demethylase, and fluconazole (FLC) is the most commonly used azole drug to treat *C. albicans* in prophylaxis and therapy. Although contemporary antifungal medications are still effective, the usefulness of these drugs is compromised by the frequent emergence of high-grade resistance.[1–6] This acquired drug resistance has been rapidly increasing worldwide and posed a grave threat to human health. Therefore, the development of new and more potent antifungal drugs becomes even more urgent.

Natural products are considered as great sources for the development of novel pharmaceuticals and their scaffolds are also recognized as "privileged structures" for further modifications. Recently, it has been reported that flavones and isoflavones, such as flavone (**1**) and formononetin (**2**) (Fig. 1) exhibited moderate antifungal activity, which could be used as the precursor for the discovery of novel antifungal agents.[7–10] In addition, Furlan and Gu have revealed a strategy to generate chemically engineered extracts through chemical diversification of natural product mixtures.[11,12] Specifically, the extract of flavones was reacted with hydrazine monohydrate, and the following bioactivity-guided fractionation of the semisynthetic mixture led to the isolation of 3,5-diaryl-1*H*-pyrazole **3**, which displayed excellent antifungal activity (Fig. 2).[11] Moreover, it was reported that the isoflavones could also react with hydrazine to generate 3,4-diaryl-1*H*-pyrazoles in one step with good-to-excellent yields.[13,14] Furthermore, pyrazole was considered as an important antifungal pharmacophore, and many pyrazole derivatives have been reported to exhibit effective antifungal activity.[15,16,3] All these findings motivated us to prepare a series of 3,4-diaryl- and 3,5-diaryl-pyrazole derivatives by the reaction of flavones and isoflavones with hydrazine hydrate and substituted hydrazine hydrate, and these novel pyrazole derivatives were then evaluated for their antifungal activity.

Firstly, each of the natural isolated flavones **1**, **10**, **11**, **13**, **14** and isoflavones **2**, **4**, **5**, **12** was reacted with hydrazine monohydrate, respectively, under reflux in ethanol. Interestingly, only four pyrazoles (compounds **6**–**9**) were obtained (Scheme 1), and the starting material bearing 5-hydroxyl or 8-hydroxyl group

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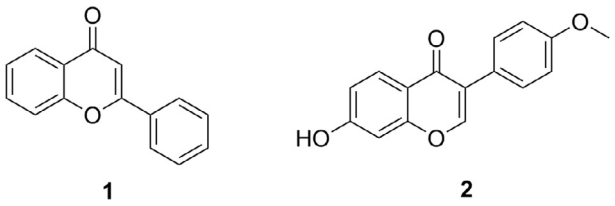


Fig. 1. The chemical structures of flavone (**1**) and formononetin (**2**).

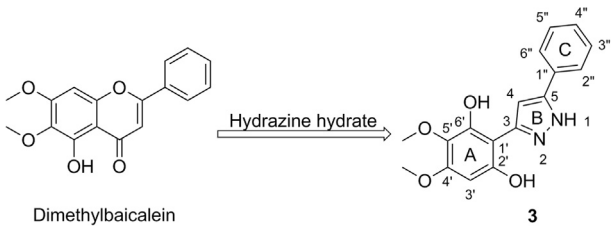
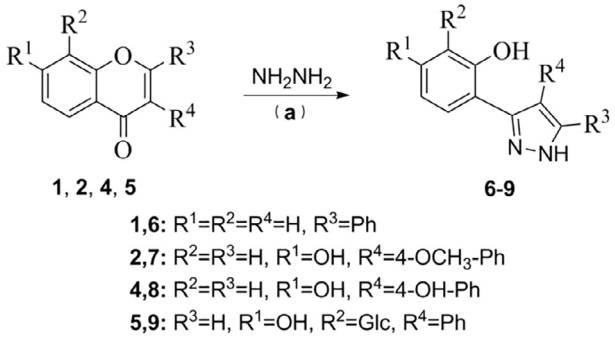


Fig. 2. Synthesis of diarylpyrazole **3**.



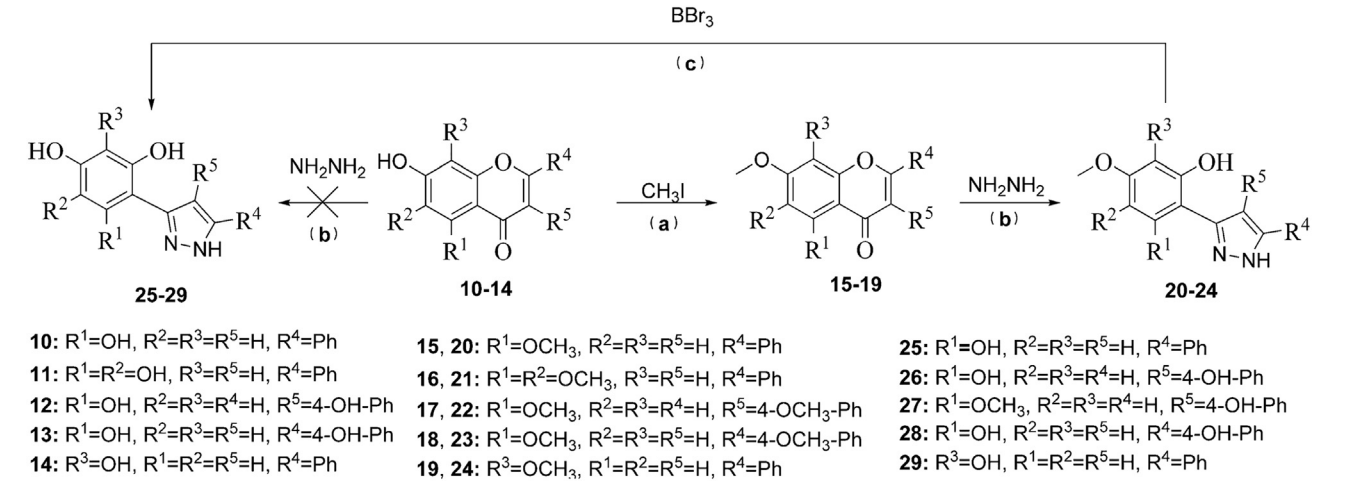
Scheme 1. Synthesis of pyrazoles derivatives **6**–**9**. Reagents and conditions: (a) EtOH, 90 °C, reflux for 8–12 h.

(compounds **10**–**14**) could not react with hydrazine under this condition, which might because of the intramolecular hydrogen bond impacting the ring-open process. Compounds **10**–**14** were then methylated by CH_3I, and the resulting intermediates **15**–**19** reacted with hydrazine hydrate to provide the pyrazoles **20**–**24** as we expected. The following demethylation by boron tribromide resulted in the formation of pyrazoles derivatives **25**–**29** in satisfied yield (Scheme 2).[17] The proposed reaction mechanism of flavones and isoflavones with hydrazine is illustrated in Fig. 3. Nucleophilic attack of hydrazine at C-2 of flavone or isoflavone followed by ring-opening afforded the intermediate VI. The further nucleophilic attack of the second nitrogen atom at the carbonyl carbon of VI and subsequent dehydration could lead to the formation of the pyrazole ring. The obtained pyrazoles may exist as mixtures of OH—N and NH—O tautomers.

Secondly, to prepare more structurally diversed pyrazoles derivatives and define their antifungal structure-activity relationship (SAR), the flavones and isoflavones **1**, **2**, **4** and **5** as well as the methylated intermediates **15**–**17** and **19** were then reacted with substituted hydrazines containing electron-donating groups, such as ethyl, phenyl, *p*-methoxyphenyl, 2-hydroxy ethyl etc, and twenty-five more novel pyrazoles derivatives were obtained (Scheme 3). However, when the substituted hydrazines bearing electron-withdrawing groups, the reaction did not work, and these observations indicated that the nucleophilic potency of hydrazine derivatives was essential for this reaction.

In addition, as shown in Scheme 4, we also studied the reaction of flavones with ethylenediamine. The compounds **1**, **14**, **15**, **18**, and **19** were treated with ethylenediamine under reflux in ethanol, and the novel 5,7-diaryl-2,3-dihydro-1,4-diazepine derivatives **47**–**51** were then obtained in satisfied yield.[18,19]

All the synthetic pyrazoles and diazepines derivatives were evaluated for antifungal activities against *C. albicans* SC5314 (the wild-type strain) and DSY654 (the Cdr1, Cdr2 efflux pumps deficient strain), and fluconazole served as the positive antifungal agent.[20] The minimum inhibitory concentrations (MICs) of active compounds are summarized in Table 1. Compounds **6**, **20** and **29** exhibited antifungal activity against the SC5314 with MIC_{80} values ranging from 8 to 16 μg/mL. Surprisingly, compounds **20**, **21**, **40b** and **43a** displayed very strong inhibitory activity against DSY654 with MIC_{80} values ranging from 1 to 4 μg/mL, and compound **20** was found to be the most potent antifungal agent (MIC_{80} = 1 μg/mL). However, the growth inhibitory activity of all the synthesized compounds against *C. albicans* SC5314 and DSY654 was less potent than the reference drug FLC, with MIC_{80} values of 2 and 0.25 μg/mL, respectively. Among all the derivatives, compound **6** displayed moderate antifungal activity against SC5314 and DSY654, with MIC_{80} values of 16 and 8 μg/mL, respectively. Introduction of two methoxyl groups at C-4′ and C-6′ on arene *A* led to the most potent compound **20** (MIC_{80} = 8 and 1 μg/mL for SC5314 and DSY654,



Scheme 2. Synthesis of pyrazoles derivatives **20**–**29**. Reagents and conditions: (a) acetone, 60 °C, reflux for overnight; (b) EtOH, 90 °C, reflux for 8–12 h; (c) DCM, -78 °C, reflux for 1 h, then reflux for overnight at rt.