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Synthesis and in vitro antifungal activities of new 3-substituted benzopyrone derivatives

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ABSTRACT

A series of new benzopyrone compounds were designed and synthesized and their antifungal activities in vitro were evaluated. The results showed that the benzopyrone derivatives with short terminal alkyl chain exhibited potent antifungal activity, which represent a novel class of promising leads for the development of novel non-azole antifungal agents. Compound **5j** is the most potent one with MIC_{80} value 1.5 μg/mL against *Trichophyton rubrum*. Flexible molecular docking was used to analyze the structure–activity relationships (SARs) of the compounds. The designed compounds interact with CA-CYP51 through hydrophobic and *van der Waals* interactions.

Over the past decades, opportunistic infection caused by fungus has displayed dramatically rising tendency, especially among the patients undergoing anticancer chemotherapy or organ transplants and patients with AIDS.[1,2] Clinically, antifungal agents can be classified as azoles (Fig. 1), polyenes, echinocandins and allylamines.[3] Azoles, possessing high therapeutic index, were broadly used as the first-line drugs in clinic for the treatment of the fungal infections. Unfortunately, the extensive use and prolonged therapy with azole antifungal agents have led to severe resistance, which significantly limited the utilization of them.[4] Therefore, the development of novel antifungal agents has been constantly required clinically.

Lanosterol 14α-demethylase (CYP51), the enzyme that catalyzes the oxidative removal of the 14α-methyl group of lanosterol to give Δ-[14,15]desaturated intermediates in ergosterol biosynthesis, is a primary target for the development of antifungal drugs.[5] Azoles inhibit CYP51 by a mechanism in which the heterocyclic nitrogen atom (N-3 of imidazole and N-4 of triazole) binds to the heme iron atom in the substrate binding site of the enzyme. The plasma membrane is made more vulnerable to be damaged by the accumulation of precursor 14α-methylated sterols, which results in an inhibition of the growth of fungi.[6] However, CYP51 is a member of the cytochrome P450 superfamily, which exists not only in fungi but also in mammals. Cases of fatal hepatotoxicity have been reported because of the ability of azoles to coordinate with the heme of a lot of host cytochrome P450 enzymes, particularly mammalian CYP3A4.[7–10] Previous studies have indicated that azole function group is not only an important pharmacophore but also a key toxicophore for hepatotoxicity. We believe that it is important to design novel non-azole antifungal compounds with good selectivity to the binding site of fungal CYP51 and without coordination binding to the heme iron atom of CYP51.

In our previous study, we have reported a series of non-azole antifungal lead compounds containing the benzopyran scaffold (Fig. 2).[11] The binding study showed that these benzopyran derivatives could interact with CYP51 without binding with heme. Flexible molecular docking model revealed that the long alkyl side chain interacted with the hydrophobic S3 subsite in the active site, which represented a narrow and hydrophobic cleft. The 7-phenolic hydroxyl group formed an hydrogen bond with the side chain hydroxyl group of catalytic residue T311 in the S1 subsite, which represents the hydrophilic hydrogen bond binding site. The benzopyran scaffold was located in S2 subsite, which is representing the core hydrophobic area.[11,12] In order to extend the structure–activity relationships (SARs) of the above antifungal leads, a series of benzopyrone compounds were designed. We first replaced the benzopyran scaffold by benzopyrone in order to investigate the effect of core scaffold on the antifungal activity. The methoxyl group was retained and long lipophilic alkyl and aromatic side chains were introduced into position 3 of benzopyrone ring, which can interact with the hydrophobic S3 subsite. As a result, a series of benzopyrone derivatives with long alkyl and aromatic ester side chains were synthesized. The design rationale was supported by

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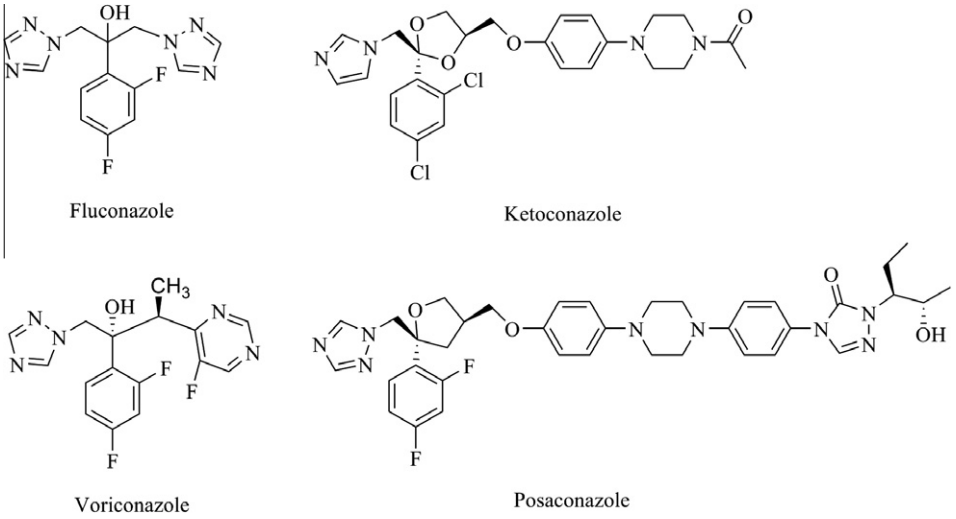


Figure 1. Chemical structures of azole antifungal agents.

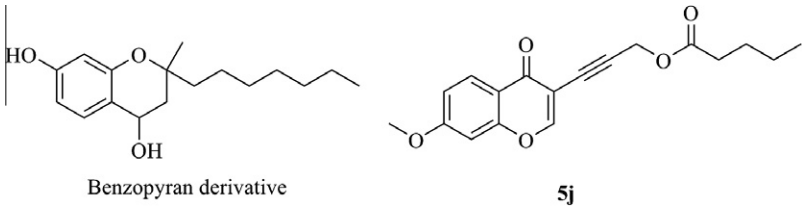
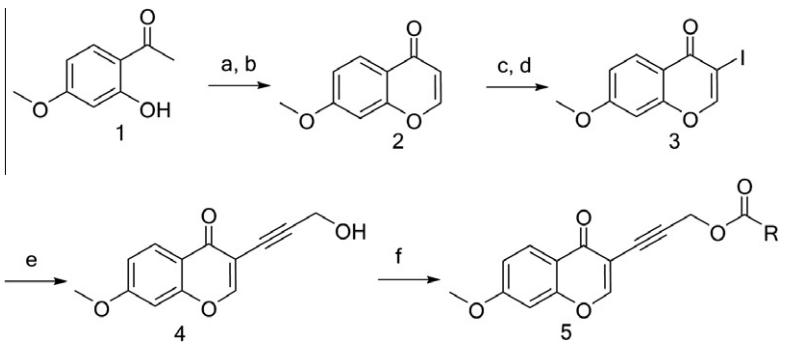


Figure 2. Lead compound and **5j**.

their antifungal activities in vitro as well as the docking model with CYP51.

The chemical synthesis of the benzopyrone derivatives was outlined in Scheme 1. As a key intermediate of our designed compounds, compound **3** was synthesized through a reported procedure via four steps from paenol.[13,14] Coupling of intermediate **3** with propynol by the Sonogashira reaction[15–17] afforded the key intermediate **4** with good yield. Intermediate **4** reacted with substituted acyl chlorides in dry DMF to afford the target compounds **5a**–**5r**. Their chemical structures were confirmed by IR, ESI-MS, and NMR spectroscopic analysis[18] and the purity of all the new compounds were determined by HPLC with two quite different systems (see Supplementary data).

In vitro antifungal activity was measured by means of the minimal inhibitory concentrations (MIC) using the serial dilution method in 96-well microtest plates according to the methods defined by the National Committee for Clinical Laboratory Standards (NCCLS).[19] *Candida albicans* and *Cryptococcus neoformans* are ATCC standard strains, and the others are clinic isolates. Fluconazole (FLZ) was used as the positive control. The MIC values are listed in Table 1. The results indicated that half of the synthesized compounds exhibited moderate antifungal activities against all the five tested human pathogenic fungus. Compounds **4**, **5a**–**5f**, and **5m**–**5o** didn't show antifungal activity or just displayed marginal activity against some of the tested pathogenic fungus (such as **5b**, **5q**, and **5r**). Compounds **5g**–**5l** exhibited broad-spectrum antifungal activities. However, their antifungal activities were lower than that of the positive control FCZ. Among the synthesized compounds, compound **5j** was the most potent one and exhibited moderate antifungal activity against the five tested fungi. The MIC_{80} value of compound **5j** against *Trichophyton rubrum* was 1.5 μg/mL, which was 30-fold more potent than that of compound **4**. From



Scheme 1. Reagents and conditions: (a) Na, ethyl formate, ice bath; (b) HCl, HOAc, reflux; (c) piperidine, MeOH, reflux; (d) DCM, I_2, rt; (e) CuI, $PdCl_2(Ph_3P)_2$, DMF, rt; (f) DMAP, DMF, substituted acyl chlorides.

Table 1
In vitro antifungal activity of the target compounds[a]

Compounds	R	*C. alb.*	*C. neo.*	*C. par.*	*T. rub.*	*M. gyp.*
4	—	>100	>100	>100	>100	>100
5a	4-Nitriophenyl	>100	>100	>100	>100	>100
5b	3,5-Dinitrophenyl	>100	>100	>100	50	50
5c	Phenyl	>100	>100	>100	>100	>100
5d	Benzyl	>100	>100	>100	>100	>100
5e	4-Chlorobenzyl	>100	>100	>100	>100	>100
5f	2-Chlorobenzyl	>100	>100	>100	>100	50
5g	Ethyl	25	25	25	6.25	12.5
5h	Propyl	12.5	50	50	12.5	25
5i	Isopropyl	50	50	12.5	3.125	25
5j	Butyl	25	25	25	1.5	25
5k	Isobutyl	>100	50	50	6.25	25
5l	*n*-Pentyl	>100	>100	50	25	12.5
5m	Cyclohexyl	>100	>100	>100	>100	>100
5n	*n*-Hexyl	>100	>100	>100	>100	>100
5o	*n*-Heptyl	>100	>100	>100	>100	>100
5p	Isoheptyl	>100	50	25	12.5	12.5
5q	2,2,3,3-Tetramethylcyclopropyl	>100	>100	>100	>100	12.5
5r	Methyl	>100	>100	>100	>100	25
FCZ	—	0.25	1	32	0.25	0.5

[a] Abbreviations: *C. alb.*: *Candida albicans*; *C. neo.*: *Cryptococcus neoformans*; *C. par.*: *Candida parapsilosis*; *T. rub.*: *Trichophyton rubrum*; *M. gyp.*: *Microsporum gypseum.*

the results of antifungal activity, the preliminary SARs were obtained. Compound **4** did not show any antifungal activity. The introduction of substituted phenyl and benzyl groups on the terminal hydroxyl, marginal activity was found. For example, compounds **5b** and **5f** were active at 50 μg/mL against *T. rubrum* and *Microsporum gypseum*. The introduction of alkyl groups on the hydroxyl resulted in obvious improvement of the activity, which suggested that the alkyl group was necessary for its binding affinity with CYP51. Moreover, the length of the alkyl group attached with the terminal hydroxyl played an important role in the antifungal activity. Compounds **5g**–**5l** containing the alkyl group within five carbon atoms exhibited the best activity among the target compounds. Compound **5j** with a 4 carbon alkyl substituent showed the best antifungal activity among the synthesized compounds. On the contrary, the loss of antifungal activity was observed for the compounds with the alkyl groups which was longer than five carbon atoms (such as the compounds **5m**–**5r**). For compound **5p**, the propyl branch of the isoheptyl could contribute to the antifungal activity a lot. For the substituted benzopyrone scaffolds represent a novel non-azole antifungal agent, it is necessary for us to make further modification in order to obtain more information about SARs.

The binding studies were carried out by the flexible docking using the affinity module within the Insight II software package.[20] Figure 3 shows the docking conformation of compound **5j** in the active site of CYP51 of *C. albicans* (CA-CYP51). Unlike azoles, the benzopyrone ring of compound **5j** did not coordinate with the heme group. Instead, its conformation is parallel to the heme group and stack with it. In addition, the benzopyrone heterocycle ring was located in S2 hydrophobic pocket interacting with the surrounding residues lined with Leu376, Val509, and Ile379. The alkyl side chain of the compound was oriented into the S3 pocket and formed hydrophobic and *van der Waals* interactions with Phe126, Ala114, Phe145, and Lys143. Due to the steric limitation of this pocket, benzyl, phenyl and long alkyl substituent groups would form stereo-collision with the active site. The docking conformation could explain activity tendency of this series of compounds, though the methoxyl group did not form H bonds with the residue of the subsites effectively. In the future study, various alkyl side chains will be introduced into position 3 of benzopyrone and some hydrophilic groups will be employed to substitute the methoxyl group in order to obtain more SAR information.

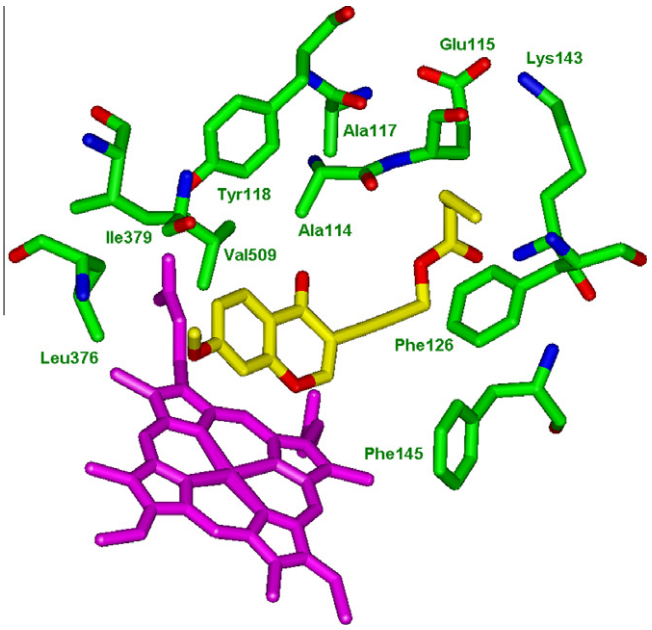


Figure 3. The docking conformation of compound **5j** in the active site of CA-CYP51.

In summary, a series of new benzopyrone derivatives were designed and synthesized. In vitro antifungal activity assay indicated that the target compounds had potent antifungal activity against the five tested human pathogenic funguses. The results indicated that the alkyl chain with 2–5 carbon atoms exhibited better effective than the others. The mode of action of benzopyrone molecules showed that the affinity of the lead molecules for CA-CYP51 was mainly attributed to their non-bond interactions with the residues of CYP51 without coordinating with the heme. The novel structure reported here represented a new scaffold type for the development of novel non-azole antifungal agents.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.072.

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18. *Representative analytical data for compound* **4**: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.914 (3H, s, CH_3–O), 4.518 (2H, d, J = 6 Hz, CH_2–O), 6.843 (1H, d, J = 2.4 Hz, Ar-H), 6.981 (1H, dd, J_1 = 2.4 Hz, J_2 = 9 Hz, Ar-H), 8.099 (1H, s, C═CH–O), 8.145 (1H, d, J = 9 Hz, Ar-H), 10.302 (1H, d, J = 6 Hz, OH); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 52.08, 55.89, 89.40, 100.44, 110.40, 115.03, 117.39, 127.68, 157.68, 158.36, 164.36, 174.58; LC–MS, m/z: 231.1 $(M+1)^+$. *Compound* **5b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.919 (s, 3H, CH_3–O), 5.314 (2H, s, CH_2–O), 6.854 (1H, d, J = 2.4 Hz, Ar-H), 6.988 (1H, dd, J_1 = 2.4 Hz, J_2 = 9 Hz, Ar-H), 8.128 (1H, s, C═CH–O), 8.158 (1H, d, J = 1.8 Hz, Ar-H), 9.217 (2H, d, J = 1.8 Hz, Ar-H), 9.247 (1H, d, J = 1.8 Hz, Ar-H); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 54.98, 55.92, 78.67, 87.28, 100.49, 109.83, 115.22, 117.25, 122.67, 127.25, 129.65, 133.17, 148.67, 157.67, 158.82, 161.90, 164.50, 174.44; LC–MS, m/z: 425.21 $(M+1)^+$. *Compound* **5j**: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.899 (3H, t, J = 7.5 Hz, CH_3CH_2), 1.328 (2H, m, $CH_3CH_2CH_2$), 1.593 (2H, m, CH_2CH_2), 2.354 (2H, t, J = 7.5 Hz, $COCH_2CH_2$), 3.911 (3H, s, CH_3–O), 4.935 (2H, s, CH_2–O), 6.839 (1H, d, J = 2.4 Hz, Ar-H), 6.977 (1H, dd, J_1 = 2.4 Hz, J_2 = 9 Hz, Ar-H), 8.107 (1H, s, C═CH–O), 8.142 (1H, d, J = 9 Hz, Ar-H); LC–MS, m/z: 315.12 $(M+1)^+$.
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