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# Novel Macrocyclic Amidinoureas: Potent Non-Azole Antifungals Active against Wild-Type and Resistant Candida Species

Maurizio Sanguinetti,[§] Stefania Sanfilippo,[†] Daniele Castagnolo,[†,‡] Dominique Sanglard,[∥] Brunella Posteraro,[§] Giovanni Donzellini,[†] and Maurizio Botta*[,†,⊥]

[†]Dipartimento Biotecnologie, Chimica e Farmacia, Università di Siena, via Aldo Moro 2, 53100 Siena, Italy

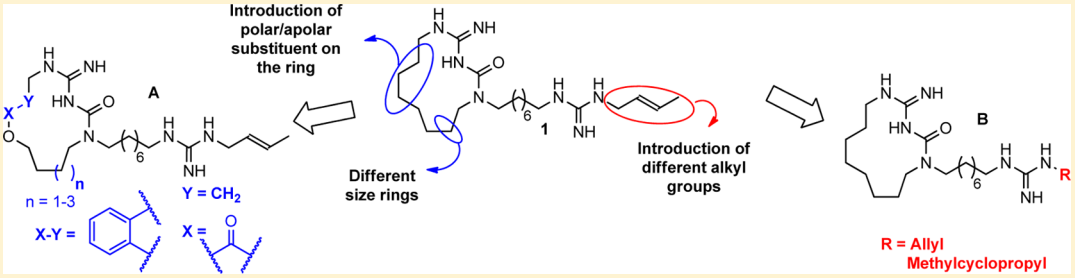
[‡]School of Applied Sciences, Northumbria University, Ellison Building, Ellison Place, NE1 8ST, Newcastle upon Tyne, United Kingdom

[§]Istituto di Microbiologia, Università Cattolica del Sacro Cuore, Largo F. Vito 1, 00168 Rome, Italy

[∥]Institute of Microbiology, University of Lausanne and University Hospital Center, Lausanne, Rue du Bugnon 48, CH-1011 Lausanne, Switzerland

[⊥]Sbarro Institute for Cancer Research and Molecular Medicine, Center for Biotechnology, College of Science and Technology, Temple University, BioLife Science Building, Suite 333, 1900 North 12th Street, Philadelphia, Pennsylvania 19122, United States

Supporting Information




**ABSTRACT:** Novel macrocyclic amidinourea derivatives **11**, **18**, and **25** were synthesized and evaluated as antifungal agents against wild-type and fluconazole resistant Candida species. Macrocyclic compounds **11** and **18** were synthesized through a convergent approach using as a key step a ring-closing metathesis macrocyclization reaction, whereas compounds **25** were obtained by our previously reported synthetic pathway. All the macrocyclic amidinoureas showed antifungal activity toward different Candida species higher or comparable to fluconazole and resulted highly active against fluconazole resistant Candida strains showing in many cases minimum inhibitory concentration values lower than voriconazole.

**KEYWORDS:** *Antifungal, amidinourea, macrocyclization, ring-closing metathesis, Candida species, fluconazole, antifungal drug-resistance*

The epidemiology of fungal infections has been an evolving issue since the late 1960s when, as a consequence of the development of antibiotic therapies, a drastic rise of mycoses was observed. Today, fungal infections represent a major global health threat and the increasing incidence of invasive and opportunistic mycoses is often associated with excessive morbidity and mortality.[1] Fungal infections have increased in incidence in recent decades often as a result of advanced medical treatments and the increase in the number of immunocompromised patients. Patients who are at risk for the development of serious fungal infections include those undergoing blood and solid-organ transplantation, gastrointestinal surgery, patients with AIDS, and premature infants. In addition, cancer chemotherapy and allogeneic bone marrow transplantation are often associated with fungal disease, and up to 30% of patients with acute leukemia experience invasive fungal infections. Given the complexity of the population of patients suffering from a mycosis and the diverse and increasing array of pathogens, fungal infections pose considerable diagnostic and therapeutic challenges. Although several species of fungi are potentially pathogenic in humans, Candida, and in particular *Candida albicans*, is the organism responsible for most fungal diseases.[2,3] Defense against Candida infections has relied on the use of a limited number of chemotherapeutic agents, including azoles, such as fluconazole and voriconazole, and polyenes, such as amphotericin B.[4] In particular, azoles have been extensively used to treat a wide range of candidiasis because of their low toxicity and high degree of bioavailability after oral administration.[5] However, despite they still constitute the preferred first line therapy, the emergence and the spread of drug resistant fungal species[6,7] draw the line at the use of these classical antifungal drugs. Today, fluconazole is poorly or not

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effective against mutant Candida species making the development of new and more active drugs a priority.[8] Recently, we reported the discovery and synthesis of a new class of potent antifungal agents, namely, guanidines and macrocyclic amidinoureas,[9−13] which proved to be strongly active against Candida species. Macrocycle **1**, bearing a crotyl moiety on the alkylguanidine side chain, emerged as the most interesting compound with a biological activity higher than fluconazole against several *Candida spp.*[14,15] Only few structure−activity relationships (SAR) arose from our previous studies, indicating that the nature of the alkyl group bound to the guanidine moiety plays a key role in the biological activity.

However, no investigations were carried out on the macrocyclic core, both in terms of size as well as regarding the effects that the incorporation of polar/apolar moieties in to the ring might have on the antifungal activity. Hence, with the aim to identify new macrocyclic amidinoureas active both on wild-type as well as mutant fungal species and to further investigate their SAR, we planned the synthesis of two series of amidinourea derivatives **A** and **B**, both preserving some structural characteristics of the lead **1** (Figure 1). The first

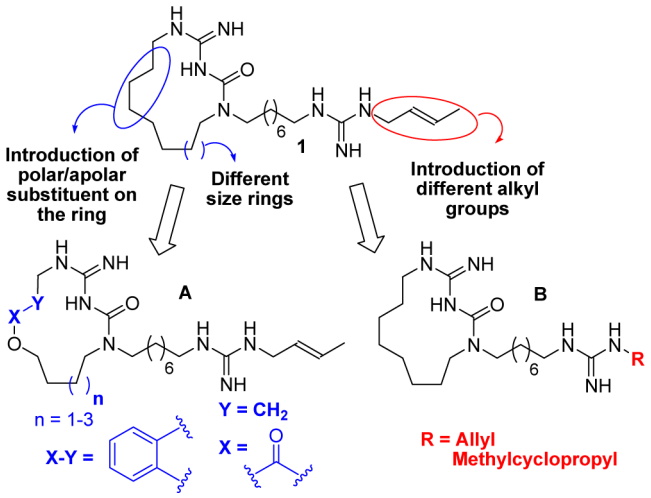


**Figure 1.** Structure of hit compound 1.

series of derivatives **A** was planned with the aim to modulate the antifungal activity introducing an ester moiety (polar group) or fusing a benzene (apolar group) with the macrocyclic core, thus potentially favoring the interaction of novel compounds with target enzymes through H-bond or $\pi$-interactions. Moreover, the synthesis of 14- and 15-membered ring amidinoureas was also planned in order to further explore the chemical space and evaluate the effects that the macrocycle size might have on the biological activity.

The synthesis of two additional derivatives **B** bearing different alkyl substituents on the guanidine moiety was also planned with the aim to evaluate the importance of an unsaturated double bond for the biological activity. Compounds **11a−c** and **18a−b** were synthesized through a convergent approach using as a key step a ring-closing metathesis (RCM) macrocyclization,[16−20] as described in Scheme 1. Aldehyde **2** was first O-alkylated with the appropriate alkenylbromide and in turn condensed with $NH_2OH$. The resulting oximes were then reduced in the presence of Zn affording the free amines **3a−c**, which were guanylated to give the guanidine building blocks **4a−c**. These latter compounds were refluxed in THF together with **6** affording amidinoureas **7a−c**. Secondary amine **6** was synthesized in a two amidation−reduction sequence starting from Cbz-aminooctanoic acid **5**, which was first coupled with allylamine followed by reduction of the resulting amide with diisobutylaluminium hydride (DIBAL-H). Macrocyclization of **7a−c** was then carried out using Grubbs' catalyst second generation at 40−80 °C leading to macrocycles **8a−c** in good-high yields,[17,21] as a mixture of *E/Z* isomers. Hydrogenation of **8a−c** allowed the reduction of the double bond and the Cbz cleavage in one single step, affording amines **9a−c**. Primary amines were then guanylated with *N*-crotyl-*S*-Me-isothiourea-*N*,*N*′-diBoc and then treated with trifluoroacetic acid (TFA), leading to desired compounds **11a−c** as trifluoroacetic salts. A similar approach was used for the synthesis of derivatives **18a−b** bearing an ester moiety on the macrocyclic ring. Beta-alanine **12** was first guanylated in the presence of trimethylsilyl chloride (TMSCl) to protect in situ the carboxylic moiety,[22] and the resulting acid was esterified using the appropriate alcohol to afford guanidines **13a−b**. Allylamine **6** was then reacted with **13a−b** in refluxing tetrahydrofuran (THF) leading to dienes **14a−b** in high yields. RCM macrocyclization of these latter compounds was accomplished with Grubbs' catalyst in DCM at 40 °C, affording a mixture of *E/Z* alkenes **15a−b**. Hydrogenation of **15a−b** followed by guanylation and TFA mediated Boc deprotection led finally to desired derivatives **18a−b**. In our previous paper, we described the synthesis of lead **1** starting from the commercially available 1,17-diamino-9-azaheptadecane. However, because of the removal of this building block from the market, we were forced to design a new synthetic pathway. Thus, macrocyclic derivatives **25a−b** were synthesized as outlined in Scheme 2. The coupling between amine **19** and acid **20** led to the formation of amide **21**, which was in turn reduced with DIBAL-H affording the secondary amine **22**.[23] Refluxing of **22** in THF led to the formation of the desired macrocycle **23** in good yield. The Cbz protecting group was then cleaved by hydrogenation affording the corresponding primary amine. This latter compound was then guanylated with two different *S*-Me-alkyl-thioureas **26a−b** leading, after Boc cleavage, to the desired derivatives **25a−b**. Compounds **11a−c**, **18a−b**, and **25a−b** were then assayed against a total of 116 clinical isolates of seven different wild-type Candida species (oral, vaginal, anorectal, urine, stool, blood, central venous catheter, and respiratory tract specimen with each strain representing a single isolate from a patient). In Table 1, the MIC90 medium values for each species of Candida were reported. Macrocycles **25a** and **25b** proved to be the most active compounds with MIC values better than fluconazole against most of the Candida strains.

In particular, **25b** showed to be strongly active against *C. krusei*, a species resistant to common azole drugs, and against *C. tropicalis*. Compounds **11** and **18** retain antifungal activity as well, in many cases with MIC values better than fluconazole. In general, compounds **11a−c** possessing an aromatic substitution on the macrocyclic core proved to be more active when compared to **18a−b** bearing an ester moiety. The increase of the ring size in compounds **11** from 13 to 15 carbon atoms evidenced an enhancement of activity toward all Candida species. However, it is noteworthy that the 14-membered ring **11b** showed the highest activity toward *C. albicans*, *C. guillermondii*, and *C. parapsilosis*, while the larger macrocycle **11c** proved to be more active against *C. tropicalis* and *C. kefyr*. In all cases, **11b−c** resulted more than or as active as


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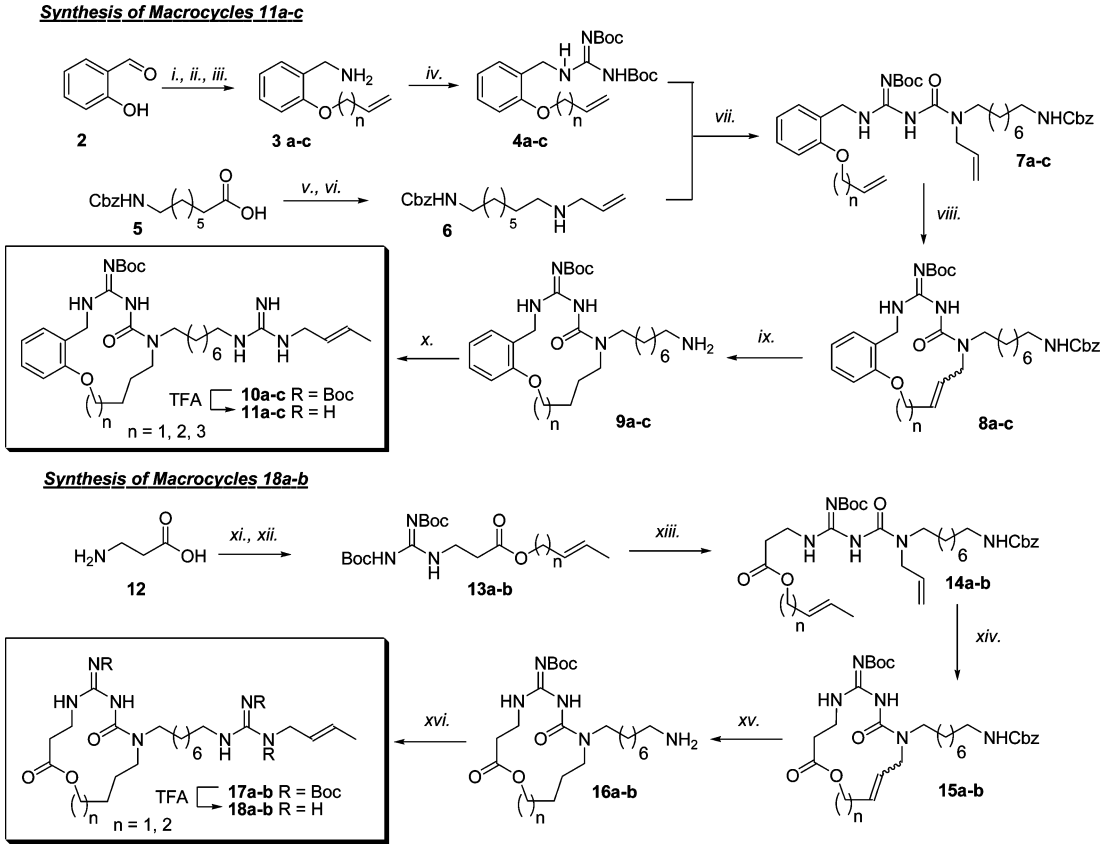
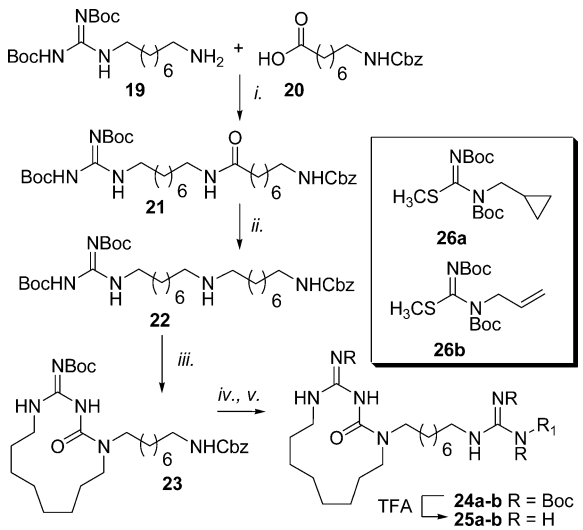
**Scheme 1. Synthesis of Derivatives 11a−c and 18a−b**[a]




[a]Reagents and conditions: (i) $Br(CH_2)_nCH{=}CH_2$, $K_2CO_3$, DCM, reflux; (ii) $NH_2OH$, Py, EtOH, reflux; (iii) Zn, HCl, THF, reflux; (iv) $(BocNH)_2C{=}NTf$, $Et_3N$, DCM; (v) $AllylNH_2$, EDC, HOBt, DIPEA, DMF; (vi) DIBAL-H, DCM, r.t.; (vii) THF, reflux, 12 h; (viii) Grubbs' Cat. second gen., toluene or DCM 2−10 mM, 40−80 °C; (ix) $H_2$, Pd/C, EtOH; (x) CrotylNBoc(C=NBoc)SMe, THF, reflux, 12 h; (xi) TMSCl, $Et_3N$, DCM, reflux; (xii) DMAP, DCC, $HO(CH_2)_nCH{=}CHCH_3$, DCM, rt 24 h; (xiii) **6**, THF, reflux, 12 h; (xiv) Grubbs' Cat. second gen., DCM 2 mM, 40 °C; (xv) $H_2$, Pd/C, EtOH; (xvi) CrotylNBoc(C=NBoc)SMe, THF, reflux, 12 h.

**Scheme 2. Synthesis of Derivatives 25a−b**[a]




[a]Reagents and conditions: (i) EDC, HOBt, DIPEA, DMF; (ii) DIBAL-H, DCM, r.t.; (iii) THF, reflux, 12 h; (iv) $H_2$, Pd/C, EtOH; (v) **26a−b**, THF, reflux, 12 h.

fluconazole, while the 13-membered ring **11a** proved to be less active. However, compounds **11** bearing a benzene fused with the macrocyclic core resulted less active than the unsubstituted analogue **25**. In general, the introduction of an apolar moiety leads to the decrease of the antifungal activity, with the exception of compound **11b**, which showed a stronger activity against *C. glabrata*, a species resistant to common azoles, with a minimum inhibitory concentration (MIC) = 8 $\mu$g/mL. Compounds **18a−b** present a lower activity than **25** and **11** toward all Candida species. Again the larger macrocycle **18b** resulted more active than **18a**. As a general rule, the introduction of a substituent on the macrocyclic ring results in a slight decrease of antifungal activity. However, the presence of a fused aromatic ring seems to be more beneficial than the introduction of an ester moiety while larger rings lead to an increase of biological activity. Azole drugs are known to act as antifungals inhibiting the fungal enzyme 14$\alpha$-demethylase, which produces ergosterol, an important component of the fungal plasma membrane. At the molecular level, different mechanisms contribute to resistance against azole agents.[24] The principal mechanism regards the alterations of 14-$\alpha$-demethylase due to the overexpression and mutations of the gene ERG11 coding for the enzyme. As a consequence, a higher intracellular azole concentration is needed to complex the increased number of demethylase enzymes present in the fungal cells.[25] In addition, it has been demonstrated that mutations in ERG11 prevents binding of azoles to the enzymatic site. A second major mechanism leading to azole resistance is the overexpression of plasma membrane efflux pumps. This


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**Table 1. Antifungal Activity (MIC90, μg/mL)[a]**

| species (No. strains tested) | F[b] | 11a | 11b | 11c | 18a | 18b | 25a | 25b | 1[c] |
|---|---|---|---|---|---|---|---|---|---|
| *C. albicans* (22) | 2 | 16 | 4 | 4 | 32 | 16 | 1 | 1 | 2.5 |
| *C. guillermondii* (10) | 4 | 16 | 2 | 2 | 32 | 8 | 2 | 2 | n.d. |
| *C. krusei* (13) | 256 | 32 | 8 | 4 | 64 | 32 | 4 | 0.5 | 5 |
| *C. parapsilosis* (24) | 0.5 | 8 | 2 | 2 | 32 | 8 | 2 | 4 | 5 |
| *C. tropicalis* (11) | 2 | 8 | 2 | 1 | 32 | 16 | 0.5 | 0.5 | 1.25 |
| *C. kefyr* (10) | 1 | 4 | 4 | 2 | 32 | 16 | 4 | 4 | n.d. |
| *C. glabrata* (26) | 16 | 64 | 8 | 16 | 64 | 32 | 16 | 32 | 20 |

[a]MIC90 values were determined at 24 h both visually and spectrophotometrically. [b]Fluconazole. [c]Activity values expressed as MIC50.

**Table 2. Antifungal Activity on *C. albicans* and *C. glabrata* Fluconazole Resistant Strains**

| | | | MIC90 (μg/mL)[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| strains | species | resistance mechanism | F[b] | VOR[c] | 11a | 11b | 11c | 18a | 18b | 25a | 25b |
| DSY284 | *C. albicans* | mutation in the ERG11 gene; increased expression of the CDR1 and CDR2 genes | 256 | 4 | 16 | 2 | 9 | 16 | 8 | 1 | 1 |
| DSY296 | *C. albicans* | mutation in the ERG11 gene; increased expression of the CDR1 and CDR2 genes | 128 | 8 | 16 | 4 | 10 | 16 | 16 | 2 | 2 |
| DSY289 | *C. albicans* | mutation in the ERG11 gene; increased expression of the CDR1 and CDR2 genes | 256 | 8 | 8 | 2 | 5 | 8 | 8 | 1 | 1 |
| DSY348 | *C. albicans* | mutation in the ERG11 gene; increased expression of the CDR1 and CDR2 genes | 32 | 0.25 | 8 | 2 | 5 | 8 | 8 | 2 | 1 |
| DSY292 | *C. albicans* | mutation in the ERG11 gene; increased expression of the MDR1 | 64 | 2 | 16 | 4 | 10 | 16 | 16 | 2 | 2 |
| DSY735 | *C. albicans* | increased expression of the CDR1 and CDR2 genes | 64 | 2 | 8 | 2 | 5 | 8 | 16 | 1 | 1 |
| DSY775 | *C. albicans* | mutation in the ERG11 gene; increased expression of the CDR1 and CDR2 genes | 128 | 8 | 16 | 4 | 10 | 16 | 16 | 2 | 2 |
| DSY751 | *C. albicans* | increased expression of the MDR1 gene; mutation in the ERG11 gene | 256 | 0.25 | 16 | 4 | 10 | 16 | 16 | 2 | |
| DSY530 | *C. glabrata* | increased expression of the CgCDR1 genes | 64 | 0.5 | 64 | 8 | 36 | 64 | 32 | 16 | 16 |
| DSY754 | *C. glabrata* | increased expression of the CgCDR1 genes | 64 | 2 | 64 | 16 | 40 | 64 | 32 | 16 | 32 |
| DSY756 | *C. glabrata* | increased expression of the CgCDR1, CgCDR2, and CgSNQ2 genes | 128 | 4 | 64 | 8 | 36 | 128 | 64 | 16 | 16 |
| DSY2254 | *C. glabrata* | increased expression of the CgCDR1 and CgCDR2 genes | 128 | 2 | 64 | 16 | 40 | 128 | 64 | 32 | 32 |
| DSY2271 | *C. glabrata* | increased expression of the CgCDR2 genes | 64 | 0.125 | 64 | 8 | 36 | 64 | 32 | 16 | 32 |

[a]MIC values were determined at 24 h both visually and spectrophotometrically. [b]Fluconazole. [c]Voriconazole.

**Table 3. Antifungal Activity on Amphotericin Susceptible and Resistant Candida Strains**

| | | MIC90 (μg/mL)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| strains | species | AmB[b] | 11a | 11b | 11c | 18a | 18b | 25a | 25b |
| ATCC 200955 | *C. albicans* | 2 | 8 | 1 | 5 | 8 | 4 | 1 | 1 |
| ATCC 200950 | *C. lusitaniae* | 1 | 8 | 2 | 5 | 8 | 8 | 4 | 2 |
| ATCC 200951 | *C. lusitaniae* | 1 | 8 | 2 | 5 | 4 | 4 | 2 | 2 |
| ATCC 200952 | *C. lusitaniae* | 2 | 8 | 2 | 5 | 4 | 4 | 1 | 2 |
| ATCC 200953 | *C. lusitaniae* | 1 | 16 | 4 | 5 | 8 | 8 | 2 | 4 |
| ATCC 24348 | *C. lusitaniae* | 0.125 | 8 | 2 | 5 | 4 | 8 | 2 | 1 |
| ATCC 60247 | *C. lusitaniae* | 0.125 | 16 | 4 | 5 | 4 | 4 | 4 | 2 |
| ATCC 200956 | *C. tropicalis* | 8 | 16 | 4 | 10 | 16 | 16 | 2 | 1 |

[a]MIC values were determined at 24 h both visually and spectrophotometrically. [b]Amphotericin B.

mechanism is mediated by two types of transporters, the major facilitators (encoded by MDR genes in *C. albicans*) and the ABC transporters (encoded by CDR genes in *C. albicans*).[26] Upregulation of the CDR genes confers resistance to multiple azoles in *C. albicans*, whereas upregulation of MDR1 alone leads to fluconazole resistance exclusively. Compounds **11**, **18**, and **25** were thus assayed against mutant *C. albicans* and *C. glabrata* strains, and voriconazole, an azole active on fluconazole resistant strains, was chosen as the reference compound. All the tested derivatives proved to be active against *C. albicans* and *C. glabrata* fluconazole-resistant strains (Table 2). Among them macrocycles **25a–b** showed better activity values against resistant *C. albicans* strains than voriconazole (MIC = 1–2 μg/mL) with the exception of DSY348 and DSY751 strains. Compound **11b** resulted more active than voriconazole itself against mutant strains DSY289, DSY296, DSY284, and DSY775 as well as **11c** against DSY289. Derivatives **18** were more active than fluconazole but in general less active than **11** and voriconazole, except for DSY289 strain. Analogues **11a**, **11c**, and **18a–b** resulted inactive against *C. glabrata* resistant strains. However, the 14-membered macrocycle **11b**, as well as compounds **25a–b**, showed a good activity proving to be more active than fluconazole against all *C. glabrata* strains and showing a MIC value against DSY756 close to voriconazole. In addition all these compounds are fully


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active against Candida isolates reported as resistant to amphotericin B (Table 3).[27]

In conclusion, novel macrocyclic amidinoureas **11a−c**, **18a−b**, and **25a−b** were synthesized and biologically assayed as antifungal agents. Derivatives **11** and **18** were obtained through an innovative convergent synthetic pathway whose key step was represented by a RCM macrocyclization. All the new macrocycles showed potent antifungal activity against different wild-type Candida species and fluconazole- or Amphotericin B-resistant Candida strains. In particular, **25a−b** and **11b** resulted in the most interesting potential antifungal agents. Macrocyclic amidinoureas proved to be excellent non-azole lead compounds able to act both on classic as well as resistant fungal infections. Because of their innovative structure, it is reasonable to hypothesize for macrocyclic amidinoureas a mechanism of action different from that of classical azole and polyenic drugs. Further studies to disclose the mechanism of action are currently in progress in our laboratories.

## ■ ASSOCIATED CONTENT

### Supporting Information

Synthetic methods and characterization of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION

### Corresponding Author

*(M.B.) Phone: +39 0577234306. E-mail: botta.maurizio@gmail.com.

### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS

DCM, dichloromethane; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt, hydroxybenzotriazole; DIPEA, *N*,*N*-diisopropylethylamine; DIBAL-H, diisobutylaluminium hydride; TMS, trimethylsilyl chloride; DMAP, 4-dimethylaminopyridine; TFA, trifluoroacetic acid; DCC, dicyclohexylcarbodiimide; Cbz, benzyl carbamate; Boc, *tert*-butoxy carbamate; MIC, minimum inhibitory concentration; MDR, multidrug resistance; CDR, Candida drug resistance; RCM, ring closing metathesis; gen, generation

## ■ REFERENCES

(1) Groll, A. H.; Lumb, J. New developments in invasive fungal disease. *Future Microbiol.* **2012**, *7*, 179−184.

(2) Mishra, N. N.; Prasad, T.; Sharma, N.; Payasi, A.; Prasad, R.; Gupta, D. K.; Singh, R. Pathogenicity and drug resistance in *Candida albicans* and other yeast species. A review. *Acta Microbiol. Immunol. Hung.* **2007**, *54*, 201−235.

(3) Chamilos, G.; Kontoyiannis, D. P. The rationale of combination antifungal therapy in severely immunocompromised patients: empiricism versus evidence-based medicine. *Curr. Opin. Infect. Dis.* **2006**, *19*, 380−385.

(4) Owen, J. N.; Skellley, J. W.; Jeffrey, A. K. The fungus among us: an antifungal review. *U.S. Pharm.* **2010**, *35*, 44−56.

(5) Saag, M. S.; Dismukes, W. E. Azole antifungal agents: emphasis on new triazoles. *Antimicrob. Agents Chemother.* **1988**, *32*, 1−8.

(6) Ahmad, A.; Khan, A.; Manzoor, N. Reversal of efflux mediated antifungal resistance underlies synergistic activity of two monoterpenes with fluconazole. *Eur. J. Pharm. Sci.* **2013**, *48*, 80−86.

(7) Klepser, M. E. Candida resistance and its clinical relevance. *Pharmacotherapy* **2006**, *26*, 68S−75S.

(8) Casalinuovo, I. A.; Di Francesco, P.; Garaci, E. Fluconazole resistance in *Candida albicans*: a review of mechanisms. *Eur. Rev. Med. Pharmacol. Sci.* **2004**, *8*, 69−77.

(9) Castagnolo, D.; Schenone, S.; Botta, M. Guanylated diamines, triamines, and polyamines: chemistry and biological properties. *Chem. Rev.* **2011**, *111*, 5247−5300.

(10) Dreassi, E.; Zizzari, A. T.; D'Arezzo, S.; Visca, P.; Botta, M. Analysis of guazatine mixture by LC and LC−MS and antimycotic activity determination of principal components. *J. Pharm. Biomed. Anal.* **2007**, *43*, 1499−1506.

(11) Raffi, F.; Corelli, F.; Botta, M. Efficient synthesis of iminoctadine, a potent antifungal agent and polyamine oxidase inhibitor (PAO). *Synthesis* **2007**, *19*, 3013−3016.

(12) Castagnolo, D.; Raffi, F.; Giorgi, G.; Botta, M. Macrocyclization of di-Boc-guanidino-alkylamines related to guazatine components: discovery and synthesis of innovative macrocyclic amidinoureas. *Eur. J. Org. Chem.* **2009**, *3*, 334−337.

(13) Castagnolo, D. *New Strategies in Chemical Synthesis and Catalysis*; Wiley-VCH Verlag GmbH & Co. KGaA: Berlin, Germany, 2012; pp 97−126.

(14) Botta, M.; Raffi, F.; Visca, P. Linear and cyclic guanidine derivatives as antifungal agents and their method of preparation. US patent WO2009113033A2, 2009.

(15) Manetti, F.; Castagnolo, D.; Raffi, F.; Zizzari, A. T.; Rajamaki, S.; D'Arezzo, S.; Visca, P.; Cona, A.; Fracasso, M. E.; Doria, D.; Posteraro, B.; Sanguinetti, M.; Fadda, G.; Botta, M. Synthesis of new linear guanidines and macrocyclic amidinourea derivatives endowed with high antifungal activity against *Candida spp.* and *Aspergillus spp*. *J. Med. Chem.* **2009**, *52*, 7376−7379.

(16) Furstner, A.; Langemann, K. Conformationally unbiased macrocyclization reactions by ring closing metathesis. *J. Org. Chem.* **1996**, *61*, 3942−3943.

(17) Han, S.-Y.; Chang, S. *Handbook of Metathesis*; Wiley-VCH Verlag GmbH: Berlin, Germany, 2003; pp 5−127.

(18) Furstner, A.; Davies, P. W. Alkyne metathesis. *Chem. Commun.* **2005**, *42*, 2307−2320.

(19) Gradillas, A.; Perez-Castells, J. Macrocyclization by ring-closing metathesis in the total synthesis of natural products: reaction conditions and limitations. *Angew. Chem., Int. Ed.* **2006**, *45*, 6086−6101.

(20) Kulkarni, A. A.; Diver, S. T. Ring synthesis by stereoselective, methylene-free enyne cross metathesis. *J. Am. Chem. Soc.* **2004**, *126*, 8110−8111.

(21) The 13-membered macrocycle **8a** was obtained in toluene (10 mM solution) at 80 °C. When the RCM was performed at lower temperature (DCM, 40 °C) compound **8a** was not formed and diene **7a** was entirely recovered. Macrocycles **8b** and **8c** were obtained in DCM (2 mM solution) at 40 °C

(22) Lal, B.; Gangopadhyay, A. K. A practical synthesis of free and protected guanidino acids from amino acids. *Tetrahedron Lett.* **1996**, *37*, 2483−2486.

(23) Attempts to reduce the amide using $BH_3$·$SMe_2$, $BH_3$−THF, or $NaBH_4$/$TiCl_4$ led to the cleavage of Boc protecting groups.

(24) Peman, J.; Canton, E.; Espinel-Ingroff, A. Antifungal drug resistance mechanisms. *Expert Rev. Anti-Infect. Ther.* **2009**, *7*, 453−460.

(25) Sanglard, D.; Odds, F. C. Resistance of Candida species to antifungal agents: molecular mechanisms and clinical consequences. *Lancet Infect. Dis.* **2002**, *2*, 73−85.

(26) Perea, S.; Lopez-Ribot, J. L.; Kirkpatrick, W. R.; McAtee, R. K.; Santillan, R. A.; Martinez, M.; Calabrese, D.; Sanglard, D.; Patterson, T. F. Prevalence of molecular mechanisms of resistance to azole antifungal agents in *Candida albicans* strains displaying high-level fluconazole resistance isolated from human immunodeficiency virus-infected patients. *Antimicrob. Agents Chemother.* **2001**, *45*, 2676−2684.

(27) Cantón, E.; Pemán, J.; Gobernado, M.; Viudes, A.; Espinel-Ingroff, A. Patterns of amphotericin B killing kinetics against seven Candida species. *Antimicrob. Agents Chemother.* **2004**, *48*, 2477−2482.