

Discovery of a Novel Broad-Spectrum Antifungal Agent Derived from Albaconazole

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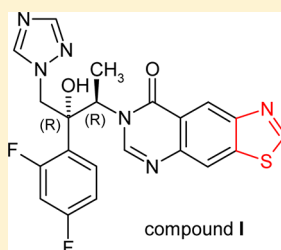
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S Supporting Information

ABSTRACT: Synthesis of a strict structural analogue of albaconazole in which the quinazolinone ring is fused by a thiazole moiety led to the discovery of a new triazole with broad-spectrum antifungal activity. Compound I exhibited high in vitro activity against pathogenic *Candida* species and filamentous fungi and showed preliminary in vivo antifungal efficacy in a mice model of systemic candidiasis.



Strict analogue of albaconazole

In vitro antifungal activities against yeasts and moulds

In vivo antifungal efficacy in a mice model of systemic candidiasis

KEYWORDS: *Candida*, *Aspergillus*, zygomycetes, antifungal agents, azoles, thiazolo[4,5-g]quinazolin-8(7H)-one

Azoles (fluconazole, itraconazole, voriconazole, and posaconazole) are important drugs for the treatment of invasive fungal infections (IFIs), which continue to be a major cause of morbidity and mortality in immunocompromised or severely ill patients.¹ These compounds target the biosynthesis of ergosterol by inhibiting the cytochrome P450-dependent lanosterol 14 α -demethylase (Erg11p, CYP51), encoded by the *ERG11* gene, resulting in accumulation of toxic methylsterols in membranes that may culminate in fungistatic effect or fungal death.² Most azoles are orally active, show a broad-spectrum against most yeasts and filamentous fungi, and are relatively nontoxic. Unfortunately, the increasing number of fungal infections, coupled with emerging resistance, has resulted in a need to develop new, more effective agents. Among these molecules, albaconazole (UR-9825, Palau Pharma S.A., Figure 1)³ is a new oral triazole antifungal agent with broad-spectrum antifungal activity against resistant and emerging pathogens as compared with fluconazole and itraconazole, good pharmacokinetics, and excellent oral bioavailability.⁴ It has demonstrated high in vitro activities against pathogenic yeasts, dermatophytes, and other filamentous fungi and has been shown to be effective in animal models of systemic aspergillosis, candidiasis, cryptococcosis, and scedosporiosis.⁴ Albaconazole has been assayed in several clinical trials, including phase I/II studies in candidal vulvovaginitis, tinea pedis, and onychomycosis (clinicaltrials.gov: NCT00199264, NCT00509275, and NCT00730405). However, phase III studies are not available until now, and the lack of an intravenous form makes studies in the acute infection setting very difficult.⁵ As part of our research

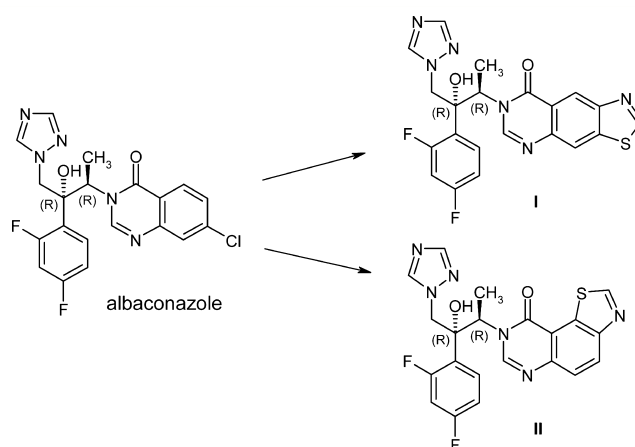


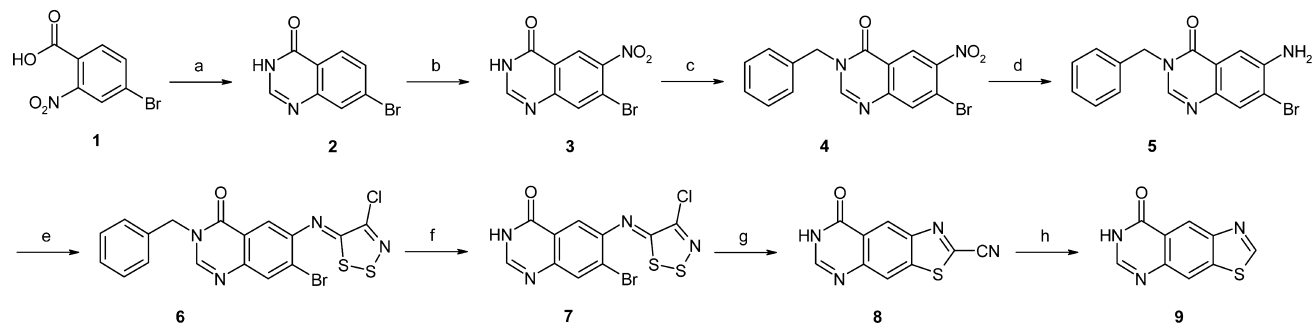
Figure 1. General structures of synthesized compounds.

program focused on the synthesis and SAR studies of novel broad-spectrum antifungal agents,^{6–10} we aimed to develop a strict structural analogue of albaconazole in which the quinazolinone ring will be replaced by a thiazoloquinazolinone scaffold (compounds I and II, Figure 1) via Appel salt chemistry. Our goal was to slightly increase the size of the inhibitor to cover a larger area within the active site of fungal

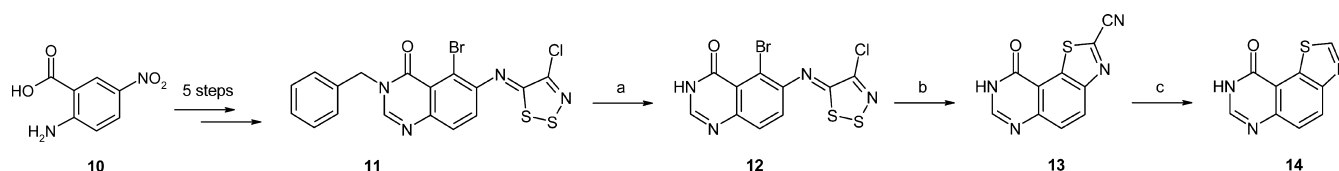
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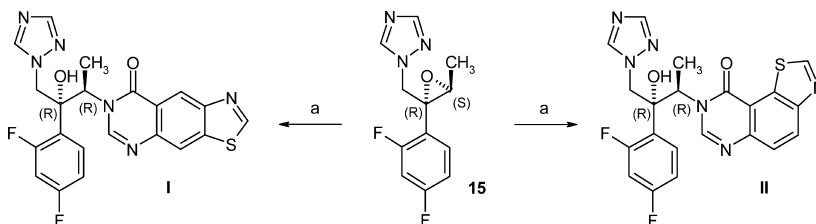
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Scheme 1. Synthesis of Compound 9^a

^aReagents and conditions: (a) H₂NCHO, InCl₃, 150 °C (mW), 40 min, 81%. (b) HNO₃, H₂SO₄, 100 °C, 1 h, 76%. (c) NaH, benzyl bromide, DMF, 80 °C (mW), 30 min, 84%. (d) Fe, AcOH, EtOH, reflux, 1 h, 93%. (e) Appel salt, CH₂Cl₂, pyridine, rt, 3 h, 58%. (f) AlCl₃, toluene, 65 °C, 30 min, 90%. (g) CuI, pyridine, 115 °C (mW), 15 min, 86%. (h) HBr (48%), 115 °C (mW), 30 min, 93%.

Scheme 2. Synthesis of Compound 14^a

^aReagents and conditions: (a) AlCl₃, toluene, 65 °C, 30 min, 85%. (b) CuI, pyridine, 115 °C (mW), 15 min, 72%. (c) HBr (48%), 115 °C (mW), 30 min, 95%.

Scheme 3. Synthesis of Compounds I and II^a

^aReagents and conditions: (a) Thiazolo[4,5-g]quinazolin-8(7H)-one (9) or thiazolo[5,4-f]quinazolin-9(8H)-one (14), K₂CO₃, *N*-methyl-2-pyrrolidone, 80 °C, 3 days, 45–47%.

CYP51 while improving calculated logP values (clogP) as compared to the parent molecule (compound I: clogP = 1.44; albaconazole: clogP = 2.15; BioByte, Bio-Loom). In addition, we wanted to check the effect of the position of the thiazole ring on the biological antifungal activities.

Scheme 1 outlines the synthesis of original 1,3-thiazolo[4,5-g]quinazolin-8(7H)-one (9) started from 4-bromo-2-nitrobenzoic acid (1), which reacted with formamide, in the presence of indium(III) chloride,¹¹ under microwave irradiations, to give 7-bromo-3H-quinazolin-4-one (2) in 81% yield. Nitration of 2 with a mixture of nitric acid and sulfuric acid at 100 °C afforded compound 3 in 76% yield,¹² which was N-benzylation in position 3 of the quinazolin-4-one skeleton by reaction with sodium hydride and benzyl bromide in anhydrous DMF (compound 4). Subsequent reduction of the nitro group was accomplished with iron/acetic acid in refluxing ethanol¹³ to give the corresponding amine 5 (yield 93%). This compound was then condensed with 4,5-dichloro-1,2,3-dithiazolium chloride (Appel salt) in dichloromethane at room temperature, followed by the addition of pyridine, to give the intermediate imino-1,2,3-dithiazoloquinazolinone 6 in 58% yield. Debenzylation was carried out using aluminum chloride (AlCl₃) in toluene at 65 °C, leading to compound 7 in excellent yield.

Fusion of the thiazole ring onto the quinazolinone moiety was realized by a thermolysis procedure at 115 °C, in the presence of copper(I) iodide (CuI), in pyridine (compound 8). Decyanation (via hydrolysis and decarboxylation) of the thiazolo-2-carbonitrile ring was performed using 48% hydrobromic acid under microwave irradiations to give compound 9. Scheme 2 outlines the synthesis of thiazolo[5,4-f]quinazolin-9(8H)-one (14), which was already prepared from 2-amino-5-nitrobenzoic acid (10) by Besson and co-workers.^{14–16} The main difference consisted of the debenzoylation of compound 11 before the formation of the thiazole ring as described in Scheme 1. Finally, thiazoloquinazolinones 9 and 14 were condensed onto the previously described chiral oxirane 15 [synthesized in seven steps from (*R*)-methyl lactate]¹⁷ in the presence of K₂CO₃ in *N*-methyl-2-pyrrolidone (NMP) at 80 °C to afford the expected compounds I and II in 47 and 45% yield, respectively (Scheme 3).

In vitro antifungal activities of compounds I and II against *Candida* species (yeasts) and filamentous fungi (molds) were compared with those of fluconazole, voriconazole, itraconazole, and albaconazole. Evaluations against *Candida* spp. and *Aspergillus fumigatus* strains were realized by Le Pape's previously reported method¹⁸ and those against other

filamentous fungi according to the CLSI Broth Microdilution Susceptibility Method (M38).¹⁹ The MICs of compounds I and II and reference drugs are summarized in Tables 1 and 2.

Against the fluconazole-susceptible *C. albicans* CAAL93 and CAAL97 isolates (Table 1), compounds I and II displayed a high level of activity with MIC values ranging from 0.001 to 0.011 $\mu\text{g mL}^{-1}$, comparable to voriconazole and albaconazole values. These compounds were further evaluated against DSY735 and DSY292 fluconazole-resistant isolates. DSY735 exhibited a gain of function mutation in *TAC1* (transcriptional activator of *CDR* genes) responsible for *Cdr1/2p* transporter overexpression and a presence of the isochromosome 5L leading to higher copy numbers of *TAC1* and *ERG11* on chromosome 5.²⁰ DSY292 showed a gain of function mutation in *MRR1* (transcriptional activator of *CaMDR1* gene) and Y132H, G464S, and R467K amino acid substitutions on *CYP51*.²¹ Compound I exhibited high antifungal activities similar to those of voriconazole and slightly inferior to those of albaconazole, showing that this compound could overcome overexpression of efflux pumps and *Erg11p* (*CYP51*) but also some specific point substitutions in the *CYP51* enzyme. However, against the DSY292 isolate, albaconazole appears to be less affected by the G464S and R467K mutations, which are close to the position of the fifth heme ligand (*Cys470*), than either voriconazole or compound I. This could be explained by tighter affinity and/or favorable compensatory adjustments of albaconazole within the active site, even if besides this argument on the *CYP51* active site, we cannot discard either a possible slight increase in *MDR1* efflux for voriconazole and compound I as opposed to albaconazole. On the other hand, a significant broad-spectrum antifungal activity was also observed for compound I against *C. krusei*, *C. glabrata*, and *C. parapsilosis* isolates with MIC values ranging from <0.005 (for CAKR8) to 0.182 $\mu\text{g mL}^{-1}$ (for CAKR7), confirming its interest for fluconazole low-susceptible strains and fluconazole intrinsically resistant *Candida* species. In particular, for the acquired-resistant *C. parapsilosis* CAPA1 and CAPA2 isolates, compound I was almost as active as albaconazole and 7–70-fold more active than voriconazole. Compound II has a narrow spectrum of activity with a high level of potency against *C. glabrata* and *C. parapsilosis* strains (MICs ranging from 0.001 to 0.095 $\mu\text{g mL}^{-1}$) but not against *C. krusei* strains (MICs > 2.5 $\mu\text{g mL}^{-1}$).

The antifungal activities of compounds I and II and reference drugs against clinical filamentous fungal isolates are shown in Table 2. *A. fumigatus*, the most frequent agent of invasive fungal infections in immunocompromised patients,²² and some emerging non-*Aspergillus* molds such as *Scedosporium* sp. (SCSP1) and zygomycetes (*Rhizomucor pulsillus* RHPU1 and *Rhizomucor miehei* RHMI1) were used in this study. Compound I was active against *A. fumigatus* isolates susceptible (ASFU7) or resistant to itraconazole (ASFU13, ASFU17, ASFU19, ASFU20, and ASFU23), with MIC values ranging from 0.27 (for ASFU23) to 4.5 $\mu\text{g mL}^{-1}$ (for ASFU20), which were comparable to those of albaconazole and slightly superior to those of voriconazole, the first-line agent for the treatment of invasive aspergillosis.²³ Because compound II was not active against azole-susceptible ASFU7, this compound was not evaluated against the other filamentous fungi. Against the emerging fungi (SCSP1, RHPU1, and RHMI1), compound I exhibited also MIC values (1–2 $\mu\text{g mL}^{-1}$) globally close to those observed for albaconazole and voriconazole.

The mechanism of action was investigated by studying inhibition of *C. albicans* CAAL93 ergosterol biosynthesis after

Table 1. Susceptibilities of Clinical Isolates of *Candida* Species to Compounds I and II and Reference Antifungal Agents

comps	MIC values ($\mu\text{g mL}^{-1}$) ^a									
	CAAL93	CAAL97	DSY735	DSY292	CAKR7	CAKR8	CAGL2	CAPA1	CAPA2	
I	0.011 ± 0.006	<0.001	0.118 ± 0.009	0.94 ± 0.15	0.182 ± 0.045	<0.005	0.068 ± 0.020	0.013 ± 0.016	0.127 ± 0.020	
II	0.003 ± 0.002	<0.001	1.43 ± 0.42	5.0 ± 4.0	5.0 ± 3.0	2.5 ± 2.0	0.095 ± 0.009	0.006 ± 0.001	<0.001	
fluconazole	0.062 ± 0.052	0.018 ± 0.002	11.0 ± 0.2	21.0 ± 7.0	>30	12.9 ± 0.9	7.7 ± 0.1	>30	>30	
voriconazole	0.005 ± 0.001	<0.003	0.101 ± 0.005	1.1 ± 0.1	0.549 ± 0.244	<0.003	0.061 ± 0.020	0.925 ± 0.120	0.905 ± 0.291	
albaconazole	0.003 ± 0.002	<0.001	0.063 ± 0.009	<0.043	0.022 ± 0.010	0.005 ± 0.001	0.065 ± 0.012	0.018 ± 0.003	0.011 ± 0.001	

^aValues represent the mean ± SD of experiments performed in triplicate. *C. albicans* (CAAL93, CAAL97, DSY735, and DSY292), *C. krusei* (CAKR7 and CAKR8), *C. glabrata* (CAGL2), and *C. parapsilosis* (CAPA1 and CAPA2).

Table 2. Susceptibilities of Clinical Isolates of Filamentous Fungi to Compounds I and II and Reference Antifungal Agents

comps	MIC values ($\mu\text{g mL}^{-1}$) ^a								
	ASFU7	ASFU13	ASFU17	ASFU19	ASFU20	ASFU23	SCSP1 ^b	RHPU1 ^b	RHMI1 ^b
I	0.28 ± 0.01	3.1 ± 1	2.2 ± 0.2	2.1 ± 0.1	4.5 ± 2	0.27 ± 0.02	1	1	2
II	>43	NT	NT	NT	NT	NT	NT	NT	NT
itraconazole	0.42 ± 0.04	>71	>71	>71	>71	>71	NT	NT	NT
voriconazole	0.15 ± 0.01	<0.35	0.25 ± 0.3	3.0 ± 0.3	<0.35	0.18 ± 0.01	0.125	2	8
albaconazole	0.23 ± 0.01	1.1 ± 0.3	2.1 ± 0.1	2.0 ± 0.1	1.2 ± 0.3	0.22 ± 0.01	0.5	0.5	1

^aValues represent the mean ± SD of experiments performed in triplicate. ^bMICs were determined by the M38 method from the CLSI, and each test was performed twice. *A. fumigatus* (ASFU7, ASFU13, ASFU19, ASFU20, and ASFU23), *Scedosporium* sp. (SCSP1), *Rhizomucor pusillus* (RHPU1), and *Rhizomucor miehei* (RHMI1). NT = not tested.

treatment by compound I. As shown in Table 3, at the concentrations of 4.5 and 22.0 ng mL⁻¹, surrounding the MIC

Table 3. Effect of Compound I on the Sterol Composition of *C. albicans* CAAL93

sterols ^a	control	compd I (ng mL ⁻¹)	
		4.5	22.0
lanosterol	9.2	29.9	41.6
eburicol	0.3	4.4	20.4
zymosterol	8.9	2.0	
episterol	13.0	2.9	
fecosterol	9.1		
14-methylfecosterol		3.8	11.4
14-methylpisterol		5.9	15.7
14-methyl-3,6-diol			5.4
ergosterol	55.3	51.1	5.4

^aSterols of interest were identified by their mass spectrum. The area under curve (AUC) of each peak was used to calculate a ratio: sterol AUC/sum of sterols AUC. Values are the result of one experiment.

value (11.0 ng mL⁻¹), compound I inhibited ergosterol biosynthesis in a dose-dependent manner, while lanosterol, eburicol, and methylated sterols at C14 position accumulated, confirming an inhibition of the *C. albicans* CYP51 enzyme.

Finally, we compared the in vivo activity of compound I with that of albaconazole against a lethal systemic infection in mice caused by *C. albicans* (Figure 2). This study will be considered in more detail elsewhere.

Transiently neutropenic swiss mice (CE Janvier, Le Genest St. Isle, France) were injected intravenously with fluconazole-susceptible *C. albicans* (CAAL93) blastoconidia. One hour after infection, mice were treated per os once daily with 15 mg/kg

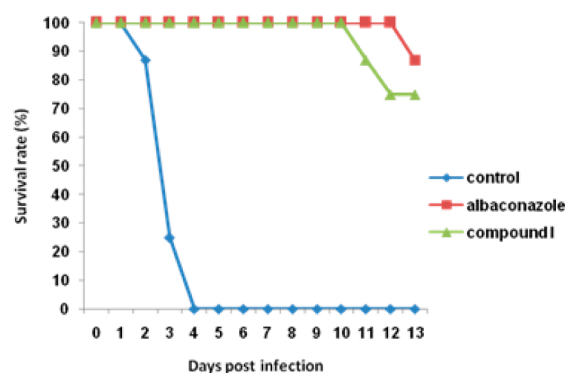


Figure 2. Effects of compound I and albaconazole against systemic infection caused by *C. albicans* (CAAL93) in neutropenic mice ($n = 8$).

body weight of compound I or albaconazole solubilized in 5% DMSO for 5 consecutive days. The control group received 100 μL of 0.9% saline solution with 5% DMSO. The efficacy of the compound I and albaconazole was expressed as the survival rates 13 days after infection. On day 13 after infection, 75 and 87% of mice were still alive in compound I and the albaconazole groups, respectively, while all control mice died on day 5, demonstrating a high and significant ($p < 0.001$, Log rank test) efficacy against murine invasive candidiasis and suggesting a possible action of this compound against other invasive fungal infections.

In summary, we have synthesized a novel antifungal agent derived from albaconazole with broad-spectrum in vitro antifungal activity against pathogenic *Candida* species and filamentous fungi, including clinical isolates that are resistant to fluconazole and itraconazole. This new CYP51 inhibitor also displayed preliminary in vivo antifungal efficacy in a mice model of systemic candidiasis. All of the biological results are close to those observed for voriconazole or albaconazole. This work confirms the promising interest of a new azole compound bearing an original fused tricyclic scaffold. Moreover, we can expect that its lower clogP value as compared to the parent molecule could modulate the characteristics of this albaconazole-derived compound on either various types of invasive fungal infections or the route of administration. Further optimizations are ongoing, and the results will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures for assay protocols, synthesis, and characterization of compounds I and II. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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