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Synthesis and antifungal activities in vitro of novel pyrazino [2,1-*a*] isoquinolin derivatives

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ABSTRACT

A series of novel pyrazino[2,1-*a*]isoquinolin compounds were designed and synthesized, and their antifungal activities in vitro were evaluated. The results showed that all of the compounds exhibited antifungal activities. Some of them exhibited stronger antifungal activities than that of lead compounds and among them compound **11b** was the most potent one, which showed more potent than that of the active control fluconazole to the four of the five tested fungi. The studies presented here provide a new structural type for the development of novel antifungal agents.

Over the past three decades, the incidence of systemic fungal infections has increased dramatically due to an increase in the number of immunocompromised hosts.[1–4] Although the arsenal of antifungal drugs has expanded, currently available antifungal drugs do not meet the increasing requirements of managing infection in the complex patient populations. The development of new antifungal drugs has been constantly required in the clinical therapy.

Lanosterol 14α-demethylase (CYP51) is one of the key enzymes of sterol biosynthesis in fungi and also a prime target for the development of antifungal drugs. Azole antifungals, such as fluconazole, itraconzole, and voriconazole (Fig. 1), are the mainly CYP51 inhibitors used in the clinical antifungal therapy,[5,6] which exert antifungal activity through inhibiting the lanosterol 14α-demethylase (CYP51) of fungi by a mechanism in which the heterocyclic nitrogen atom (N-3 of imidazole and N-4 of 1,2,4-triazole) binds to the sixth coordination position of the heme iron atom of the prophyrin in the substrate-binding site of the enzyme.[7] Extensive use and prolonged therapy with azole antifungals have led to resistance. The search of new non-azole CYP51 inhibitors is meaningful.

We have focused on the search of non-azole CYP51 inhibitors for several years. In the previous study,[8–10] we reported the design of non-azole lead molecules with a tetrahydroisoquinoline scaffold based on the constructed three-dimensional model of *Candida albicans* CYP51 by coupling structure-based de novo design. The binding study showed that tetrahydroisoquinoline scaffold was located on the substrate-binding site of fungal CYP51, and affinity of the lead molecule for CYP51 was mainly attributed to their nonbonding interaction with the residues of apoprotein but without binding with the heme, which was different from that of azole antifungals. L-6 was the most potent compound discovered (Fig. 1). Lansterol is the natural substrate for CYP51 with a skeleton of four rings in its structure, and located on the same substrate-binding site of CYP51 with the tetrahydroisoquinoline compounds. Considering that the

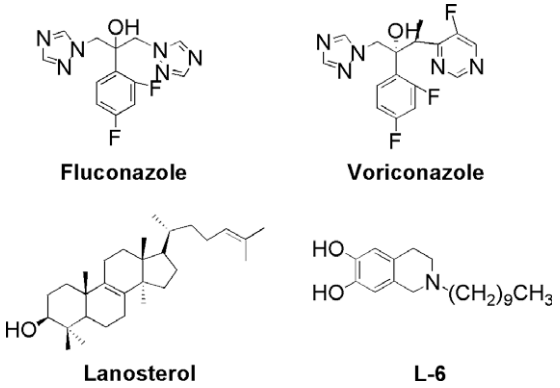

Figure 1. Chemical structures of fluconazole, voriconazole, lanosterol, and lead compound **L-6**.

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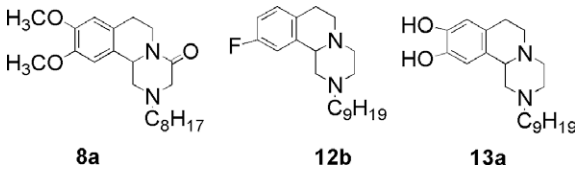


Figure 2. Chemical structures of the target compounds.

tetrahydroisoquinoline scaffold is smaller in the active site than that of lansterol, the possible increase of affinity with CYP51 and antifungal activities of target compounds is expected by expanding the tetrahydroisoquinoline scaffold of lead molecules. On the basis of the above hypothesis, the novel pyrazino [2,1-*a*] isoquinolin derivatives were designed (Fig. 2), and their antifungal activities in vitro were evaluated.

The chemical synthesis of pyrazino [2,1-*a*] isoquinolin derivatives was outlined in Scheme 1. As a key intermediate of our designed compounds, **4a**–**4b** was synthesized respectively according to the paper.[11,12] Intermediate **4a**–**4b** was allowed to react with different alkyl halides in the presence of potassium carbonate in ethanol at 80 °C, and then the hydrogen chloride gas was added to form compounds **5a**–**5b**, **8a**–**8b**, **11a**–**11b**, **and 14a**–**14b**. Reduction of **5a**–**5b**, **8a**–**8b**, **11a**–**11b**, and **14a**–**14b** with lithium aluminum hydride in THF yields the target compounds **6a**–**6b**, **9a**–**9b**, **12a**–**12b**, and **15a**–**15b**. Cleavage of the methoxy groups of **6a**, **9a**, **12a**, **15a** in HBr/CH_3COOH provided *N*-substituted-1,2,3,6,7,11b-hexahydro-4*H*-pyrazino [2,1-*a*] isoquinoline-9,10-diol hydrobromide **7a**, **10a**, **13a**, **16a**. All the target compounds were obtained as racemates.

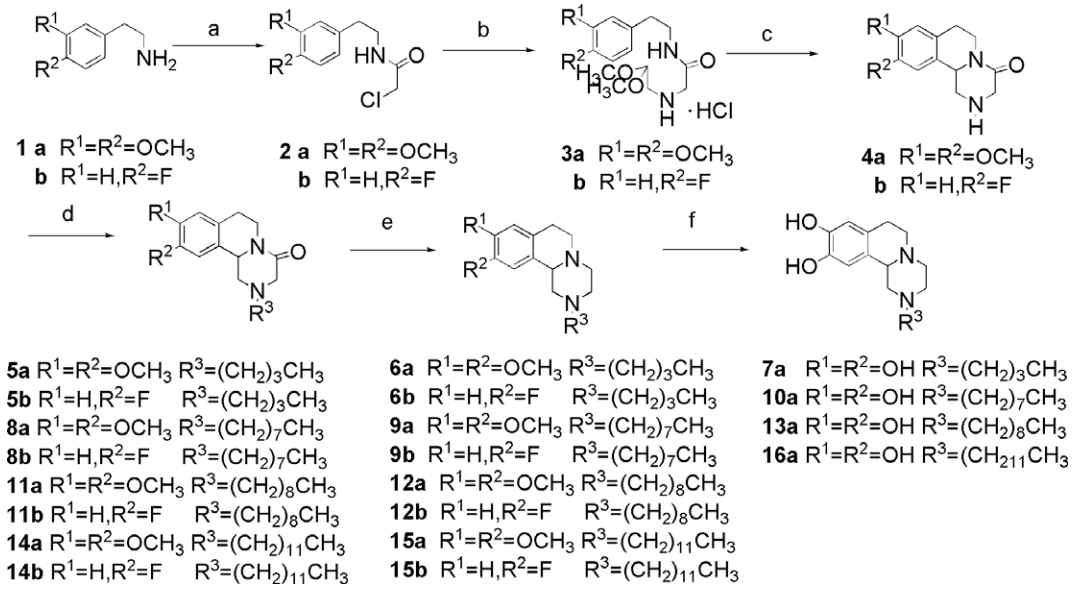
The in vitro minimal inhibitory concentrations (MIC_{80}) of the compounds were determined by the micro-broth dilution method in 96-well microtest plates according to the methods defined by the National Committee for Clinical Laboratory Standards (NCCLS).[13] The tested fungi species included five pathogenic fungi, which were found in dermatomycoses (*Trichophyton rubrum*, and *Microsporum gypseum*) and systemic mycoses (*Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*). The in vitro antifungal activities of all the title compounds were listed in Table 1, in which fluconazole and **L-6** were used as the controls. In general, all the target compounds showed potent activities

Table 1
In vitro antifungal activity MIC_{80} (μg/ml)[a]

Compound	*C. alb.*	*C. neo.*	*T. rub*	*M. gyp*	*A. fum*
5a	>64	16	>64	>64	>64
5b	>64	32	64	>64	>64
6a	>64	>64	>64	>64	>64
6b	64	>64	>64	4	>64
7a	>64	4	>64	>64	>64
8a	16	8	32	8	64
8b	>64	16	16	16	32
9a	32	8	16	4	8
9b	16	4	8	4	32
10a	16	2	16	32	64
11a	64	4	32	2	32
11b	32	2	4	0.5	16
12a	32	4	8	2	8
12b	8	4	8	2	16
13a	8	4	8	8	64
14a	>64	32	>64	64	>64
14b	>64	>64	64	64	32
15a	>64	16	>64	32	>64
15b	>64	64	64	4	>64
16a	64	16	16	16	64
L-6	64	16	32	16	64
FCZ	2	4	32	2	>64

[a] Abbreviations: *C. alb.*, *Candida albicans*; *C. neo.*, *Cryptococcus neoformans*; *T. rub.*, *Trichophyton rubrum*; *M. gyp.*, *Microsporum gypseum*; *A. fum.*, *Aspergillus fumigatus*; FCZ: Fluconazole.

against most the test fungal pathogens. The MIC_{80} values indicated that the compounds **8a**, **9a**, **9b**, **11b**, **12a**–**12b**, **and 13a** showed more excellent antifungal activities against four pathogenic fungi than that of **L-6**. Noticeably, the MIC_{80} value of compounds **11b**, **12a and 12b** showed higher activities against nearly all the test fungi except *Candida albicans* than that of fluconazole. *Cryptococcus neoformans* has a worldwide distribution and is the most common cause of life-threatening fungal infections. Compounds **7a**, **9b**, **10a**, **11a**–**11b**, and **13a** exhibited comparable or stronger inhibitory activities against *Cryptococcus neoformans* than fluconazole. Fluconazole is not effective against *Aspergillus fumigatus*, while most of our compounds showed potent activities. For example, the MIC_{80} values of compounds **9a** and **12a** against *Aspergillus fumigatus* are 8 μg/mL. On the *Trichophyton rubrum* strains, compound **9b**, **11b**, **12a**–**12b**, and **13a** showed better activities than fluconazole. On



Scheme 1. Synthesis of the target compounds **5a**–**16a**. Reagents and conditions: (a) chloroacetyl chloride, $NaHCO_3$, CH_2Cl_2; room temperature; (b) 2,2-dimethoxyethanamine toluene, reflux; (c) H_2SO_4, CH_2Cl_2; (d) RX, K_2CO_3/KI; (e) $LiAlH_4$, THF; (f) HBr/CH_3COOH reflux.

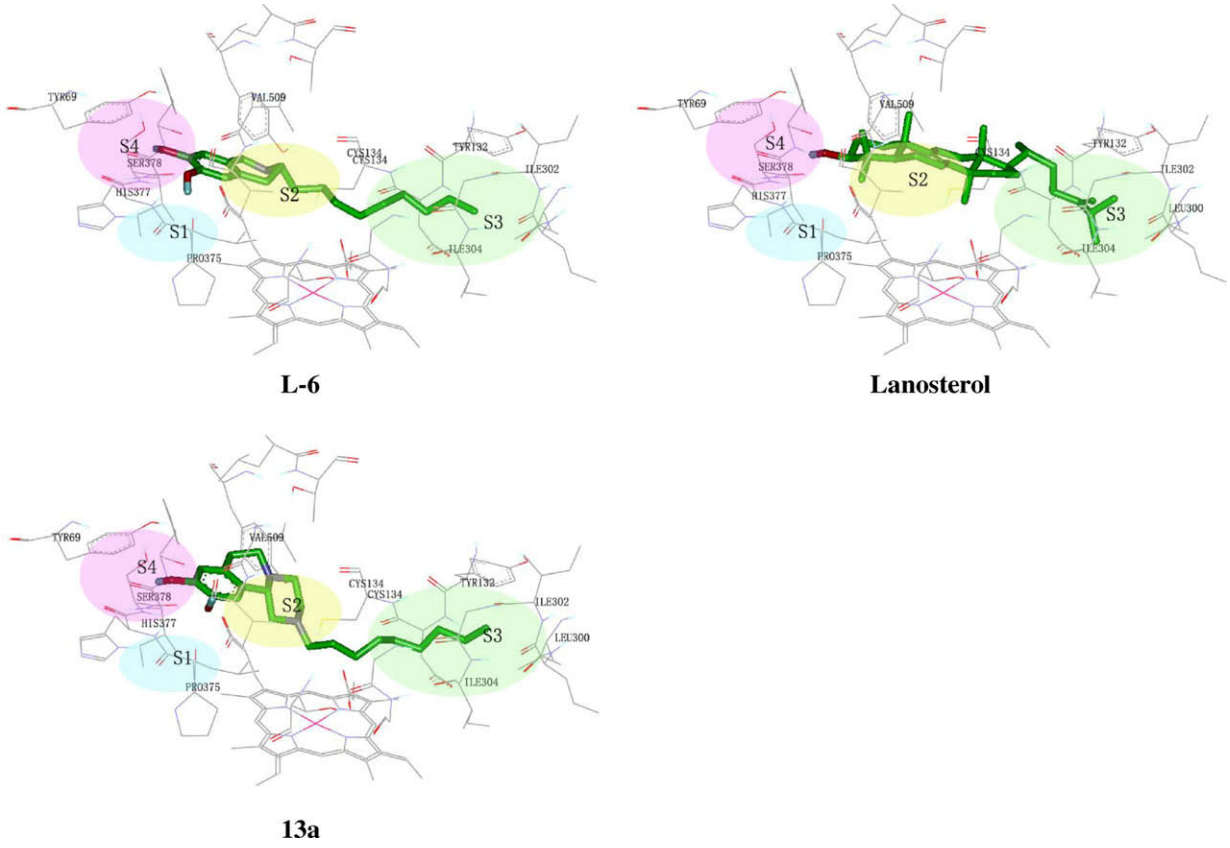


Figure 3. The mode of action of compound **L-6**, lanosterol, and compound **13a** with the active site of CYP51 of *Candida albicans* (Blue indicates the S1 subsite; Yellow indicates the S2 subsite; green indicates the S3 subsite; Pink indicates the S4 subsite).

Table 2
A comparison of the calculated interaction energies of **L-6** and **13a** with CYP51 of *Candida albicans*

Compounds	L-6	13a
Energy (kcal/mol)	−9.91	−11.66
MIC_{80} (μg/ml)	64	8

the *Microsporum gypseum* strains, most of the compounds showed inhibitory activity and compound **12a** showed stronger inhibitory activities than fluconazole with MIC_{80} values of 0.5 μg/mL. In particular, compounds **8a**, **9a**–**12b**, and **13a** exhibited strong in vitro antifungal activities with broad antifungal spectrum, which were worthy of further evaluation.

The binding studies were carried out by the flexible docking using the Affinity module within the Insight II software package.[14] The mode of action of lead molecule **13a** with the active site of CYP51 of *Candida albicans* (Fig. 3) showed that the pyrazino [2,1-*a*] isoquinolin ring interacted with the hydrophobic S2 subsite. The hydroxy group in the benzene ring formed H-bonding interactions with the residues HIS377 and Ser378 in the S4 subsite. The lipophilic alkyl side chain at position 2 of the compound interacted with the hydrophobic S3 subsite. The docking model showed that the appropriate length of the substituents on the amino group of target derivatives was important for antifungal activities. For example, compounds **14a**–**16a** were less potent because the alkyl side chain was too long to be accommodated by the hydrophobic S3 subsite. On the other hand, compounds **5a**–**7a** were less potent because the alkyl side chain was too short to interact firmly with the hydrophobic S3 region, although it was able to enter the active site. The substituents with hydrogen bond donor or acceptor groups on the benzene ring seemed to have little effect on the antifungal activities. Compounds with hydrogen bonding donor substituents at positions 9 and 10, such as **7a**, **10a**, **13a**, and **16a**, exhibited similar antifungal activities with that of those compounds with hydrogen bonding acceptor substituents at positions 9 and 10 of pyrazino [2,1-*a*] isoquinolin ring, such as **6b**, **9b**, **12b**, and **15b**. The interaction energies of title compounds **13a** and lead compound **L-6** were in good qualitative agreement with their antifungal activities in vitro (Table 2). All of the results gave us the suggestion to further design and synthesize their derivatives.

In summary, a series of novel pyrazino [2,1-*a*] isoquinolin compounds were successfully designed and synthesized. In vitro antifungal activity assay indicated that the target compounds had potent antifungal activity against both systemic pathogenic fungi and dermatophytes. In particular, the most active compounds **8a**, **9a**–**12b**, and **13a** exhibited broad antifungal spectrum, and showed potent activities against *Aspergillus fumigatus* not inhibited effectively by fluconazole, which are worthy of further evaluation. Moreover, the mode of action of the pyrazino [2,1-*a*] isoquinolin molecules showed that the affinity of the lead molecules for CYP51 was mainly attributed to their nonbonding interaction with the residues of apoprotein but without binding with the heme, which was different from that of azoles. The studies presented here provide a new structural type for the development of novel antifungal agents.

Acknowledgments

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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.2009.12.050.

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