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SAR studies of pyridazinone derivatives as novel glucan synthase inhibitors

Gang Zhou [a,*], Pauline C. Ting [a], Robert Aslanian [a], Jianhua Cao [a], David W. Kim [a], Rongze Kuang [a], Joe F. Lee [a], John Schwerdt [a], Heping Wu [a], R. Jason Herr [b], Andrew J. Zych [b], Jinhai Yang [b], Sang Lam [b], Samuel Wainhaus [c], Todd A. Black [d], Paul M. McNicholas [d], Yiming Xu [d], Scott S. Walker [d]

[a] Department of Chemical Research, Merck Research Laboratories, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA
[b] Albany Molecular Research Institute, Albany, NY 12203, USA
[c] Department of Drug Metabolism, Merck Research Laboratories, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA
[d] Department of Biological Research, Merck Research Laboratories, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA

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ABSTRACT

A novel series of pyridazinone analogs has been developed as potent β-1,3-glucan synthase inhibitors through structure–activity relationship study of the lead 5-[4-(benzylsulfonyl)piperazin-1-yl]-4-morpholino-2-phenyl-pyridazin-3(2*H*)-one (**1**). The effect of changes to the core structure is described in detail. Optimization of the sulfonamide moiety led to the identification of important compounds with much improved systematic exposure while retaining good antifungal activity against the fungal strains *Candida glabrata* and *Candida albicans*.

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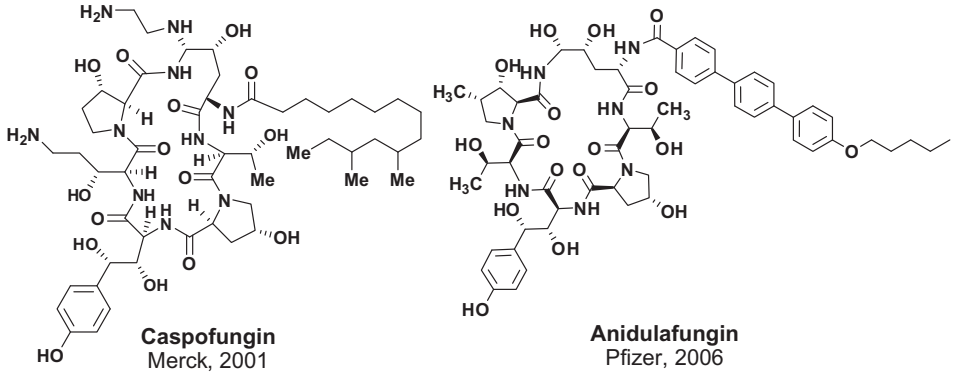
Over the past decades, several different series of antifungal agents, such as the azoles or polyenes, have been widely used for the treatment of invasive fungal infections caused by *Candida albicans* and *Aspergillus fumigatus*.[1] However, to overcome their off-target toxicity, fungistatic activity and emerging resistance, a new class of macrocyclic lipopeptidolactones[2] represented by caspofungin and anidulafungin have been developed as potent fungicidal agents. The lipopeptidolactones[2] exert their fungicidal activity through a totally different mechanism of action from the azoles and polyenes by disrupting the fungal cell wall polymer synthesis through inhibition of β-1,3-glucan synthase (GS). Consequently, they have attracted considerable attention. A major drawback of this new class of compounds, however, is that they are only deliverable via the intravenous (iv) route so an orally bioavailable small molecule inhibitor is desirable. Unfortunately, only a few small molecule GS inhibitors have been reported to date.[3] Herein, we describe the SAR optimization of a novel pyridazinone series of small molecule glucan synthase inhibitors.



Caspofungin
Merck, 2001

Anidulafungin
Pfizer, 2006

* Corresponding author. Tel.: +1 908 740 3724; fax: +1 908 740 7152.
E-mail address: gang.zhou@merck.com (G. Zhou).

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In the course of our program pursuing orally active antifungal compounds, we have previously reported the identification of compound **1** as a GS inhibitor with modest activity against the yeast strain *C. albicans* via high throughput screening of the legacy Schering–Plough compound collection.[4] Initial SAR studies towards improving the biological activity of this series have been described.[5] In this Letter, we would like to provide further information on our efforts to explore alternative core structures and to optimize both the antifungal and pharmacokinetic profiles.

Preliminary examples of modification of the original pyridazinone core are summarized in Table 1. From our previous report, introducing an ether or aryl chain at the C-4 position in place of the morpholine ring in **1** produces a 5- to 10-fold improvement in activity against *C. glabrata* and *C. albicans*. (compounds **2** and **8**). Further introduction of an alkyl or ether chain at the C-6 position (compounds **3** and **4**) reduces antifungal activity dramatically. While replacement of the pyridazinone core with a simple benzene or pyridazine ring (compounds **5** and **6**) removes all antifungal activity, the pyrimidin-4-one and pyridinone (compounds **7** and **9**) showed antifungal activity against fungal strain *C. glabrata*. A comparisons of several different core series demonstrated that the C-6 unsubstituted pyridazinone was the best core structure in terms of antifungal activity.

In our previous report on the SAR of pyridazinone series, a cyclopentyloxy or cyclohexyloxy group at C-4 shows relatively optimal activity in combination with a 3-F/Cl substituted phenyl group at the N-2 position.[5] Since the sulfonamide moiety on the right side of the structure was shown to be essential for antifungal activity after screening of a variety of different moieties, we next decided to optimize the sulfonamide tail. A large variety of sulfonamide groups have been introduced into the pyridazinone (**10**) and are summarized in Table 2. Simple and substituted alkyl sulfonamides (compounds **10a**–**d**) and aryl sulfonamides (compounds **10f**–**h**) displayed relatively low activity against *C. albicans* except for the thienyl compound (**10e**). The benzyl compound (**10i**) and benzothiazolmethyl compound (**10m**) exhibited the best antifungal activity. Activity against fungal strains *C. glabrata* and *C. albicans* was increased 20- to 50-fold in comparison to the morpholine compound (**1**). Extension of the benzyl sulfonamide to the phenethyl (**10k** and **l**), or phenylpropyl (**10n**) result in a decrease in antifungal activity.

The biggest challenge remaining for the sulfonamide series was the very low plasma exposure shown in the rat upon oral dosing mainly due to low solubility and high clearance.[5] Therefore, significant optimization was still necessary in order to obtain in vivo activity.

We selected the most active benzylsulfonamide (compound **10i**, AUC = 0 μg h/mL) for further optimization. Since cold metabolite ID has shown the benzylsulfonamide moiety is a major site for metabolism, our initial approach was to block potential metabolic sites of the benzylic position by F or Me, which did not improve the PK profile and resulted in a deleterious effect on antifungal activity (data not shown). Functional groups such as F, Me, CF_3, CN, and CO_2Et were also introduced into the benzene ring (compounds **11a**–**e**), but did not show any improvement over compound **10i** in rat pharmacokinetic studies (Table 3). An alternative approach is to improve the hydrophilicity of the benzylsulfonamide series by introducing polar group on the sulfonamide benzene ring since this type of substitution on other parts of the molecule is not tolerated. Substitution on the phenyl ring with hydrophilic groups such as a boronic acid in **11f**, or a nitro group in **11h** all displayed no improvement in rat blood levels. Introduction of a carboxylic acid group in **11g** gave some improvement in rat PK but lost antifungal activity. Replacement of the phenyl ring with the pyridine as in **11i** showed a small but measureable rat AUC (0–6 h) of 0.06 μg h/mL at 10 mg/kg po (MC vehicle) while retaining similar antifungal activity to the parent. Most interesting, a hydrogen bond donor amino group in the *ortho*, *meta*, or *para* position (compounds **11j**–**l**) exhibited a slight improvement in rat AUC (0–6 h) at 10 mg/kg po (MC vehicle): 0.29 μg h/mL for **11j**, 0.05 μg h/mL for **11k**, and 0.08 μg h/mL for **11l**. Only substitutions at the *ortho* and *para* position retain good antifungal activity. Furthermore, by replacing the cyclopentyloxy with the 1-ethyl-1-methylcyclopropanyloxy at the

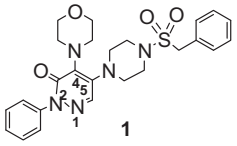

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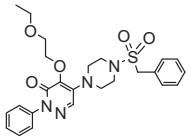
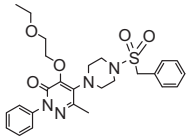
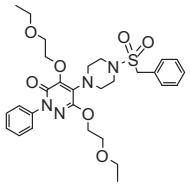
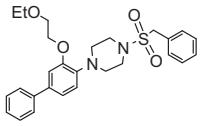
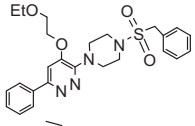
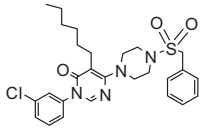
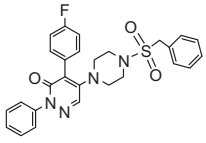
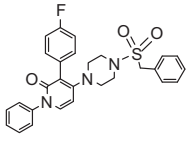
Table 1
SAR on core modification with pyridazinone

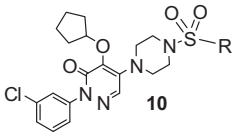
Compd	Structure	GS IC_{50}[a] (μg/mL)	*C. glabrata* MIC_{100}[b] (μg/mL)	*C. albicans* MIC_{100}[b] (μg/mL)
2		0.9	0.10	3.13
3		71	25	>50
4		4.2	6.25	>50
5		138	>50	> 50
6		37	>25	>25
7		1.31	9.00[c]	>25
8		NA	0.05	6.25
9		2.4	0.78	>50

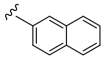
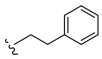
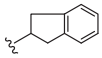
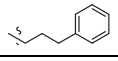
[a] GS activity (*C. albicans* membranes, strain BWP17) IC_{50} values are the average of at least two independent determinations.

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

[c] *C. albicans* (strain C693) MIC_{50} values are the average of at least two independent determinations.

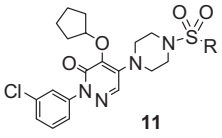
Table 2
SAR of sulfonamide substitution on pyridazinones

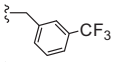
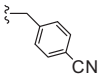
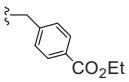
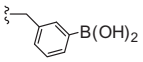
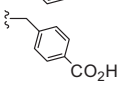
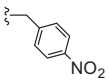
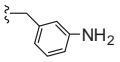
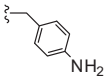
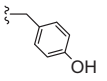
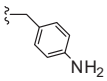


Compd	R	GS IC_{50}[a] (μg/mL)	*C. glabrata* MIC_{100}[b] (μg/mL)	*C. albicans* MIC_{100}[b] (μg/mL)
10a		0.41	0.10	0.78[c]
10b		0.62	0.10	3.13
10c		0.43	0.20	3.13
10d		109.3	>50.0	>50.0
10e		0.42	0.05	0.78
10f		NA	0.30	3.1
10g		0.074	1.04	33.4
10h		0.16	0.20	0.78[c]
10i		0.20	0.02	0.39
10j		0.22	0.02	2.10
10k		0.80	0.08	5.00
10l		0.29	0.10	1.56
10m		0.08	0.05	0.78
10n		23.0	3.13	>50.0

[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] *C. albicans* (strain C693) MIC_{50} values are the average of at least two independent determinations.

Table 3
SAR of substitution on pyridazinone benzylsulfonamide



Compd	R	*C. glabrata* MIC_{100}[b] (μg/mL)	*C. albicans* MIC_{100}[b] (μg/mL)	Rat $AUC_{(0-6\ h)}$ (μg h/mL) (MC)[c]
10i		0.02	0.39	0
11a		0.05	1.56	0
11b		0.08	0.31	0
11c		0.78	>50.0	NT
11d		0.02	0.13	0
11e		0.78	6.25	NT
11f		0.42	13.4	0
11g		25.0	>50.0	0.13
11h		0.05	0.78	0
11i		<0.02	0.20	0.06
11j		0.03	0.35	0.29
11k		0.39	3.13	0.05
11l		0.20	0.78	0.08
11m[a]		0.13	0.52	0.70
11n[a]		0.26	1.04	8.10

[a] 1-Ethyl-1-methylcyclopropanyloxy instead of cyclopentyloxy at the C-4 position.
[b] *C. galbrata* (strain C624) and *C. albicans* (strain C693) MIC_{100} values are the average of at least two independent determinations.
[c] Rat PK at 10 mg/kg po with methylcellulose vehicle.

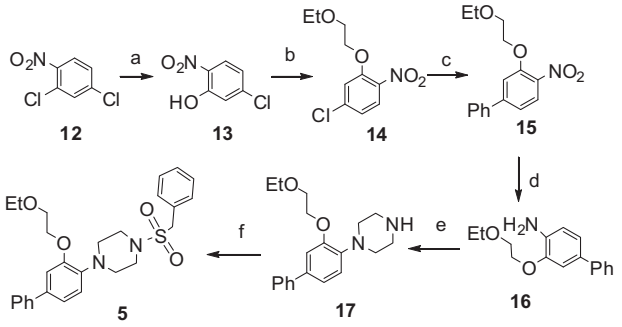
C-4 position, a dramatic increase in drug plasma exposure was observed with the *p*-NH_2 benzylsulfonamide (**11n**) while the *p*-OH compound (**11m**) showed little effect on the rat PK profile. Compound **11n** exhibited rat hepatocyte clearance of 6.6 μL/min/M cells, minimal hERG activity (12% inhibition at 5 μg/mL in the rubidium assay), and no CYP450 2D6 and 2C9 liver enzyme issues with IC_{50} >30 μM although CYP3A4 was inhibited with an IC_{50} = 0.3 μM.

A common synthetic route to the synthesis of pyridazinone analogs **10a**–**n** and **11a**–**n** has been depicted previously.[5]

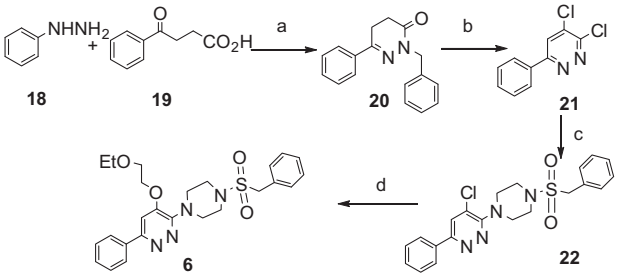
Benzene sulfonamides **5** were prepared using the approach described in Scheme 1 via a six step sequence starting from dichloronitrobenzene **12** (Scheme 1).[6] The chlorine *ortho* to the nitro group in **12** was first transformed to phenol **13** then the ether chain was installed at the C3 position via the Mitsunobu alkylation to give **14**. Stille coupling of **14** provided the necessary intermediate **15**. A further three-step manipulation converted the nitro compound **15** into the target **5**.

The construction of pyridazine **6** followed the synthetic route depicted in Scheme 2.[7] Condensation of a substituted hydrazine **18** with keto acid **19** gave the core pyrimidine **20**. Treatment of **20** with $POCl_3$ gave the dichloride **21**. Subsequent displacement of the two chlorines first with *N*-benzylsulfonyl and alcohol respectively at the 5- and 6-position generated the desired pyridazine **6**.

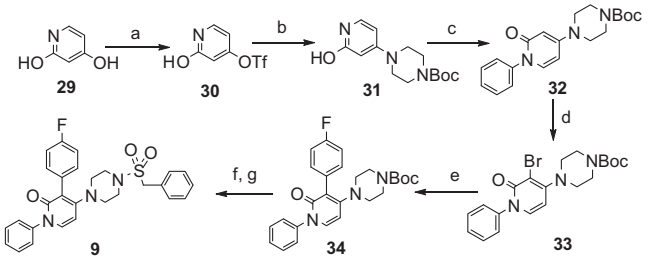
The conversion of thiourea **23** to the final primidin-4-one **7** was straightforward using a general synthetic process depicted in Scheme 3. Thiourea **23** was first condensed with malonic methyl ester **24** to generate **25**. After conversion of **25** to the triflate **26**,



Scheme 1. Reagents and conditions: (a) *t*BuOH, KOH, Δ, 10 h; (b) DEAD, Ph_3P, $EtOCH_2CH_2OH$, THF, rt, 12 h; (c) Bu_3SnPh, $Pd(Ph_3P)_2Cl_2$, dioxane, 80 °C, 18 h; (d) Fe, 2 N HCl in water, ethanol, 3.5 h; (e) $(ClCH_2CH_2)_2NH$, K_2CO_3, diglyme, 180 °C, 72 h; (f) RSO_2Cl, Et_3N, CH_2Cl_2, rt, 10 h.

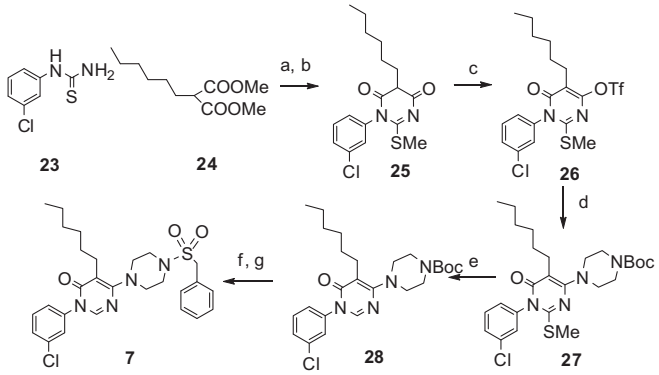


Scheme 2. Reagents and conditions: (a) NaOAc, EtOH, reflux; (b) $POCl_3$, PCl_5; (c) *N*-benzylsulfonylpiperazine, DIPEA, DMSO, MeOH, Δ; (d) $EtOCH_2CH_2OH$, NaH, THF, Δ.



Scheme 4. Reagents and conditions: (a) CF_3SO_3NPh, NaH, DMF, 5 h; (b) *N*-Boc-piperazine, DMF, 80 °C, 6 h; (c) $PhB(OH)_2$, $Cu(OAc)_2$, Et_3N, DCM, rt, 72 h; (d) NBS, AcOH, rt, 3 h; (e) Bu_3SnPhF, $Pd(PPh_3)_4$, LiCl, THF, reflux, 42 h; (f) 4 N HCl in dioxane, CH_2Cl_2; (g) RSO_2Cl, Et_3N, CH_2Cl_2, rt, 10 h.

Boc-piperazine was used to displace the triflate to produce **27**. Further reduction of **27** gave intermediate **28**. Removal of the Boc protecting group and sulfonylation produced the target compound **7**.



Scheme 3. Reagents and conditions: (a) NaOMe, MeOH, reflux; (b) MeI, MeOH, 50 °C; (c) Tf_2O, 2,6-lutidine, DCM, −78 °C; (d) *N*-Boc-piperazine, DIPEA, chlorobenzene, μw, 140 °C; (e) $NaBH_4$, $NiCl_2$, MeOH, THF, 0 °C, 1 h; (f) 4 N HCl in dioxane, CH_2Cl_2; (g) RSO_2Cl, Et_3N, CH_2Cl_2, rt, 10 h.

Following the seven step synthetic sequence as shown below, pyridinone analog **9** has been synthesized using 2,4-dihydroxypyridine **29** as starting material (Scheme 4).

In summary, we have identified a new series of GS inhibitors with good in vitro antifungal activity. The effect of core structure modifications on antifungal activity has been studied in detail. Further optimization of the sulfonamide moiety led to the identification of key aniline compounds with much improved systematic exposure while retaining good antifungal activity. Further in vivo study and SAR optimization of compound **11n** to improve both pharmacokinetic and biological activity will be discussed in the near future.

References and notes

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