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Scaffold hopping of sampangine: Discovery of potent antifungal lead compound against *Aspergillus fumigatus* and *Cryptococcus neoformans*

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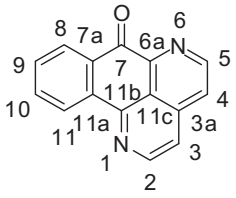
ABSTRACT

Discovery of novel antifungal agents against *Aspergillus fumigatus* and *Cryptococcus neoformans* remains a significant challenge in current antifungal therapy. Herein the antifungal natural product sampangine was used as the lead compound for novel antifungal drug discovery. A series of D-ring scaffold hopping derivatives were designed and synthesized to improve antifungal activity and water solubility. Among them, the thiophene derivative **S2** showed broad-spectrum antifungal activity, particularly for *Aspergillus fumigatus* and *Cryptococcus neoformans*. Moreover, compound **S2** also revealed better water solubility than sampangine, which represents a promising antifungal lead compound for further structural optimization.

Recently, the rapid increase in immunocompromised patients has led to severe invasive fungal infections (IFIs).[1] IFIs are often life-threatening and the associated mortality is very high. *Candida albicans* (mortality: 20–40%), *Cryptococcus neoformans* (mortality: 20–70%), and *Aspergillus fumigatus* (mortality: 50–90%) represent three major human fungal pathogens.[2,3] Despite the high mortality of IFIs, effective and safe antifungal agents are very limited. Clinically available antifungals (Fig. 1) for **IFIs** can be classified into: polyenes (e.g., amphotericin B), fluorinated pyrimidines (e.g., 5-fluorocytosine), azoles (e.g., fluconazole and voriconazole), and echinocandins (e.g., caspofungin, micafungin and anidulafungin).[4,5] However, clinical efficacy of them is far from satisfactory. These antifungal agents generally suffered from limited efficacy, narrow spectrum, low bioavailability, high toxicity and particularly severe drug resistance.[6] Therefore, it is highly desirable to discover and develop antifungal agents with novel chemotype and fungal-specific mode of action.

Natural products provide a rich source for the discovery of novel antifungal agents.[7,8] For example, polyenes and echinocandins, two important classes of antifungal agents, are derived from natural products.[7] Discovery of effective and safe antifungal agents from natural products is becoming an active area of research interests.[6,8] Sampangine (Fig. 1) is an azaoxoaporphine alkaloid extracted from the stem bark of *Cananga odorata*.[9] Sampangine showed potent antifungal strong inhibitory activity against a variety of human fungal pathogens including *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigates*.[10] Although the molecular target of sampangine was still unknown, inhibition of heme biosynthesis and production of reactive oxygen species (ROS) was reported to be its primary mode of action.[11,12] Due to the broad-spectrum antifungal activity of sampangine, it is of particular interest as an



Sampangine

Figure 1. Chemical structure of sampangine.

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antifungal lead compound. Peterson et al. reported several A- and B-ring-substituted sampangine derivatives and found that the introduction of a 3-methoxy, 3-methyl, or 4,5-benzo group was favorable for the antifungal activity.[13] Mink and Bracher designed and synthesized hetero analogues of sampangine.[14] These derivatives showed similar antifungal activity to sampangine when replacing its C7-carbonyl group with hetero atoms (O, S, N, SO). The results indicated that the scaffold of sampangine could be modified without loss of the antifungal activity. More recently, Kimpe and co-workers reported novel sampangine derivatives with excellent anti-tuberculosis activities, which expanded the therapeutic application of sampangine.[15]

Although sampangine has shown promising future as an antifungal lead compound, information of its structure–activity relationship (SAR) is still limited. Moreover, sampangine showed poor water solubility because of its aromatic tetracyclic scaffold. Continuing our efforts on novel antifungal drug discovery,[6,16–18] herein scaffold hopping studies of sampangine were performed. A potent derivative **S2** with improved antifungal activity and aqueous solubility was successfully identified.

As shown in Figure 2, scaffold hopping of sampangine was focused on the D-ring. Various heterocycles were used to replace the D-ring phenyl group of sampangine to improve the water solubility and provide new SAR information. As a result, a series

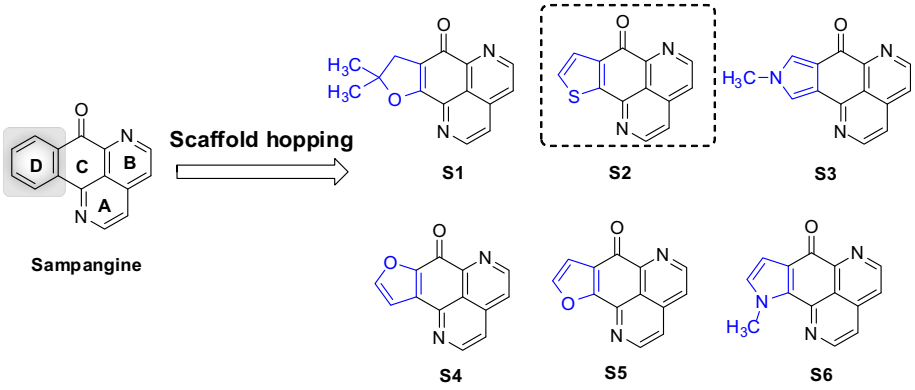
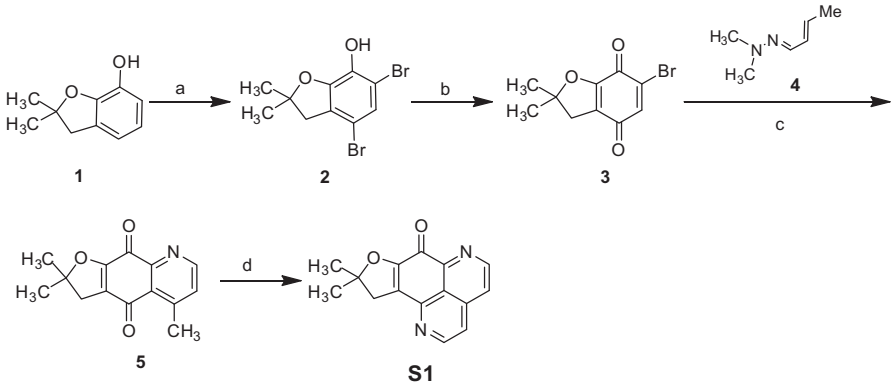
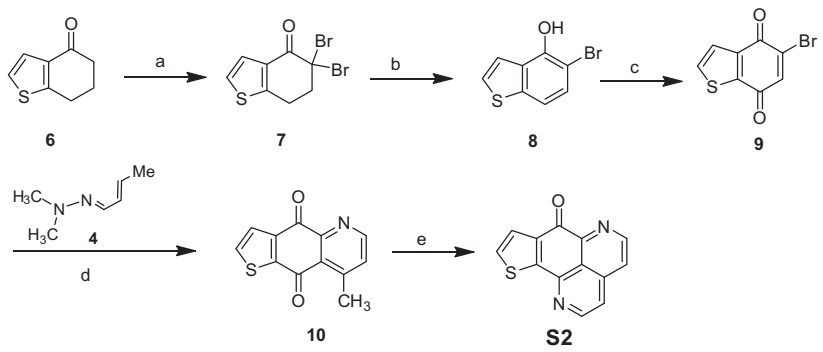


Figure 2. Scaffold hopping design of sampangine.



Scheme 1. Reagents and conditions: (a) $Br_2/CHCl_3$, 25 °C, 2 h, 67%; (b) CrO_3, HOAc/H_2O, 25 °C, 1 h, 51%; (c) xylene, 140 °C, 6 h, 48%; (d) (1) DMFDMA, DMF, 120 °C, 2 h, (2) NH_4Cl, HOAc, 120 °C, 1 h, 68% (2 steps).

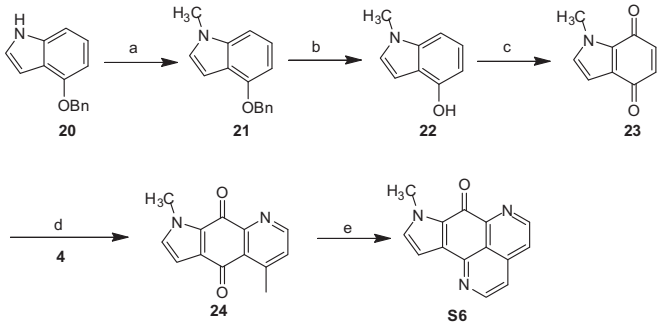


Scheme 2. Reagnents and conditions: (a) $CuBr_2$/EtOAc/$CHCl_3$, 80 °C, 12 h, 97%; (b) Li_2CO_3, DMF, 100 °C, 6 h, 96%; (c) $PhI(OAc)_2$, HOAc/TFA, 0–25 °C, 0.5 h, 81%; (d) $NaHCO_3$, EtOH, 4 h, 73%; (e) (1) DMFDMA, DMF, 120 °C, 2 h, (2) NH_4Cl, HOAc, 120 °C, 1 h, 58% (2 steps).

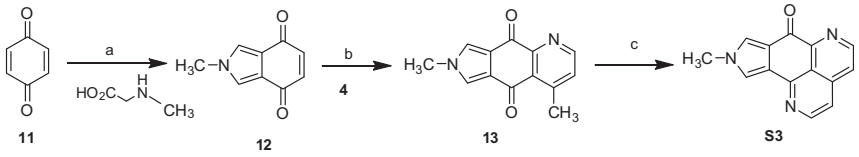
of furan (**S1**, **S4**, **S5**), thiophene (**S2**) and pyrrole (**S3**, **S6**) derivatives were designed and synthesized. Sampangine was synthesized according to the procedure of Peterson et al.[13] The synthetic route of sampangine derivatives were depicted in Schemes 1–5. Starting from 2,2-dimethyl-2,3-dihydrobenzofuran-7-ol (**1**), it was bromized by Br_2 to give dibromo intermediate **2**, which was subsequently oxidized to quinone intermediate **3**. BCD ring intermediate **5** was obtained through the hetero Diels–Alder reaction of quinone **3** with hydrazone **4**, followed by an in situ elimination of dimethylammonium bromide from the cycloadduct. The condensation of **5** with dimethylformamide dimethyl acetal provided furan derivative **S1** in good yield. Other sampangine derivatives were obtained by a similar synthetic strategy using various starting materials (Schemes 2–5). Based on compound **S2**, bromination and chlorination using *N*-bromosuccinimide (NBS) and *N*-chlorosuccinimide (NCS) afforded target compounds **S2a–d** (Scheme 6). Subsequently, treating compound **S2b** with 1-methylpiperazine or CH_3ONa yielded compounds **S2e** and **S2f**, respectively. Catalyzed by HOAc, 2-substituted derivatives **S2g–j** were obtained by condensation of intermediate **10** with various aromatic aldehydes and NH_4Cl (Scheme 6).

In vitro antifungal assay[18] revealed that most of the target compounds, except compound **S3**, showed broad-spectrum inhibitory activity against the tested human fungal pathogens (Table 1). Clinically, the treatment of *Aspergillus fumigatus* infection still remains a significant challenge in current antifungal therapy. For example, the first-line antifungal agent fluconazole is inactive against *Aspergillus fumigatus* (MIC >64 μg/mL). In contrast, compound **S1** and **S2** showed good inhibitory activity (MIC = 1 μg/mL), which was also more potent than lead compound sampangine. *Cryptococcus neoformans* infections frequently involve the brain and are often life-threatening. Compound **S2** revealed excellent inhibitory activity against *Cryptococcus neoformans* with a MIC value of 0.25 μg/mL, whose activity was comparable to fluconazole (MIC = 0.25 μg/mL) and superior to sampangine (MIC = 2 μg/mL). On the strains of *Candida* spp., the sampangine derivatives generally showed decreased activity. Only compound **S1** was highly active against *Candida glabrata* (MIC = 0.125 μg/mL). For the dermatophytes, most of the compounds were moderately active, which was similar to fluconazole and sampangine. Compound **S2** was more active than fluconazole and sampangine for *Trichophyton rubrum* (MIC = 2 μg/mL) and *Microsporum gypseum* (MIC = 2 μg/mL). SAR analysis revealed that the replacement of D-ring phenyl group of sampangine by 2,2-dimethyl-2,3-dihydrofuran and thiophene was favorable for the antifungal activity. Particularly, the thiophene derivative **S2** showed potent fungistatic activity with a broad spectrum (MIC range: 0.25–8 μg/mL). In contrast, other furan and pyrrole analogues generally showed decreased antifungal activity. Moreover, water solubility was also determined for several scaffold hopping derivatives (Table 1). Most of the compounds showed better solubility than sampangine. Particularly, compound **S2**, the most active one, also exhibited the best solubility (48 μg/mL), which was almost four fold higher than that of sampangine (12.6 $\mu g/mL^{-1}$).

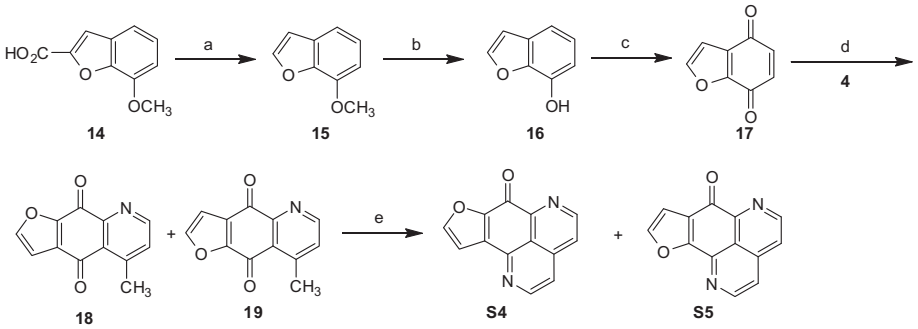
Due to potent and broad-spectrum antifungal activity of thiophene derivative **S2**, it was subjected to further SAR analysis (Table 2). First, various groups were introduced at positions 4 and 9 (**S2a–S2f**). The results of antifungal assay revealed that the attachment of bromine on position 4 (**S2b**) or 9 (**S2a**) resulted in the decreased antifungal activity. Similarly, the presence of 4-methylpiperazinyl (**S2e**) or 4-methyloxyl (**S2f**) groups at position 4 was also unfavorable for the antifungal activity. Interestingly,



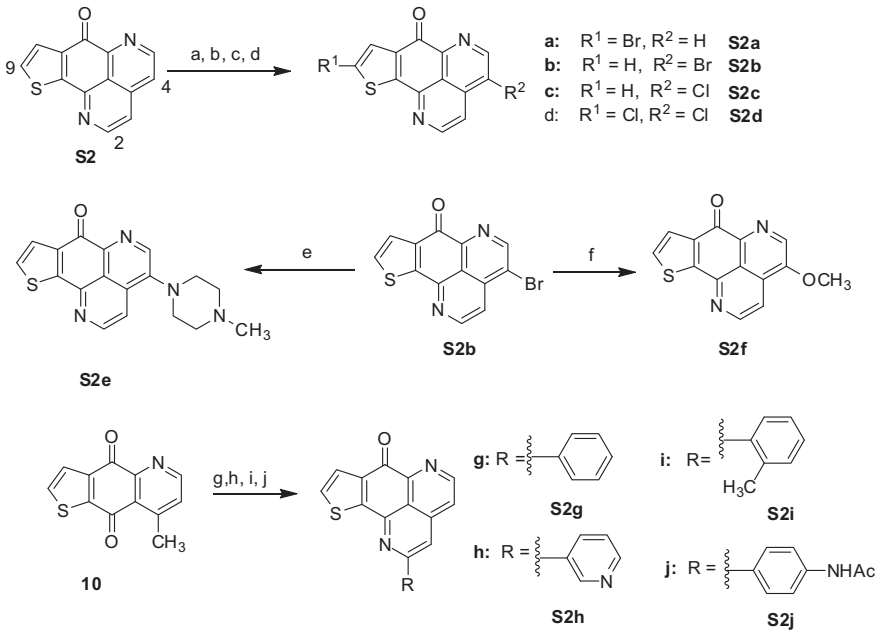
Scheme 5. Reagents and conditions: (a) MeI, NaH, DMF, 25 °C, 5 h, 55%; (b) 5% Pd-C, MeOH, 40 psi, 25 °C, 4 h, 91%; (c) Fremy's salt, KH_2PO_4 aq, acetone/H_2O, 3 h, 52%; (d) xylene, 140 °C, 6 h, 45%; (e) (1) DMFDMA, DMF, 120 °C, 2 h, (2) NH_4Cl, HOAc, 120 °C, 1 h, 42% (2 steps).



Scheme 3. Reagents and conditions: (a) sarcosine, paraformaldehyde, toluene, reflux (Dean Stark), 4.5 h, 15%; (b) xylene, 140 °C, 6 h, 39%; (c) (1) DMFDMA, DMF, 120 °C, 2 h, (2) NH_4Cl, HOAc, 120 °C, 1 h, 62% (2 steps).



Scheme 4. Reagents and conditions: (a) Cu, quinoline, 240 °C, 2 h, 64%; (b) BBr_3, DCM, −78 °C, 2 h, 50%; (c) Fremy's salt, KH_2PO_4 aq, acetone/H_2O, 1 h, 42%; (d) xylene, 140 °C, 6 h, 35%; (e) (1) DMFDMA, DMF, 120 °C, 2 h, (2) NH_4Cl, HOAc, 120 °C, 1 h, 58% (2 steps).



Scheme 6. Reagents and conditions: (a) NBS, DMF, 140 °C, 5 h, 25%; (b) NBS, HOAc, $CHCl_3$, 80 °C, 2 h, 57%; (c/d) NCS, HOAc, $CHCl_3$, 80 °C, 3 h, 49%; (e) 1-methylpiperazine, $K_3PO_4.H_2O$, CuI, $Me_2NCH_2CH_2OH$, 25 °C, 12 h, 38%; (f) Cs_2CO_3, MeOH, 40 °C, 1 h, 25%. (g) benzaldehyde, NH_4Cl, HOAc, DMF, 120 °C, 3 h, 45%; (h) nicotinaldehyde, NH_4Cl, HOAc, DMF, 120 °C, 3 h, 36%; (i) 2-methyl benzaldehyde, NH_4Cl, HOAc, DMF, 120 °C, 3 h, 40%; (j) *N*-(4-formylphenyl)acetamide, NH_4Cl, HOAc, DMF, 120 °C, 3 h, 42%.

Table 1
In vitro antifungal activity (MIC_{80}, μg/mL) and kinetic solubility (μg/mL) of D-ring modified sampangine analogues[a]

Compounds	*A. fum.*	*C. neo.*	*C. alb.*	*C. par.*	*C. gla.*	*T. rub*	*M. gyp*	Solubility[b]
S1	1	2	>64	>64	0.125	8	>64	27
S2	1	0.25	4	8	4	2	2	48
S3	>64	>64	>64	>64	>64	>64	>64	17
S4	32	16	32	>64	32	8	16	-
S5	32	8	32	>64	64	8	16	-
S6	64	8	64	>64	32	16	>64	22
Sampangine	16	2	0.5	4	0.125	16	>64	12.6
Fluconazole	>64	0.25	0.5	2	0.25	4	16	-

[a] Abbreviations: *A. fum.*, *Aspergillus fumigatus*; *C. neo.*, *Cryptococcus neoformans*; *C. alb.*, *Candida albicans*; *C. par.*, *Candida parapsilosis*; *C. gla.*, *Candida glabrata*; *T. rub.*, *Trichophyton rubrum*; *M. gyp.*, *Microsporum gypseum*.
[b] Kinetic solubility (PBS buffer, pH = 7.4).

Table 2
In vitro antifungal activity of S2 derivatives (MIC_{80}, μg mL^{-1})

Compounds	*C. neo.*	*A. fum.*	*C. alb.*	*C. par.*	*C. gla.*	*T. rub*	*M. gyp*
S2a	4	4	8	64	4	2	1
S2b	4	8	4	16	4	2	0.5
S2c	0.5	2	32	>64	0.5	2	0.125
S2d	>64	>64	32	>64	>64	>64	8
S2e	64	>64	>64	>64	64	>64	>64
S2f	>64	>64	>64	>64	>64	>64	>64
S2g	4	>64	32	>64	>64	16	>64
S2h	32	>64	32	>64	>64	>64	32
S2i	>64	>64	32	>64	>64	32	>64
S2j	>64	>64	32	>64	>64	>64	>64
Fluconazole	1	>64	0.5	1	2	0.5	16

Abbreviations: *C. neo.*, *Cryptococcus neoformans*; *A. fum.*, *Aspergillus fumigatus*; *C. alb.*, *Candida albicans*; *C. par.*, *Candida parapsilosis*; *C. Gla.*, *Candida glabrata*; *T. rub.*, *Trichophyton rubrum*; *M. gyp.*, *Microsporum gypseum*.

the 4-chloro derivative **S2c** showed excellent activity against *Cryptococcus neoformans* (MIC = 0.5 μg/mL), *Candida glabrata* (MIC = 0.5 μg/mL), *Microsporum gypseum* (MIC = 0.125 μg/mL), which was comparable or superior to **S2**, sampangine and fluconazole. In contrast, almost loss of antifungal activity was observed when introducing aromatic groups (**S2g–j**) on the position 2 of **S2**.

In summary, a series of D-ring modified sampangine derivatives were designed and synthesized and new SAR results were obtained. After two rounds of drug design, several compounds (e.g., **S1**, **S2** and **S2c**) showed potent antifungal activity with a broad spectrum and improved water solubility. Particularly, compound **S2** was highly active against *Aspergillus fumigatus* and *Cryptococcus neoformans*, which are difficult to treat in current antifungal therapy. Compound **S2** has a novel scaffold that differs from that of all reported antifungal agents and represents a promising antifungal lead compound for further structural optimization.

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Supplementary data

Supplementary data associated (experimental protocols and structural characterization of target compounds.) with this article

can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.07.064.

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