Bioorganic & Medicinal Chemistry Letters 21 (2011) 1819–1822

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

The synthesis and structure–activity relationship of pyridazinones as glucan synthase inhibitors

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ARTICLE INFO

Article history:
Received 10 December 2010
Revised 11 January 2011
Accepted 13 January 2011
Available online 21 January 2011

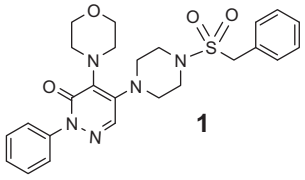
Keyword:
Glucan synthase inhibitor

ABSTRACT

A structure–activity relationship study of the lead 5-[4-(benzylsulfonyl)piperazin-1-yl]-4-morpholino-2-phenyl-pyridazin-3(2*H*)-one **1** has resulted in the identification of 2-(3,5-difluorophenyl)-4-(3-fluorocyclopentyloxy)-5-[4-(isopropylsulfonyl)piperazin-1-yl]-pyridazin-3(2*H*)-one **11c** as a β-1,3-glucan synthase inhibitor. Compound **11c** exhibited significant efficacy in an in vivo mouse model of *Candida glabrata* infection.

Fungal infections are a major risk associated with organ transplant, hematopoietic stem cell transplant, cancer chemotherapy, HIV infection, venous catheterization, or intensive care hospitalization. The standard of care for invasive fungal infections is either the azoles or amphotericin B, both of which disrupt the fungal cell membrane function. A relatively new class of antifungal agents are the echinocandins, such as caspofungin, which disrupt the fungal cell wall by inhibiting β-1,3-glucan synthase (GS). The potent candicidal activity of the echinocandins offers a treatment option for azole resistant *Candida* spp., and their safety allows the clinician to avoid the significant side effects of amphotericin B. However, since the echinocandins are lipopeptides available only by IV dosing, an oral GS inhibitor will be an important contribution to the arsenal of antifungal treatment.[1–5] The successful identification and development of caspofungin as a marketed antifungal agent is a prime example of using natural products as a source for the discovery of new drugs.[6,7]

High throughput screening of the legacy Schering–Plough compound collection identified compound **1** as an in vitro inhibitor of *Candida albicans* GS (strain BWP17) with an IC_{50} of 4.6 μg/mL.[8] Although compound **1** exhibited reasonable in vitro antifungal activity against the yeast *Candida glabrata* (strain C624), it was inactive against the yeast *C. albicans* (strain C693) and produced no measurable blood levels in the rat upon oral dosing.[9] Therefore, significant optimization was required.



1

Analogs were synthesized according to the route depicted in Scheme 1.[10] Condensation of a substituted hydrazine with mucochloric acid gave the core pyridazinone **3**. *N*-BOC piperazine added selectively to the 5-position. A number of different moieties were then introduced at the 4-position. Removal of the BOC protecting group and sulfonylation produced the target compounds **6**.

Initial SAR studies of the 4-position are summarized in Table 1. While replacement of the morpholine ring with a cyclohexyl ring in **7a** removed all antifungal activity, the cyclohexyloxy **7b** and cyclopentyloxy **7c** showed significantly improved antifungal activity. The 4-position also tolerated a long acyclic chain (analogs 7d–**j**) where the preferred linker was oxygen (**7f–g**) > sulfur (**7h**) > nitrogen (**7d**) or carbon (**7e**).

In contrast, the piperazine ring was not open to modification (Table 2). Introduction of a methyl group in **8a–c** reduced both GS and antifungal activity while the constrained diaza-bicyclo[2.2.1]heptane was completely inactive. Replacement of the piperazine ring with an acyclic chain was also not tolerated (data not shown).

The sulfonamide moiety is critical for antifungal activity. All attempts to replace the sulfonamide with a variety of groups

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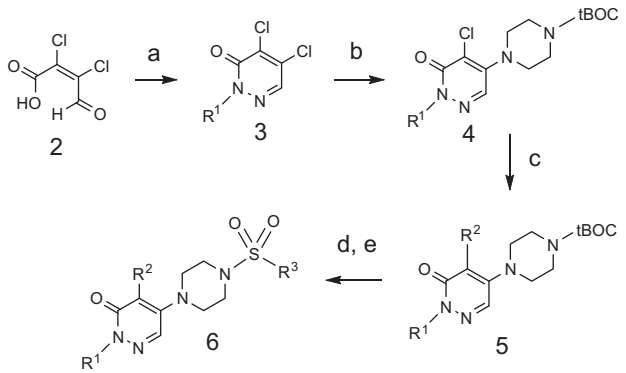
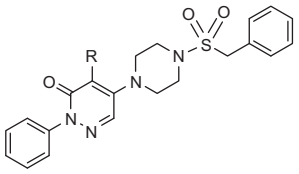
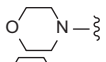
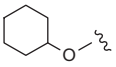
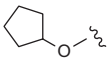
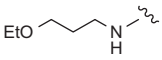
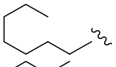
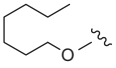
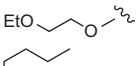
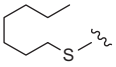
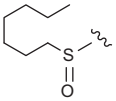
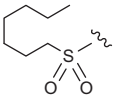
doi:10.1016/j.bmcl.2011.01.058



Scheme 1. Reagents and conditions: (a) R^1NHNH_2–HCl, NaOH, 100 °C; (b) *N*-BOC–piperazine, Et_3N, EtOH, Δ, 93%; (c) ROH, $NaN(TMS)_2$, THF or RSH, NaOMe, MeOH or amine, toluene, Δ or amine, K_2CO_3, $Pd(OAc)_2$, BINAP, toluene or $RB(OH)_2$, Na_2CO_3, $PdCl_2(PPh_3)_2$, CH_3CN, H_2O, Δ;(d) 4 N HCl in dioxane, CH_2Cl_2; (e) R^3SO_2Cl, Et_3N, CH_2Cl_2.

Table 1
Antifungal profile of 4-substituted pyridazinones **7a–j**



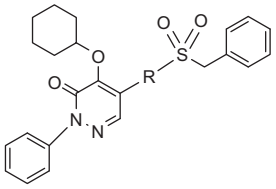
Compd	R	GS IC_{50} [a] (μg/mL)	*C. glabrata* MIC_{100} [b] (μg/mL)	*C. albicans* MIC_{100} [b] (μg/mL)
1		4.6	0.39	>50
7a		2.2	>20	>20
7b		0.53	0.05	0.78
7c		0.18	0.03	0.26
7d		2.3	0.78	>50
7e		1.5	0.78	>50
7f		0.12	<0.10	0.78
7g		0.90	0.10	3.1
7h		1.1	0.78	3.1[c]
7i		3.0	0.59	25
7j		>10	1.6	>50

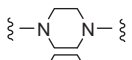
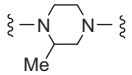
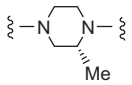
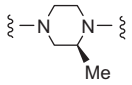
[a] GS activity (*C. albicans* membranes, strain BWP17) IC_{50} values are the average of at least two independent determinations.

[b] *C. galbrata* (strain C624) and *C. albicans* (strain C693) MIC_{100} values are the average of at least two independent determinations.

[c] In general, 100% inhibition of fungal growth is observed for MIC_{100} determination. Compound **7h** exhibits 50% inhibition of fungal growth resulting in a MIC_{50} for *C. albicans* (strain C693) as the average of at least two independent determinations.

Table 2
Antifungal profile of piperazine-substituted pyridazinones **8a–d**



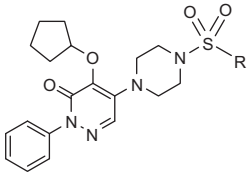
Compd	R	GS IC_{50} [a] (μg/mL)	*C. glabrata* MIC_{100} [b] (μg/mL)	*C. albicans* MIC_{100} [b] (μg/mL)
7b		0.53	0.05	0.78
8a		2.2	0.39	6.3
8b		2.8	0.78	19[c]
8c		4.3	0.39	2.0[c]
8d		>10	>50	>50

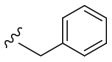
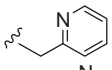
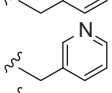
[a] GS activity (*C. albicans* membranes, strain BWP17) IC_{50} values are the average of at least two independent determinations.

[b] *C. galbrata* (strain C624) and *C. albicans* (strain C693) MIC_{100} values are the average of at least two independent determinations.

[c] In general, 100% inhibition of fungal growth is observed for MIC_{100} determination. Compounds **8b** and **8c** exhibit 50% inhibition of fungal growth resulting in a MIC_{50} for *C. albicans* (strain C693) as the average of at least two independent determinations.

Table 3
Antifungal profile of sulfonamide-substituted pyridazinones **9a–f**



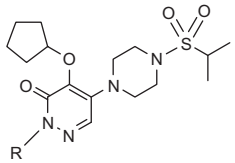
Compd	R	GS IC_{50} [a] (μg/mL)	*C. glabrata* MIC_{100} [b] (μg/mL)	*C. albicans* MIC_{100} [b] (μg/mL)
7c		0.18	0.03	0.26
9a		0.59	0.06	8.4
9b		0.20	0.13	8.4
9c		>10	0.15	0.78[c]
9d		1.3	0.63	5.0
9e	Me	2.2	0.63	>20
9f	*i*Pr	1.7	0.20	25[c]

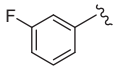
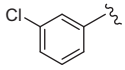
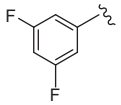
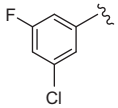
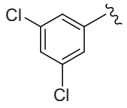
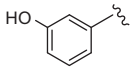
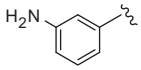
[a] GS activity (*C. albicans* membranes, strain BWP17) IC_{50} values are the average of at least two independent determinations.

[b] *C. galbrata* (strain C624) and *C. albicans* (strain C693) MIC_{100} values are the average of at least two independent determinations.

[c] In general, 100% inhibition of fungal growth is observed for MIC_{100} determination. Compounds **9c** and **9f** exhibit 50% inhibition of fungal growth resulting in a MIC_{50} for *C. albicans* (strain C693) as the average of at least two independent determinations.

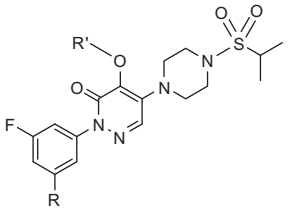
Table 4
Antifungal profile of 2-substituted pyridazinones **10a–g**



Compd	R	GS IC_{50} [a] (μg/mL)	*C. glabrata* MIC_{100} [b] (μg/mL)	*C. albicans* MIC_{100} [b] (μg/mL)
9f		1.7	0.20	25[c]
10a		8.8	0.78	>50
10b		6.1	1.6	>50
10c		>10	8.4	>20
10d		1.0	0.10	6.3
10e		0.52	0.10	3.1
10f		0.62	0.40	6.3
10g		0.25	0.06	6.3
10h		0.26	0.20	3.1
10i		0.90	2.5	>20
10j		1.9	25	>50

[a] GS activity (*C. albicans* membranes, strain BWP17) IC_{50} values are the average of at least two independent determinations.
[b] *C. galbrata* (strain C624) and *C. albicans* (strain C693) MIC_{100} values are the average of at least two independent determinations.
[c] In general, 100% inhibition of fungal growth is observed for MIC_{100} determination. Compound **9f** exhibits 50% inhibition of fungal growth resulting in a MIC_{50} for *C. albicans* (strain C693) as the average of at least two independent determinations.

Table 5
Antifungal profile of cyclopentyl-substituted pyridazinones **11a–d**



Compd	R	R'	GS IC_{50} [a] (μg/mL)	*C. glabrata* MIC_{100} [b] (μg/mL)	*C. albicans* MIC_{100} [c] (μg/mL)	*A. fumigatus* MEC[d] (μg/mL)	Rat $AUC_{(0-6\ h)}$ 10 mg/kg po μg h/mL (MC)
10b	H		1.0	0.10	6.3	1.6	0.67
11a	H		0.42	0.13	16.7	4.2	0.30
11b	H		1.1	0.78	6.3	3.1	0.47
11c	F		0.62	0.10	6.3	1.6	2.1
11d	F		0.60	0.05	12.5	3.1	0.44

[a] GS activity (*C. albicans* membranes, strain BWP17) IC_{50} values are the average of at least two independent determinations.
[b] *C. galbrata* (strain C624) MIC_{100} values are the average of at least two independent determinations.
[c] *C. albicans* (strain C693) MIC_{100} values are the average of at least two independent determinations.
[d] *A. fumigatus* (strain ND158) MEC values are the average of at least two independent determinations.

including amides, ureas, carbamates, and phosphoamides were not successful (data not shown). In general, the benzylsulfonamides such as **7c** exhibited the best antifungal activity, but zero blood levels in the rat (10 mg/kg po) due to both metabolism and absorption issues. Conversion of the benzyl sulfonamide to the pyridylmethyl (**9a–b**), phenyl (**9c**), or pyridyl (**9d**) all resulted in a decrease in antifungal activity (Table 3). Although the alkyl sulfonamides (**9e–f**) also exhibited reduced antifungal activity, the isopropyl sulfonamide **9f** was the first compound to show moderate rat $AUC_{(0-6\ h)}$ of 1.6 μg h/mL at 10 mg/kg po (methylcellulose, MC vehicle).

Since compound **9f** possessed a moderate PK profile, it was the basis for further SAR optimization of the 2-phenyl substituent (Table 4). Replacement of the phenyl ring with a cyclohexyl (**10a**) or heterocycle (pyridine **10b** or thiazole **10c**) decreased antifungal activity. Substitution on the phenyl ring was preferred at the *meta* position with one electron withdrawing halogen (**10d–e**) or with two halogens (**10f–h**) rather than a hydrogen bonding group (**10i–j**). The meta halogen substituted analogs all displayed equivalent rat $AUC_{(0-6\ h)}$ at 10 mg/kg po (MC vehicle): 0.67 μg h/mL for **10d**, 0.49 μg h/mL for **10e**, and 0.49 μg h/mL for **10g**.

Since the cyclopentyl ring was identified as a site for metabolism, the mono and disubstituted fluorocyclopentyl analogs 11a–**d** were synthesized (Table 5). The mono substituted fluoro compounds **11a** and **11c** are a mixture of enantiomers. Analogs **11a–d** also exhibited activity against the mold *Aspergillus fumigatus* (strain ND158), resulting in the morphologic effects on hyphal growth typical of GS inhibitors.[11] Compound **11c** possessed the best rat $AUC_{(0-6\ h)}$ of 2.1 μg h/mL at 10 mg/kg po (MC vehicle). In addition, compound **11c** exhibited MICs to other clinical isolates of *C. albicans* (strain C43) and *C. glabrata* (strain C454) of 12.5 μg/mL and 1.0 μg/mL, respectively, and showed no inhibition of the P450 liver enzymes 2D6, 3A4, and 2C9 at 30 μM; low activity in the herg voltage clamp assay with 22% inhibition at 1 μM; reasonable dog $AUC_{(0-8\ h)}$ of 3.5 μg h/mL at 2 mg/kg po (MC/HPBCD vehicle); and low hepatocyte clearance of 2.4 μL/min/M cells in rat, 1.7 μL/min/M cells in dog, and 1.0 μL/min/M cells in human.

As proof of concept, compound **11c** was tested in a mouse model of systemic *C. glabrata* infection. Immunocompromised mice were infected with *C. glabrata* strain C624 (10^7 colony forming units, CFU/mouse) and treated with compound **11c** at 10 and 30 mg/kg po QD for seven days. Following treatment, fungal kidney burdens were significantly (p <0.001) reduced at the 30 mg/kg dose ($10^{1.30}$ CFU/kidneys), but not at the 10 mg/kg dose ($10^{4.16}$ CFU/kidneys) relative to vehicle treated mice ($10^{4.28}$ CFU/kidneys). Analysis of plasma levels suggested that at 10 mg/kg, the concentration of

compound **11c** was not above the MIC for sufficient time (4 h) versus the 30 mg/kg dose (10 h).

In conclusion, a new series of GS inhibitors has been discovered. Optimization of the original lead compound **1** has led to the identification of compound **11c** which exhibits oral efficacy against *C. glabrata* in the mouse. Further SAR optimization of compound **11c** in terms of *C. albicans* activity and PK profile as well as additional details of the biological properties for this GS inhibitor series will be reported in due course.[12]

References and notes

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