Bioorganic & Medicinal Chemistry Letters 20 (2010) 4624–4626

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and evaluation of novel antifungal agents-quinoline and pyridine amide derivatives

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ARTICLE INFO

Article history:
Received 4 March 2010
Revised 28 May 2010
Accepted 1 June 2010
Available online 8 June 2010

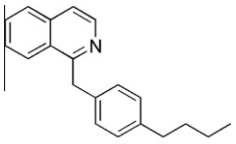
Keywords:
GPI-anchored protein
GWT1
Candia albicans
Aspergillus fumigatus

ABSTRACT

Quinoline amide, azaindole amide and pyridine amides were synthesized and tested for in vitro antifungal activity against fungi. These synthesized amides have potent antifungal activity against *Candida albicans* and *Aspergillus fumigatus*. Our results suggest that hetero ring amides may be potent antifungal agents that operate by inhibiting the function of Gwt1 protein in the GPI biosynthetic pathway.

The overall frequency of fungal infections has continued to increase in immunocompromised patients receiving immunosuppressive or anticancer therapy; the most common opportunistic pathogen causing these infections continues to be *Candida albicans*. The occurrence of infections due to non-*C. albicans* species is also increasing in frequency and invasive pulmonary aspergillosis is a leading cause of mortality in bone marrow transplant recipients. Amphotericin B has been the most common treatment for infections due to these species, but newer therapies including azoles and candins that are active against *Candida* and *Aspergillus* species have recently been reported. Seven of these newer treatments (fluconazole, itraconazole, voriconazole, posaconazole, caspofungin, micafungin, and anidulafungin) are already in clinical use. While these agents offer potential advantages, including reduced toxicity and a broad therapeutic index, their activities are still limited. Resistance to the azole-class of drugs is increasing and candins are not intrinsically active against *Cryptococcus* spp. as well as some clinically important filamentous fungi. Thus, the development of new drugs is still necessary in order to ensure successful clinical treatment of opportunistic fungal infections.[1–11]

Glycosylphosphatidylinositols (GPIs) are glycolipids that play a role in attaching cell surface proteins to eukaryotic plasma membranes, including those in fungi. Fungal pathogens are thought to utilize their cell wall glucan-anchored mannoproteins to initiate the binding to target tissues and begin the process of invasion. GPI-anchored proteins are part of a highly conserved pathway that exports, assembles, and anchors surface structures. In yeast and other fungi, GPI-anchored proteins play important roles in cell wall biosynthesis and maintenance of homeostasis. As GPI-anchored proteins are one of the major cell wall components of eukaryotic microorganisms, it may be possible to design drugs that target this biosynthetic pathway in fungi without causing adverse effects in human cells. Previously, we have reported that the GWT1 gene product is a target of the novel antifungal derivative, 1-[4-butylbenzyl-isoquinoline] (**1**, BIQ), which inhibits cell wall localization of GPI-anchored mannoproteins in *Saccharomyces cerevisiae* (Fig. 1). Moreover, we have shown that compound **1** has moderate antifungal activity against *C. albicans* (minimum inhibitory concentration (MIC): 1.56 μg/mL). However, BIQ was not active against *Aspergillus fumigatus*, one of the clinically important pathogenic



BIQ(1)

Figure 1. Antifungal 1-[butylbenzyl]isoquinoline.

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doi:10.1016/j.bmcl.2010.06.005

fungi, and metabolically unstable as a drug. In this study we used several techniques to attempt to synthesize compounds with greater antifungal activity than compound **1**.[12,13]

We first carried out chemical modifications to increase the antifungal activity of compound **1**; however, we found no benzylisoquinolines that possessed more potent antifungal activity. Next, we screened an in-house library of compounds in order to identify a lead compound that had antifungal activity. As a result, we successfully obtained an active nicotine amide derivative (MIC: 3.13 μg/mL: *C. albicans*) with a structure different from benzylisoquinoline. Finally, we synthesized quinoline amides and pyridine amides, and tested their antifungal activities against *C. albicans* and *A. fumigatus*.

In this study, we report new quinoline, azaindole, and pyridine amide derivatives (**8**, **9**, **10a–d** and **15**) that show potent antifungal activity against *C. albicans* and *A. fumigatus* that act by inhibiting the function of the Gwt1 protein in an early step of the GPI biosynthetic pathway.

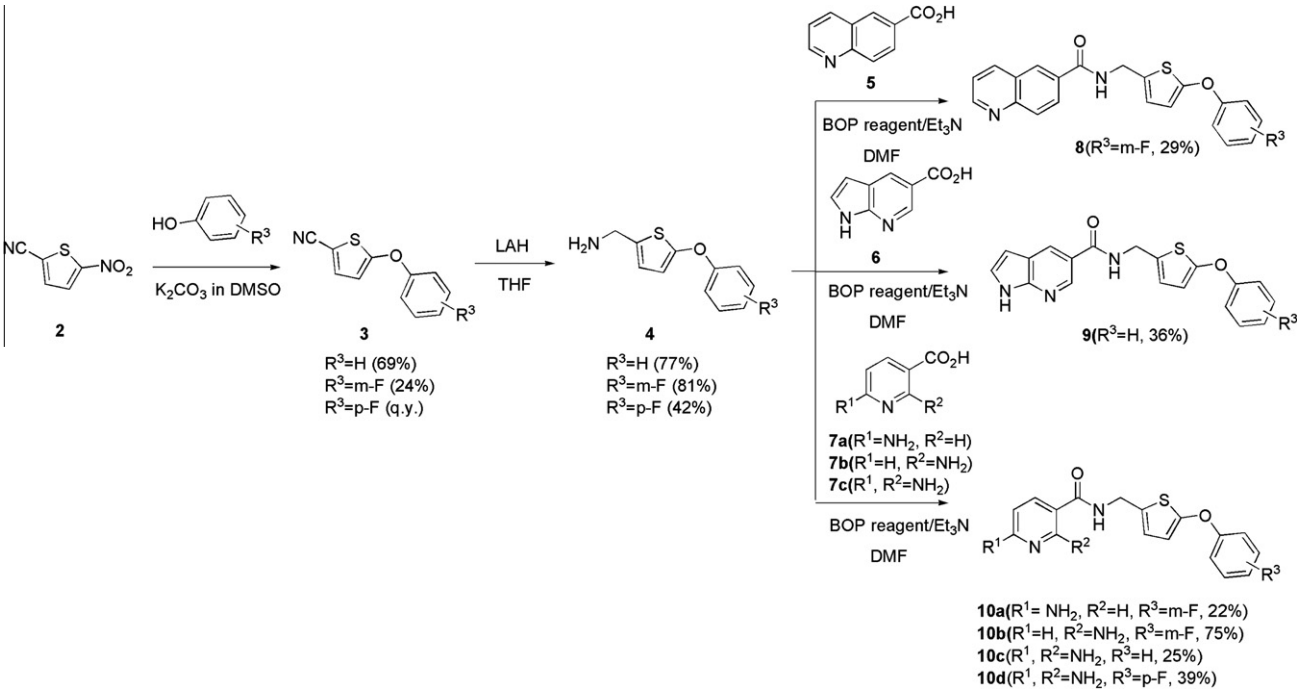
We synthesized target molecules **8**, **9**, **10a–d**, and **15** by using coupling reactions of amines **4** and **12** with carboxylic acids **5**, **6**, and **7a–c**. The synthesis of amine **4** began with 5-nitro-2-thiophenecarbonitrile (Scheme 1).[14] Subsequent treatment of compound **2** with substituted phenols afforded substituted phenoxythiophenes **3**. Reduction of the nitrile functionality of compound **3** with LAH in THF gave the alkylamine **4**. We then prepared the amidemethylene compounds **8**, **9**, and **10a–d** by using coupling reactions of amine **4** with carboxylic acids **5**, **6**, and **7a–c** by using BOP reagent as the coupling agent in DMF at room temperature.

Alternatively, we synthesized benzylthiophene **14** from 1-bromo-5-(1,-dioxolan-2-yl)thiophene (Scheme 2). First, a coupling reaction of bromothiophene **11** with benzylbromide in the presence of *n*-BuLi in THF at −78 °C resulted in compound **12**, and subsequent deprotection with citric acid provided the corresponding aldehyde **13**. Hydrogenation of aldehyde **13** over Raney Ni in aqueous NH_3 resulted in amine **14**. Finally, amide formation between **14** and **7b** resulted in amidemethylene compound **15**.

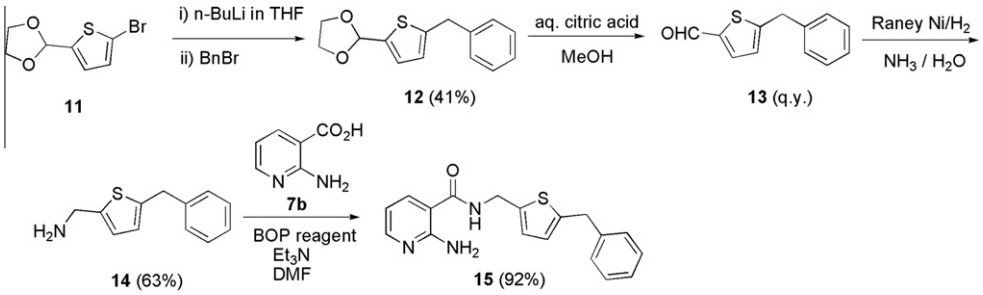
After completing the synthesis of quinoline amide **8**, azaindole amide **9**, and pyridine amides **10a–d** and **15**, we tested their inhibitory activity on the growth of key pathogenic fungi. The MIC values of these compounds were comparable to the most commonly used antifungal agents fluconazole and amphotericin B in a standard in vitro growth inhibition assay (Table 1).[15] The synthesized quinoline amide **8** and azaindole amide **9**, as well as compounds **10a–d** and **15**, showed potent antifungal activity against *C. albicans*. In addition, compounds **8**, **9**, **10b–d**, and **15** had more potent antifungal activity than compound **1**. The effectiveness of these compounds (MIC: 0.05–0.78 μg/mL) was 2–31-fold greater than that of amphotericin B, one of the most commonly prescribed antifungal treatments; compounds **8**, **10b**, and **15** also had more potent activity than fluconazole. Compounds **8**, **9**, **10b–d**, and **15** showed in vitro antifungal activity against *A. fumigatus*. The in vitro antifungal activity of compounds **10b**, **10d**, and **15** against *A. fumigatus* was the same as that of amphotericin B.

We tested the antifungal activities of most of these compounds against a GWT1-overexpressing strain of *S. cerevisiae* (KE744) (Table 2).[15] The MICs of these compounds for the KE744 strain were at least eightfold higher than those for a control strain (KE743). Thus, the overexpression of GWT1 conferred resistance to these compounds and was not associated with cross-resistance to amphotericin B or fluconazole. These results support our hypothesis that GWT1 is the target molecule for these compounds.

In conclusion, quinoline amide **8**, azaindole amide **9**, and pyridine amides **10a–d** and **15** were synthesized by coupling quinoline carboxylic acid, azaindole carboxylic acid, and amino nicotinic acid with benzylthiophene amine and phenoxythiophene amines. Among those tested, compounds **8**, **9**, **10b–d**, and **15** show potent antifungal activity against *C. albicans* and *A. fumigatus*. This is the first report on amides that target the biosynthetic pathway of GPI-anchored proteins. These amide derivatives may be promising leads for the development of new antifungal agents. Moreover, these results suggest that the synthesis of amide analogs could provide antifungal properties that might prove to be better than the currently approved therapies.



Scheme 1. Synthesis of phenoxythiophene amide derivatives.



Scheme 2. Synthesis of benzylthiophene amide derivative.

Table 1
Antifungal activity of **8**, **9**, **10a–d** and **15** against *C. albicans* and *A. fumigatus*

Compd	MIC (μg/ml)	
	C. albicans	*A. fumigatus*
8	0.1	1.56
9	0.39	3.13
10a	1.56	N.T.
10b	0.05	0.78
10c	0.78	1.56
10d	0.39	0.78
15	0.05	0.78
1	1.56	N.T.
Fluconazole	0.39	N.T.
Amphotericin B	1.56	0.78

Table 2
Antifungal activity of **8**, **9** and **10a–d** against GWT1-overexpressing strain of *S. cervisiae*

Compd	MIC (μg/ml)		Degree of resistance
	S. cerevisiae KE743	*S. cerevisiae* KE744	
8	0.025	3.13	128
9	0.2	>12.5	>64
10a	1.56	>12.5	>8
10b	0.1	12.5	128
10c	1.56	>12.5	>8
10d	0.78	12.5	16
Fluconazole	6.25	6.25	1
Amphotericin B	0.78	0.78	1

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15. In vitro susceptibility tests: *Candida albicans* E81022, *Aspergillus fumigatus* Tsukuba and *Saccharomyces cerevisiae* W303-1A were used in this study. *S. cerevisiae* W303-1A was transformed with empty vector (strain KE743) or multi-copy vector Yep352GAPII-GWT1, in which *S. cerevisiae* GWT1 was overexpressed in the presence of GAPDH promoter (strain KE744). The MICs were determined with the broth-microdilution method developed by the Clinical and Laboratory Standards Institute and reported in documents M27-A3 and M38-A2 with slight modification. The test compounds were dissolved in dimethyl sulfoxide (DMSO) to yield a concentration of 20 mg/mL and further serially twofold diluted with DMSO and test media to achieve a range of final concentrations from 200 to 0.05 μg/mL. The *C. albicans* strain was subcultured in Sabouraud dextrose broth medium (SDB; Becton, Dickinson and Company, Sparks, MD) at 35 °C for 1–2 days. The *A. fumigatus* strain was subcultured on potato dextrose agar (PDA; Eiken Chemical Co., Tokyo, Japan) at 35 °C for one week and the collected conidia was stored at −80 °C before testing. The strains of *S. cerevisiae* KE743 and *S. cerevisiae* KE744 were subcultured in yeast nitrogen base medium (SD ura-; Becton, Dickinson and Company) at 30 °C for 1–2 days. The wells were inoculated with 100 μL of the culture suspension diluted to a final inoculum of 2×10^3–2×10^4 cells or conidia per ml with SDB medium, RPMI 1640 medium (Nissui Pharmaceutical Co., Ltd, Tokyo, Japan) buffered to pH 7.0 with 0.165 M 3-[*N*-morpholino] propanesulfonic acid (MOPS; Wako Pure Chemical Industries, Ltd, Osaka, Japan) or SD ura- medium. The test organisms were cultured in medium containing test compounds (0.05–200 μg/mL) or 1% DMSO at 30 °C for *S. cerevisiae* or 35 °C for *C. albicans* and *A. fumigatus*. The MICs of test compounds were measured after two days of incubation, and were defined as the lowest concentrations at which a prominent decrease in turbidity could be determined visually relative to that in the control well.