ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

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

Rémi Guillon,[†] Fabrice Pagniez,*[,‡] Carine Picot,[‡] Damien Hédou,[§] Alain Tonnerre,[†] Elizabeth Chosson,[§] Muriel Duflos,[†] Thierry Besson,[§] Cédric Logé,*[,†] and Patrice Le Pape[‡]

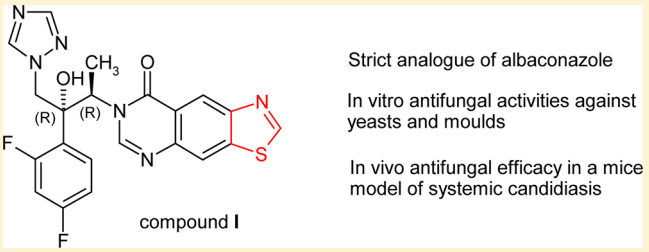
[†]Université de Nantes, Nantes Atlantique Universités, Laboratoire de Chimie Thérapeutique, Cibles et Médicaments des Infections et du Cancer, IICIMED-EA 1155, UFR Sciences Pharmaceutiques, 1 rue Gaston Veil, Nantes F-44035 Cedex 1, France

[‡]Université de Nantes, Nantes Atlantique Universités, Laboratoire de Parasitologie et Mycologie Médicale, Cibles et Médicaments des Infections et du Cancer, IICIMED-EA 1155, UFR Sciences Pharmaceutiques, 1 rue Gaston Veil, Nantes F-44035 Cedex 1, France

[§]Université de Rouen, Laboratoire C.O.B.R.A., CNRS UMR 6014 & FR 3038, Institut de Recherche en Chimie Organique Fine (I.R.C.O.F.), rue Tesnière, 76130 Mont Saint-Aignan, France

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.



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 14α-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 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

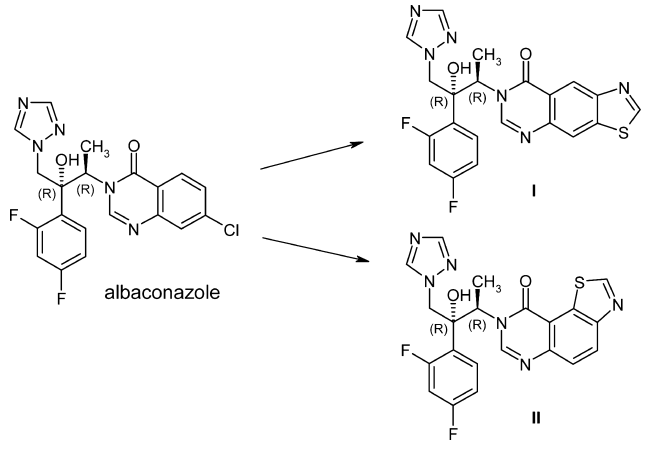
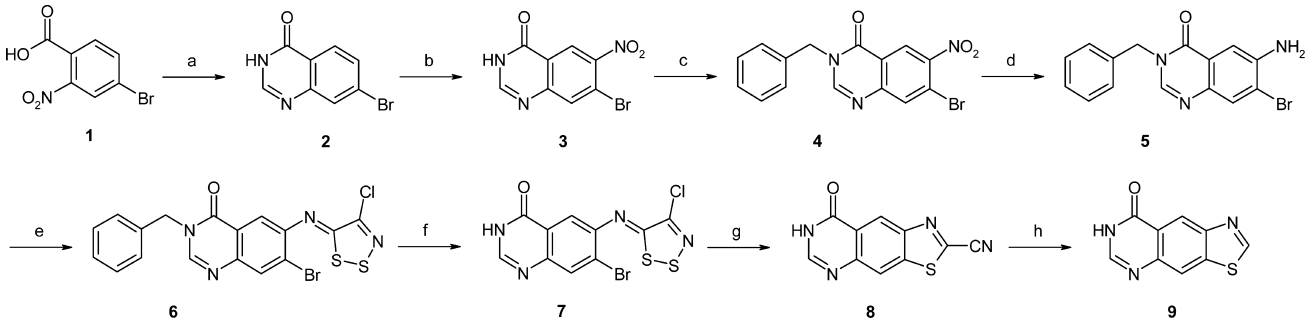


Figure 1. General structures of synthesized compounds.

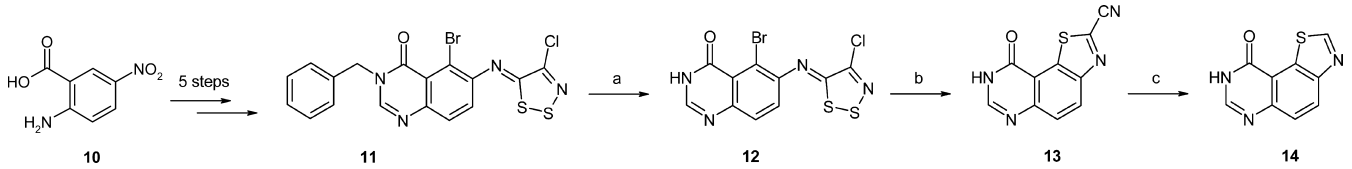
Received: November 30, 2012
Accepted: January 17, 2013
Published: January 17, 2013

Scheme 1. Synthesis of Compound 9[a]



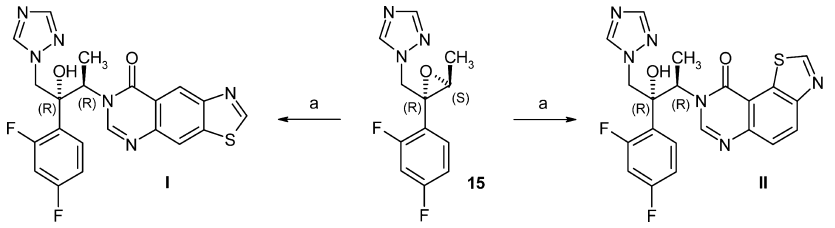
[a]Reagents and conditions: (a) H_2NCHO, $InCl_3$, 150 °C (mW), 40 min, 81%. (b) HNO_3, H_2SO_4, 100 °C, 1 h, 76%. (c) NaH, benzyl bromide, DMF, 80 °C (mW), 30 min, 84%. (d) Fe, AcOH, EtOH, reflux, 1 h, 93%. (e) Appel salt, CH_2Cl_2, pyridine, rt, 3 h, 58%. (f) $AlCl_3$, toluene, 65 °C, 30 min, 90%. (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]



[a]Reagents and conditions: (a) $AlCl_3$, toluene, 65 °C, 30 min, 85%. (b) CuI, pyridine, 115 °C (mW), 15 min, 72%. (h) HBr (48%), 115 °C (mW), 30 min, 95%.

Scheme 3. Synthesis of Compounds I and II[a]



[a]Reagents and conditions: (a) Thiazolo[4,5-*g*]quinazolin-8(7*H*)-one (**9**) or thiazolo[5,4-*f*]quinazolin-9(8*H*)-one (**14**), K_2CO_3, *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(7*H*)-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-3*H*-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-benzylated 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 ethanol[13] 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 ($AlCl_3$) in toluene at 65 °C, leading to compound **7** in excellent yield. Fusion of the thiazole ring onto the quinazoline 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(8*H*)-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 debenzylation 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 K_2CO_3 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 method[18] and those against other

dx.doi.org/10.1021/ml300429p | *ACS Med. Chem. Lett.* 2013, 4, 288−292

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-suceptible *C. albicans* CAAL93 and CAAL97 isolates (Table 1), compounds **I** and **II** displayed a high level of activity with MIC values ranging from of 0.001 to 0.011 μg 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 isochrome 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 μg 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 μg mL^{-1}) but not against *C. krusei* strains (MICs > 2.5 μg 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 μg 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 μg 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

	MIC values (μg mL^{-1})[a]								
compds	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

[a]Values 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).

dx.doi.org/10.1021/ml300429p | *ACS Med. Chem. Lett.* 2013, 4, 288−292

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

	MIC values (μg mL^{-1})[a]								
compds	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

[a]Values represent the mean ± SD of experiments performed in triplicate. [b]MICs 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 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.

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

		compd **I** (ng mL^{-1})	
sterols[a]	control	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-methylepisterol		5.9	15.7
14-methyl-3,6-diol			5.4
ergosterol	55.3	51.1	5.4

[a]Sterols 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.

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 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.

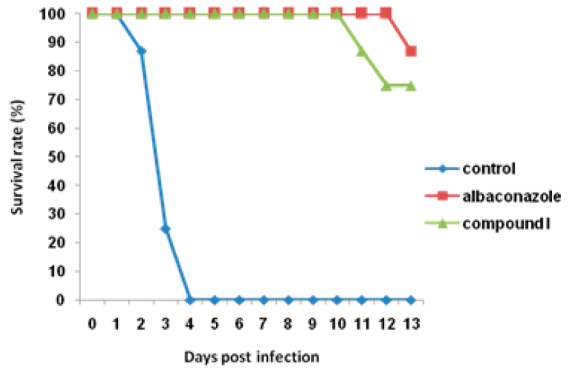


Figure 2. Effects of compound **I** and albaconazole against systemic infection caused by *C. albicans* (CAAL93) in neutropenic mice ($n = 8$).

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.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +33(0)240 412 828. Fax: +33(0)240 412 866. E-mail: fabrice.pagniez@univ-nantes.fr (F.P.). Tel: +33(0)240 411 108. Fax: +33(0)240 412 876. E-mail: cedric.loge@univ-nantes.fr (C.L.).

Funding

R.G. and D.H. acknowledge the Ministère de l'Enseignement Supérieur et de la Recherche (MESR) for their Ph.D. grants.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Pr. Dominique Sanglard (Institute of Microbiology, University of Lausanne, Switzerland) for providing strains DSY292 and DSY735 used in this study.

■ REFERENCES

(1) Groll, A. H.; Lumb, J. New developments in invasive fungal disease. *Future Microbiol.* **2012**, *7*, 179−184.
(2) Kelly, S. L.; Arnoldi, A.; Kelly, D. E. Molecular genetic analysis of azole antifungal mode of action. *Biochem. Soc. Trans.* **1993**, *21*, 1034−1038.
(3) Bartroli, J.; Turmo, E.; Alquero, M.; Boncompte, E.; Vericat, M. L.; Conte, L.; Ramis, J.; Merlos, M.; Garcia-Rafanell, J.; Forn, J. New azole antifungals: 3. Synthesis and antifungal activity of 3-substituted-4(3H)-quinazolinones. *J. Med. Chem.* **1998**, *41*, 1869−1882.
(4) Bartroli, J.; Merlos, M.; Sisniega, H. Overview of albaconazole. *Eur. Infect. Dis.* **2011**, *5*, 88−91.
(5) Türel, Ö. Newer antifungal agents. *Expert Rev. Anti-Infect. Ther.* **2011**, *9*, 325−338.
(6) Giraud, F.; Logé, C.; Pagniez, F.; Crépin, D.; Le Pape, P.; Le Borgne, M. Design, synthesis and evaluation of 1-(*N*-benzylamino)-2-phenyl-3-(*1H*-1,2,4-triazol-1-yl)propan-2-ols as antifungals agents. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1820−1824.
(7) Giraud, F.; Guillon, R.; Logé, C.; Pagniez, F.; Picot, C.; Le Borgne, M.; Le Pape, P. Synthesis and structure-activity relationships of 2-phenyl-1-[(pyridinyl- and piperidinylmethyl)amino]-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ols as antifungal agents. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 301−304.
(8) Guillon, R.; Giraud, F.; Logé, C.; Le Borgne, M.; Picot, C.; Pagniez, F.; Le Pape, P. Design of new antifungal agents: synthesis and evaluation of 1-[(1*H*-indol-5-ylmethyl)amino]-2-phenyl-3-(*1H*-1,2,4-triazol-1-yl)propan-2-ols. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5833−5836.
(9) Guillon, R.; Pagniez, F.; Giraud, F.; Crépin, D.; Picot, C.; Le Borgne, M.; Morio, F.; Duflos, M.; Logé, C.; Le Pape, P. Design, synthesis and *in vitro* antifungal activity of 1-[(4-substituted benzyl)-methyl amino]-2-(2,4-difluorophenyl)-3-(*1H*-1,2,4-triazol-1-yl)-propan-2-ols. *ChemMedChem* **2011**, *6*, 816−825.
(10) Guillon, R.; Pagniez, F.; Rambaud, C.; Picot, C.; Duflos, M.; Logé, C.; Le Pape, P. Design, synthesis and biological evaluation of 1-[(biarylmethyl)methylamino]-2-(2,4-difluorophenyl)-3-(*1H*-1,2,4-triazol-1-yl)propan-2-ols as potent antifungal agents: New insights into structure-activity relationships. *ChemMedChem* **2011**, *6*, 1806−1815.
(11) Kundu, S.; Mahindaratne, M.; Quintero, M.; Bao, A.; Negretea, G. One-pot reductive cyclization to antitumor quinazoline precursors. *Arkivoc* **2008**, *ii*, 33−42.
(12) Rewcastle, G. W.; Palmer, B. D.; Bridges, A. J.; Showalter, H. D. H.; Sun, L.; Nelson, J.; McMichael, A.; Kraker, A. J.; Fry, D. W.; Denny, W. A. Tyrosine kinase inhibitors. 9. Synthesis and evaluation of fused tricyclic quinazoline analogues as ATP site inhibitors of the tyrosine kinase activity of the epidermal growth factor receptor. *J. Med. Chem.* **1996**, *39*, 918−928.
(13) Krolski, M. E.; Renaldo, A. F.; Rudisill, D. E.; Stille, J. K. Palladium-catalyzed coupling of 2-bromoanilines with vinylstannanes. A regiocontrolled synthesis of substituted indoles. *J. Org. Chem.* **1988**, *53*, 1170−1176.
(14) Alexandre, F.-R.; Berecibar, A.; Wrigglesworth, R.; Besson, T. Efficient synthesis of thiazoloquinazolinone derivatives. *Tetrahedron Lett.* **2003**, *44*, 4455−4458.
(15) Testard, A.; Picot, L.; Lozach, O.; Blairvacq, M.; Meijer, L.; Murillo, L.; Piot, J.-M.; Thiéry, V.; Besson, T. Synthesis and evaluation of the antiproliferative activity of novel thiazoloquinazolinone kinases inhibitors. *J. Enzyme Inhib. Med. Chem.* **2005**, *20*, 557−568.
(16) Testard, A.; Logé, C.; Léger, B.; Robert, J.-M.; Lozach, O.; Blairvacq, M.; Meijer, L.; Thiéry, V.; Besson, T. Thiazolo[5,4-*f*]quinazolin-9-ones, inhibitors of glycogen synthase kinase-3. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3419−3423.
(17) Tasaka, A.; Tamura, N.; Matsushita, Y.; Teranishi, K.; Hayashi, R.; Okonogi, K.; Itoh, K. Optically active antifungal azoles. I. Synthesis and antifungal activity of (2*R*,3*R*)-2-(2,4-difluorophenyl)-3-mercapto-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol and its stereoisomers. *Chem. Pharm. Bull.* **1993**, *41*, 1035−1041.
(18) Pagniez, F.; Le Pape, P. New fluorometric screening test for possible antifungal drugs. *J. Mycol. Med.* **2001**, *11*, 73−78.
(19) *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard. CLSI Document M38-A*; CLSI: Pennsylvania, 2002.
(20) Coste, A.; Selmecki, A.; Forche, A.; Diogo, D.; Bougnoux, M. E.; d'Enfert, C.; Berman, J.; Sanglard, D. Genotypic evolution of azole resistance mechanisms in sequential *Candida albicans* isolates. *Eukaryotic Cell* **2007**, *6*, 1889−1904.
(21) Dunkel, N.; Blass, J.; Rogers, P. D.; Morschhäuser, J. Mutations in the multi-drug resistance regulator MRR1, followed by loss of heterozygosity, are the main cause of MDR1 overexpression in fluconazole-resistant Candida albicans strains. *Mol. Microbiol.* **2008**, *69*, 827−840.
(22) Ben-Ami, R.; Lewis, R. E.; Leventakos, K.; Kontoyiannis, D. P. *Aspergillus fumigatus* inhibits angiogenesis through the production of gliotoxin and other secondary metabolites. *Blood* **2009**, *114*, 5393−5399.
(23) Walsh, T. J.; Anaissie, E. J.; Denning, D. W.; Herbrecht, R.; Kontoyiannis, D. P.; Marr, K. A.; Morrison, V. A.; Segal, B. H.; Steinbach, W. J.; Stevens, D. A.; Burik, J. A.; Wingard, J. R.; Patterson, T. F. Treatment of aspergillosis clinical practice guidelines of the infectious diseases of America. *Clin. Infect. Dis.* **2008**, *46*, 327−360.