Bioorganic & Medicinal Chemistry 26 (2018) 573–580

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Novel fluconazole derivatives with promising antifungal activity

Nishad Thamban Chandrika [a], Sanjib K. Shrestha [a], Huy X. Ngo, Kaitlind C. Howard, Sylvie Garneau-Tsodikova *

University of Kentucky, Department of Pharmaceutical Sciences, Lexington, KY 40536-0596, USA

ARTICLE INFO

Article history:
Received 30 September 2017
Revised 2 December 2017
Accepted 12 December 2017
Available online 17 December 2017

Keywords:
Azoles
Clinical isolates
Cytotoxicity
Hemolysis
Time-kill studies

ABSTRACT

The fungistatic nature and toxicity concern associated with the azole drugs currently on the market have resulted in an increased demand for new azole antifungal agents for which these problematic characteristics do not exist. The extensive use of azoles has resulted in fungal strains capable of resisting the action of these drugs. Herein, we report the synthesis and antifungal activity of novel fluconazole (FLC) analogues with alkyl-, aryl-, cycloalkyl-, and dialkyl-amino substituents. We evaluated their antifungal activity by MIC determination and time-kill assay as well as their safety profile by hemolytic activity against murine erythrocytes as well as cytotoxicity against mammalian cells. The best compounds from our study exhibited broad-spectrum activity against most of the fungal strains tested, with excellent MIC values against a number of clinical isolates. The most promising compounds were found to be less hemolytic than the least hemolytic FDA-approved azole antifungal agent voriconazole (VOR). Finally, we demonstrated that the synthetic alkyl-amino FLC analogues displayed chain-dependent fungal membrane disruption as well as inhibition of ergosterol biosynthesis as possible mechanisms of action.

1. Introduction

The rise in the number of infections caused by fungi poses a serious threat to human health and life.[1] Fungal infections can be endogenous (*e.g.*, *Candida* infections) or acquired from the environment (*e.g.*, *Cryptococcus* and *Aspergillus* infections). Invasive fungal infections have become a major problem for patients with immunodeficiency syndrome (*e.g.*, AIDS), organ transplant patients, and patients receiving chemotherapeutic agents for cancer treatment.[2–4] Clinically, candidiasis, aspergillosis, and cryptococcosis are the major infections in immunocompromised patients.[5,6] *Candida* and *Aspergillus* species are responsible for the majority of documented fungal infections. Recent studies indicated an epidemiological shift towards infections caused by emerging non-*albicans Candida* and *Aspergillus* species resistant to the current antifungal drugs.[7–9] Non-*albicans Candida* species such as *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei* are more prominent now and they account for more incidence of invasive candidiasis such as candidemia than *C. albicans*.[10–13] The relative presence of these fungal strains is region dependent. For example, *C. glabrata* is the second most common species after *C. albicans* in North America.[14] Fungal strains such as *C. parapsilosis* and *C. tropicalis* are relatively more common in Europe, Australia, Latin America, and Asia.[15–17] As resistance to the currently available antifungal agents is emerging in many of these non-*albicans Candida*, there is a need for developing novel antifungals.[18]

Most of the current drugs on the market are either highly toxic (*e.g.*, amphotericin B (AmB)) or becoming ineffective due to appearance of resistant fungal strains (*e.g.*, azoles such as fluconazole (FLC) and voriconazole (VOR)) (Fig. 1).[19] Azoles are the most frequently used class of antifungals to treat fungal infections as they are inexpensive and are available for oral administration.[20] However, there is an extensive documentation of intrinsic and developed resistance to azole drugs among *C. albicans* and non-*albicans Candida* species. As the frequency of occurrence of infections caused by non-*albicans Candida* species is increasing in clinical settings, there is currently a need to improve on the existing azole scaffolds to develop novel antifungals. Various studies were reported by our and other groups, which illustrated the role of alkylation on different drug scaffolds resulting in promising antifungal activity.[21–27] There are examples of 2,4-difluoro-2-(1*H*-1,2,4-triazo-1-yl)acetophenone analogues with linear C_5-C_8

Abbreviations: AmB, amphotericin B; ATCC, American Type Culture Collection; CAS, caspofungin; CLSI, Clinical and Laboratory Standards Institute; FBS, fetal bovine serum; FLC, fluconazole; KANB, kanamycin B; mRBCs, murine red blood cells; PI, propidium iodide; SAR, structure-activity relationship; TOB, tobramycin; VOR, voriconazole.

* Corresponding author.
E-mail address: sylviegtsodikova@uky.edu (S. Garneau-Tsodikova).
[a] These authors contributed equally to this work.

https://doi.org/10.1016/j.bmc.2017.12.018
0968-0896/

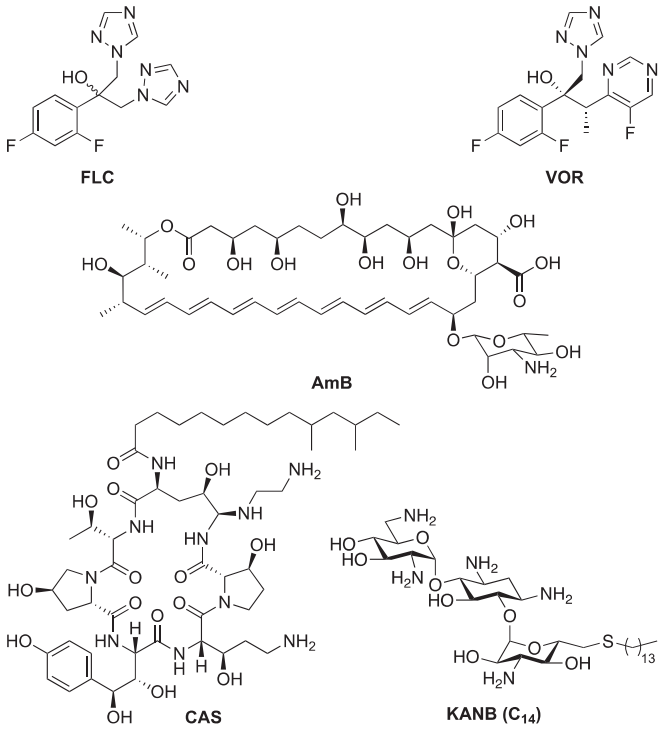

Fig. 1. Structures of all antifungal agents used as controls in this study.

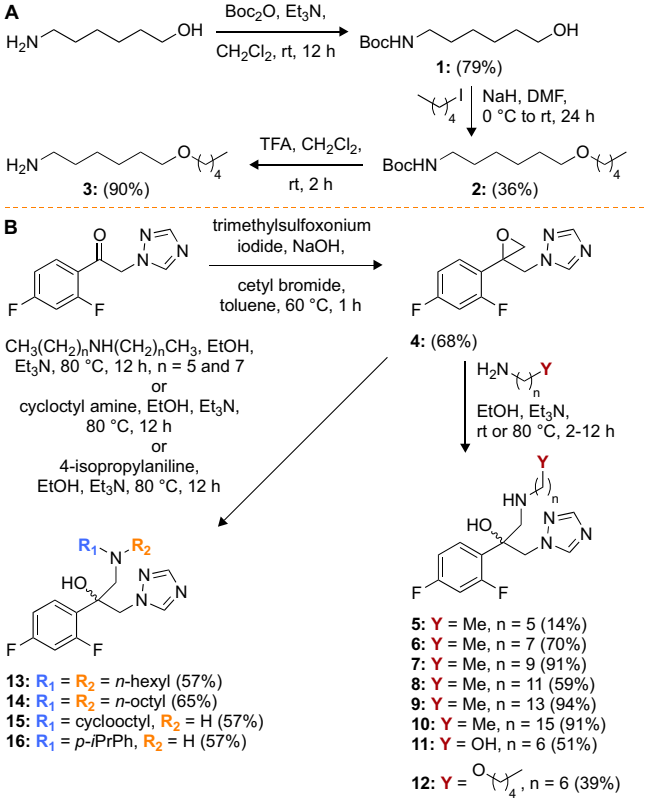

Fig. 2. Synthetic schemes for the preparation of A. amine derivative **3**, and B. novel azole analogues **5**–**16**.

alkyl chains[25] and of an *n*-alkylated ebsulfur derivative with a linear C_5 alkyl chain, which displayed strong antifungal activity.[24] Similarly, examples of aminoglycosides (*e.g.*, kanamycin B (KANB) and tobramycin (TOB)) with linear alkyl chains with 12 and 14 carbons (C_{12} and C_{14}) displaying antifungal activity were also reported.[21,22]

Based on the information provided above and the promise shown by these small molecules, herein, we decided to generate novel FLC derivatives in which the triazole ring on the carbon alpha to the dihalophenyl ring of FLC was displaced by various linear alkyl-, aryl-, dialkyl-, and cycloalkyl-amino substituents. We report the synthesis of twelve novel FLC derivatives (Fig. 2) and their antifungal activity against a variety of *C. albicans*, non-*albicans Candida*, *Aspergillus*, and *Cryptococcus* strains as established by in vitro MIC determination as well as by time-kill studies. We explore the hemolytic activity as well as cytotoxicity of these compounds against murine erythrocytes and mammalian cell lines, respectively. Finally, we investigate the potential mechanisms of action of selected compounds by probing their ability to disrupt fungal membrane or to inhibit ergosterol biosynthesis.

2. Results and discussion

2.1. Design and synthesis of antifungal agents *5*–*16*

We synthesized the alkyl-/aryl- and cycloalkyl-amino FLC derivatives **5**–**16** in two steps by using the commercially available fluorinated compound 2,4-difluoro-2-(1*H*-1,2,4-triazo-1-yl)acetophenone as a starting material (Fig. 2B). We first converted the carbonyl group of 2,4-difluoro-2-(1*H*-1,2,4-triazo-1-yl)acetophenone to an epoxide by using trimethylsulfoxonium iodide in the presence of a strong base and a surfactant to yield the oxirane intermediate **4**, which we then reacted with various amines (all commercially available, with the exception of amine **3** used in the synthesis of derivative **12**) under mild basic conditions to afford derivatives **5**–**16**. The amine **3** used for the synthesis of derivative **12** was prepared in three steps (Fig. 2A). The amino group of 6-aminohexanol was protected with Boc to yield compound **1**, which was then subjected to nucleophilic substitution reaction with 1-iodopentane. The deprotection of the Boc group of intermediate **2** yielded the desired amine **3**.

2.2. Antifungal activity and structure-activity relationship (SAR) analysis

We first evaluated the antifungal activity of the newly prepared FLC derivatives **5**–**16** against a panel of seven *C. albicans* (ATCC 10231 (**A**), ATCC 64124(R) (**B**), ATCC MYA-2876(S) (**C**), ATCC 90819(R) (**D**), ATCC MYA-2310(S) (**E**), ATCC MYA-1237(R) (**F**), and ATCC MYA-1003(R) (**G**)), three non-*albicans Candida* (*C. glabrata* ATCC 2001 (**H**), *C. krusei* ATCC 6258 (**I**), and *C. parapsilosis* ATCC 22019 (**J**)), and three *Aspergillus* (*A. flavus* ATCC MYA-3631 (**K**), *A. nidulans* ATCC 38163 (**L**), and *A. terreus* ATCC MYA-3633 (**M**)) strains using a concentration range of 0.03–31.3 μg/mL (Tables 1 and S1). We used commercially available antifungal agents such as AmB, caspofungin (CAS), FLC, and VOR as positive controls for comparison. For derivatives **5**–**16** as well as the reference drugs AmB and CAS, we reported MIC-0 values, which correspond to no visible growth. We reported MIC-2 values (*i.e.*, 50% growth inhibition) for FLC and VOR against all fungal strains tested with the exception of strain **A** by VOR. We defined antifungal activity as excellent (0.03–1.95 μg/mL), good (3.9 μg/mL), moderate (7.8–15.6 μg/mL), or poor (≥31.3 μg/mL) based on MIC values. In this manuscript, we performed all activity comparisons by using the MIC values reported in μg/mL (*Note*: the corresponding MIC values are also provided in μM in Tables S1 and S2).

By a survey of the data reported in Table 1, the following observations could rapidly be made. The introduction of a side-chain