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MK-5204: an orally active β-1,3-glucan synthesis inhibitor

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ABSTRACT

Our previously reported efforts to produce an orally active β-1,3-glucan synthesis inhibitor through the semi-synthetic modification of enfumafungin focused on replacing the C2 acetoxy moiety with an aminotetrazole and the C3 glycoside with a N,N-dimethylaminoether moiety. This work details further optimization of the C2 heterocyclic substituent, which identified 3-carboxamide-1,2,4-triazole as a replacement for the aminotetrazole with comparable antifungal activity. Alkylation of either the carboxamidetriazole at C2 or the aminoether at C3 failed to significantly improve oral efficacy. However, replacement of the isopropyl alpha amino substituent with a t-butyl, improved oral exposure while maintaining antifungal activity. These two structural modifications produced MK-5204, which demonstrated broad spectrum activity against Candida species and robust oral efficacy in a murine model of disseminated Candidiasis without the N-dealkylation liability observed for the previous lead.

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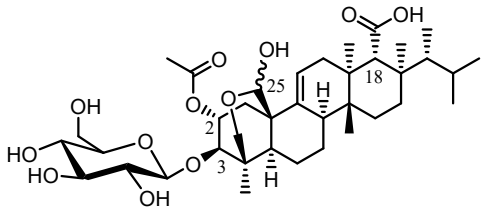
common serious fungal infections, continue to produce high mortality rates despite the development of new treatment options (azoles, echinocandins) over the past few decades.[1-6] Once confined mainly to immunocompromised patients, the incidence of fungal infections in intensive care units has increased substantially with Candida species becoming one of the leading causes of nosocomial bloodstream infections.[1-3] Only three main classes of antifungal agents (polyenes, azoles, and echinocandins) are available to treat invasive fungal infections. Even though the rate of antifungal resistant infection is low, increasing reports of azole and echinocandin resistant Candida and Aspergillus isolates are of particular concern as affected patients have limited remaining treatment options and typically result in poor clinical outcomes.[5-10] Consideration of these factors clearly indicates the need for the development of novel antifungal therapies that demonstrate benefit over existing options.

Amphotericin B (polyene), which disrupts fungal cell membranes by binding to ergosterol, was the gold standard in antifungal therapy for many years because of its broad spectrum of activity.[3,5,11] However, therapy with Amphotericin B is often limited due to nephrotoxicity, resulting from the binding of mammalian sterols.[3,5,11] The development of azole antifungals



enfumafungin **(1)**

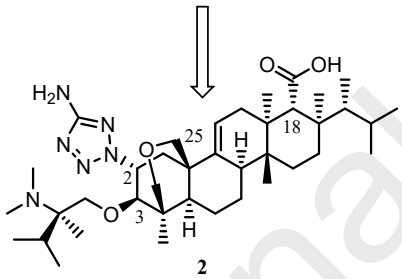


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Figure 1. C2 aminotetrazole, C3 aminoether lead

patients with azole resistant infections or needing step-down therapy from a parenteral antifungal.

Synthetic derivatization of enfumafungin[15] **(1)**, a naturally occurring GS inhibitor,[16-17] previously reported by our group culminated in the discovery of **2**, which had an aminotetrazole at C2 in place of the acetoxy group, an aminoether at C3 in place of the glycoside, and a bridging ether at C25 in place of the hemiacetal (Figure 1).[18] **2** exhibited robust oral efficacy in a 7 day murine model of disseminated Candidiasis with an ED_{99} of 9 mpk (estimated dose required to reduce fungal burden by 99%).[18] Herein we present additional optimization of the C2 and C3 substituents aimed at improving oral efficacy and mitigating the

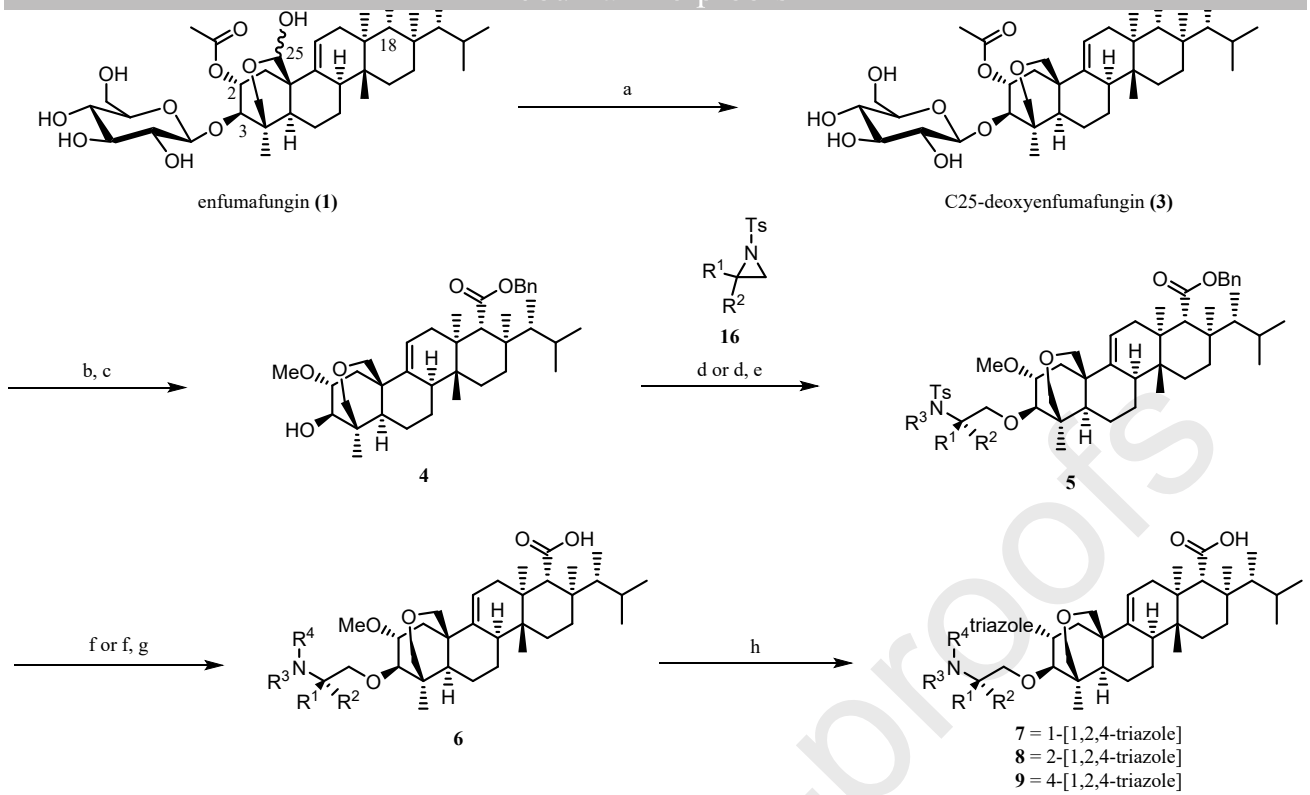
the function of the fungal cell membrane through inhibiting a fungal P450 enzyme involved in ergosterol biosynthesis, provided a safer treatment option, but their use is complicated by extensive drug-drug interactions that result from inhibition of host P450 enzymes.[3,5,11]

Conversely, the most recently developed class of antifungals, echinocandins (caspofungin, micafungin, anidulafungin), target the fungal cell wall instead of the cell membrane through the inhibition of β-1,3-glucan synthase (GS), which is responsible for the production of a key cell wall component (β-1,3 glucan). The structurally compromised cell wall leaves the fungal cell vulnerable to lysis under osmotic stress.[5,12] The echinocandins exhibit fewer mechanism based toxic side effects than the polyenes and fewer drug-drug interactions than the azoles because of their target specificity to fungal cells.[3,5,11] The improved safety profile and broad spectrum of activity has led to the adoption of echinocandins as the first line therapy of choice for invasive Candida infections in recent years.[1,3-4,10,13] The echinocandins are restricted to parenteral administration as a result of poor oral bioavailability.[11-14] An orally active GS inhibitor, retaining the safety profile of the echinocandins, would be an important addition to antifungal therapy options, particularly for

N-dealkylation observed for **2**.[18] Our original screen of C2 heterocyclic substituents with the naturally occurring glycoside at C3 indicated that 1,2,4-triazole warranted further optimization as its antifungal activity was within 2-fold of the tetrazole analog.[18] The effect of C2 substitution with 1,2,4-triazoles in combination with the optimal C3 aminoether from the tetrazole series was probed by preparing analogs as described in Scheme 1.

Reduction of the bridging hemiacetal of enfumafungin **(1)** with triethylsilane afforded C25-deoxyenfumafungin **(3)** in good yield.[18-21] Treatment of **3** with sulfuric acid in methanol hydrolyzed the C3 glycoside to the alcohol and displaced the C2 acetoxy group to give the methyl ether.[18] **4** was attained in good yield through subsequent protection of the C18 carboxylic acid as a benzyl ester. **5** was prepared *via* alkylation of the C3 alcohol of **4** with tosyl protected aziridine **16**. Deprotection of the tosyl protected aminoether at C3 and the benzyl ester at C18 was achieved through a dissolving metal reduction to give **6**. Borontrifluoride diethyletherate mediated displacement of the C2 methoxy with substituted 1,2,4-triazoles proceeded with retention of stereochemistry at C2 as previously described.[18] The distribution of regioisomeric products from the displacement reaction was substrate specific with the 1-[1,2,4-triazole] **7** typically being the major product, though the distribution was slightly tilted in favor of the 2-[1,2,4-triazole] **8** in some cases. In general, the 4-[1,2,4-triazole] **9** was only obtained as a minor product out of the displacement reaction. The regioiosomers were readily separable by reverse phase HPLC. Mono-methyl alkylation of the aminoether was achieved by treating the tosyl protected amine of **5** with iodomethane prior to global deprotection and C2 displacement. Dimethyl, ethyl, and propyl alkylations of the aminoether were installed *via* reductive amination of **6** followed by C2 displacement.[20-21]

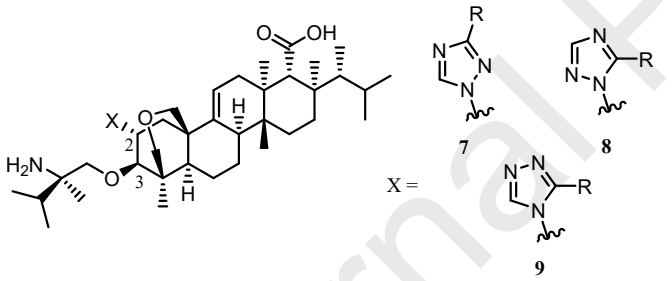
A previously reported method was employed to measure



Reagents and conditions: (a) Et_3SiH, TFA, DCM, rt; (b) H_2SO_4, MeOH, 65 °C; (c) BnBr, $NaHCO_3$, DMF, 65 °C, 76% (3 steps); (d) 1) K t-pentylate, 18-crown-6, DMA, 0 °C, or 2) KH, 18-crown-6, DME, 0 °C; (e) NaH, MeI, DMF, 50 °C; (f) Na, NH_3, THF, -35 °C; (g) RCHO, $NaBH_3CN$, AcOH, MeOH, rt; (h) triazole, $BF_3 \cdot OEt_2$, DCE, 50 °C.

Scheme 1. Synthesis of C2 triazole, C3 aminoether derivatives.

Table 1
C2 Triazole Regioisomer Derivatives

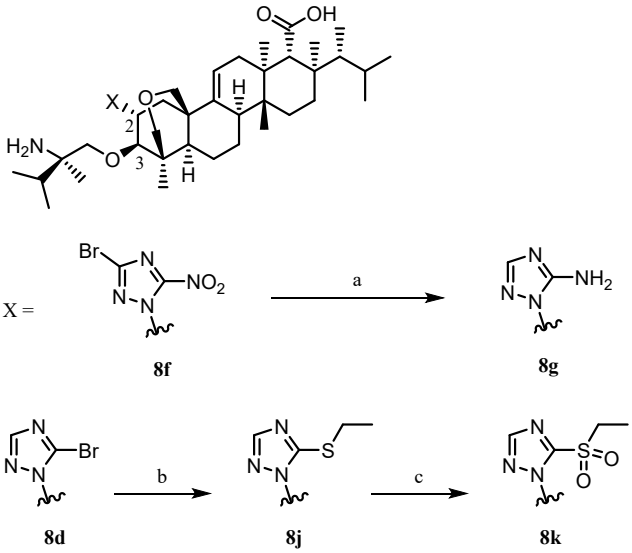


Compound	R	All concentrations in µg/mL		
		IC_{50} GS	MIC[a] + (ser) C. *albicans*	MEC[b] A. *fumigatus*
7a	Cl	0.019	8 (>32)	0.25
7b	CN	0.334	8 (>32)	4
8a	Cl	0.003	0.06 (8)	<0.03
8b	CN	0.006	0.125 (8)	<0.03
9a	Cl	>1	32 (>32)	16
9b	CN	>1	16 (>32)	8

[a]Minimum inhibitory concentration
[b]Minimum effective concentration

the inhibition of GS (IC_{50}, Table 1) by synthesized analogs in *Candida albicans* (MY1055) microsomal membrane fragements.[16] The CLSI protocols in RPMI medium reported by Onishi et al were used to determine the in vitro antifungal susceptibilities for clinically relevant fungal species (minimum inhibitory concentration for *Candida albicans* (MY1055) and minimum effective concentration for *Aspergillus fumigatus* (MF5668, Table1)).[16] Analogs of interest were further profiled by evaluating mouse pharmacokinetics (PK) and oral antifungal efficacy in the target organ kidney assay (TOKA), a murine model of disseminated Candidiasis utilizing C. *albicans* (MY1055) as the pathogen.[22]

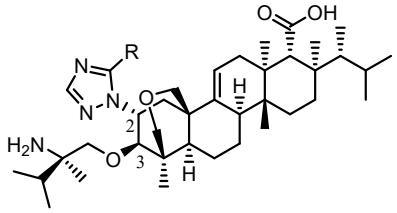
Table 1 displays the comparison of antifungal activity for the 1-[1,2,4-triazole] **7**, 2-[1,2,4-triazole] **8**, and 4-[1,2,4-triazole] **9** isomers with chloro and cyano substitution that were prepared according to the route shown in Scheme 1. These substituents were chosen for comparison because the displacement reaction produced sufficient quantities of all three regioisomers to allow for



Reagents and conditions: (a) Pd-C, H_2, EtOH, rt; (b) NaSEt, DMF, 50 °C; (c) mCPBA, DCE, rt.

Scheme 2. Post displacement triazole modification.

Tabl
C2 2-[1,2,4-triazole] Derivatives



		All concentrations in µg/mL			Mouse PK and PO Efficacy		
Compound	**R**	**IC_{50} GS**	**MIC + (ser) C. *albicans***	**MEC A. *fumigatus***	**$nAUC_{oral}$ µM·hr·kg/mg**	**F_{oral} (%)**	**TOKA Δlog CFUs[a] (dose mpk)**
8c	H	0.007	0.25 (4)	<0.03	-	-	-
8a	Cl	0.003	0.06 (8)	<0.03	0.03	9	-1.9 (25) -2.2 (12.5) -1.2 (6.25)
8d	Br	0.002	0.5 (8)	<0.03	0.17	33	-2.3 (25) -1.7 (12.5) -0.2 (6.25)
8e	I	0.005	0.5 (16)	<0.03	-	-	-
8g	NH_2	0.010	0.25 (16)	0.125	-	-	-
8b	CN	0.006	0.125 (8)	<0.03	-	-	-1.1 (25) 0 (12.5) -0.4 (6.25)
8h	$CONH_2$	0.005	<0.03 (2)	<0.03	0.11	7	-2.5 (25) -0.2 (12.5) -0.9 (6.25)
8i	COOMe	0.004	1 (32)	0.125	-	-	-
8j	SCH_2CH_3	0.009	0.5 (32)	<0.03	-	-	-
8k	$SO_2CH_2CH_3$	0.005	1 (8)	<0.003	-	-	-
8l	SO_2NH_2	0.006	0.25 (8)	<0.003	-	-	-0.7 (25) -0.8 (12.5) -0.8 (6.25)

[a]Change in colony forming units/g kidney relative to sham treated control animals.

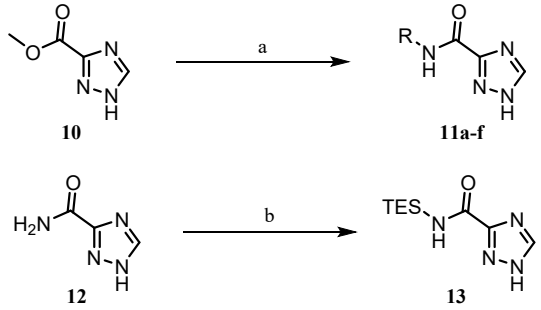
their isolation and characterization. For both the chloro and cyano substituents, the 2-[1,2,4-triazole] **8a-b** clearly exhibited superior GS inhibition and antifungal activity compared to the 1-[1,2,4-triazole] **7a-b**. This result is in contrast to the trend observed previously in the tetrazole series where distal was favored over proximal substitution.[18] Surprisingly, the regioisomeric 4-[1,2,4-triazole] **9**, almost completely lost both GS and antifungal activity.

Having identified the most active triazole regioisomer, the SAR of the 2-[1,2,4-triazole] series was investigated further. Using triazoles commercially sourced or prepared by literature procedures (**8d** and **8l**),[23-24] additional analogs with electron withdrawing and/or polar substituents were prepared according to Scheme 1. Scheme 2 illustrates the preparation of analogs that were prepared *via* modification of the triazole following the displacement reaction. The amino substituted analog **8g** was prepared *via* hydrogenation of the 3-nitro-5-bromo product **8f** obtained from the displacement reaction.[21] This additional step was necessary because the displacement reaction with 3-amino-1,2,4-triazole failed to produce enough of the 2-[1,2,4-triazole] isomer to isolate. The ethyl sulfide analog **8j** was obtained *via* displacement of the bromo substituent of **8d** with sodium ethanethiolate.[21] Subsequent treatment of **8j** with mCPBA produced the ethyl sulfone derivative **8k**.[21]

All of the 2-[1,2,4-triazole] analogs prepared in this series demonstrated potent GS inhibition (Table 2), but differences were observed in the whole cell antifungal activity. The chloro analog **8a** was 4 fold more active against C. *albicans* compared to the unsubstituted triazole **8c**. The modest efficacy of **8a** in the TOKA at 25 and 12.5 mpk is likely a result of the counter balance of excellent antifungal activity versus poor oral exposure. The larger halogen analogs **8d** and **8e** were 8 fold less active against C. *albicans* compared to **8a**, leaving them slightly less active than the unsubstituted triazole **8c**. However, the oral exposure of the bromo analog **8d** was 6 times higher than **8a**, which compensated for the loss in whole cell antifungal activity to give **8d** similarly modest oral efficacy at 25 and 12.5 mpk. The amino analog **8g** was equipotent relative to the unsubstituted triazole **8c** against C. *alblicans*, but was 4 fold serum shifted and substantially less potent against A. *fumagatus*. The cyano analog **8b** possessed similar antifungal activity to the unsubstituted triazole **8c**, but failed to show efficacy in the TOKA. The carboxamide analog **8h** exhibited superior antifungal potency, particularly in the presence of mouse serum, compared to the other 2-[1,2,4-triazole] analogs. **8h** was the only triazole analog to rival the antifungal potency of the previous C2 aminotetrazole series.[18] **8h** demonstrated reasonable efficacy at 25 mpk in the TOKA, but low oral exposure led to a precipitous drop in efficacy at 12.5 and 6.25 mpk. In contrast, the ester analog **8i** significantly lost antifungal activity relative to **8h** and **8c**. The ethyl sulfide analog **8j** showed similar antifungal potency to **8c**, but was more highly serum shifted. The ethyl sulfone analog **8k** displayed similar antifungal potency to **8j**, but was 4 fold more potent in the presence of serum. Moving from **8k** to the sulfonamide analog **8l** restored the antifungal activity 4 fold, while maintaining activity in the presence of serum. **8l** showed similar antifungal activity compared to **8c**, but failed to produce oral efficacy in the TOKA.

Given the superior antifungal activity of **8h**, particularly in the presence of serum, the carboxamide triazole substitution was chosen over the halogen triazole substitutions for further exploration. Holding the carboxamide triazole constant at C2, several modifications were evaluated with the aim of improving the oral exposure of **8h** while maintaining potent antifungal activity. First, a series of alkylated carboxamides (Table 3) were prepared according to the route described in Scheme 1. The triazoles **(11a-f)** used to prepare **8m-r** were readily obtained by neat treatment of methyl 1H-1,2,4-triazole-3-carboxylate **(10)** with the appropriate alkyl amine as shown in Scheme 3.[20-21] Potent GS inhibition was maintained across the series; however, increasing

the l…final cell antifungal activity both in the presence and absence of serum. Smaller alkyl substituents **(8m, 8o)** produced similar oral exposure compared to the unalkylated carboxamide **(8h)**, while more lipophilic substituents **(8n, 8p-r)** resulted in a 3-4X improvement in oral exposure. The efficacy of the alkylated analogs **(8m-r)** at 25 mpk in TOKA was similar to the unalkylated carboxamide **(8h)**. The alkylated analogs **(8m-q)** had improved efficacy at 12.5 mpk relative to **8h**, but failed to reach the 2 log reduction in fungal burden considered to be the threshold for robust activity. The alkylated analogs **(8m-r)** failed to significantly improve oral efficacy in TOKA over the unsubstituted carboxamide **(8h)** because the gains in oral exposure were negated by the concurrent loss of antifungal activity.



Reagents and conditions: (a) NH_2R, rt; (b) 2,6-lutidine, triethylsilyl trifluoromethanesulfonate, DCE, 50 °C, 68%.

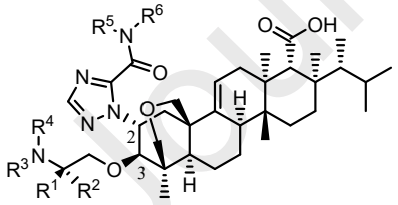
Scheme 3. Synthesis of carboxamide triazoles.

Next, a series of C3 N-alkylated (*R*)-isopropyl, methyl aminoethers were prepared with 3-carboxamide-1,2,4-triazole at C2 (Table 3). In the original synthesis of **8h**, the displacement reaction with 3-carboxamide-1,2,4-triazole only produced a 4% yield of the desired isomer. Later, it was discovered that using TES protected 3-carboxamide-1,2,4-triazole **(13)**, which was prepared by treating 3-carboxamide-1,2,4-triazole **(12)** with triethylsilyl triflormethanesulfonate (Scheme 3), improved the yield of the displacement reaction for the desired isomer to 13-51% depending on the C3 aminoether substituent.[20-21] The TES protecting group was hydrolyzed under the acidic reaction conditions to give the deprotected carboxamide directly from the displacement reaction.[20-21] The N-alkylated aminoether analogs C2 displacement step. Potent GS inhibition was preserved across the series, but the whole cell antifungal activity both with and without the presence of serum rapidly diminished as the lipophilicity of the alkyl substituent increased. The same trend was observed in the previous aminotetrazole series, but to a less severe extent.[18] The addition of each carbon to the alkyl substituent resulted in about a 3X improvement in oral exposure. N,N-dimethyl alkylation of amine **8t** increased the oral exposure 13X, compared to unalkylated amine **8h**. Unfortunately, these significant increases in oral exposure were accompanied by a concurrent loss in antifungal activity. As a result, unalkylated amine **8h** exhibited better TOKA activity than any of the alkylated analogs **(8s-v)**. Interestingly, alkylating the aminoether was more detrimental to TOKA efficacy than alkylating the carboxamide even though it produced higher oral exposure coupled with similar antifungal activity.

Finally, the alkyl substitution alpha to the amine was examined (Table 3). These analogs were prepared according to Scheme 1 using the appropriate aziridine **(16a-f)** during the alkylation step and employing **13** in the displacement reaction. The aziridines **(16)** used to prepare **8h, 8w-8aa** were synthesized by either treating olefins **14** with chloroamine-T or by cyclizing aminoalcohols **15** as shown in Scheme 4.[20-21] The aziridines used to prepare the isopropyl, methyl **(8h)** and t-butyl, methyl **(8x)** analogs were synthesized in racemic fashion and the diastereomeric alkylation products were separated by normal phase flash chromatography.[20-21] The aziridine used to prepare t-butyl analog **8w** was synthesized from a chiral aminoalcohol prepared from literature procedures.[25]

GS inhibition was maintained across the series, but t-butyl analog **8w** showed diminished antifungal potency relative to **8h**. However, the oral exposure of **8w** was 2X higher, resulting in similar TOKA efficacy at 25 mpk with marginal efficacy at 12.5 and 6.25 mpk. In contrast, t-butyl, methyl analog **8x** maintained antifungal potency and exhibited an almost 3X increase in oral exposure, producing robust TOKA efficacy at 25 and 12.5 mpk Gem-diethyl **8y** and spirocyclohexyl **8z** analogs lost antifungal activity in the presence of serum relative to **8h**. **8y** failed to improve oral exposure and produced diminished efficacy in TOKA while **8z** only displayed marginal efficacy in TOKA at 25

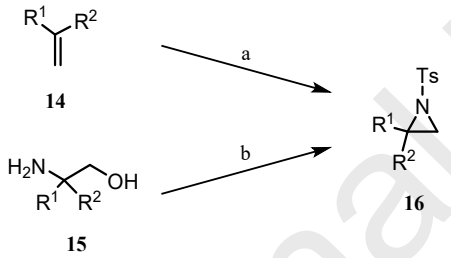
Table 3
C2 Carboxamidetriazole, C3Aminoether Derivatives



				All concentrations in µg/mL			Mouse PK and PO Efficacy		
Compound	**R^1, R^2**	**R^3, R^4**	**R^5, R^6**	**IC_{50} GS**	**MIC + (ser) C. *albicans***	**MEC A. *fumigatus***	**$nAUC_{oral}$ µM·hr·kg/mg**	**F_{oral} (%)**	**TOKA Δlog CFUs[a] (dose mpk)**
8h	iPr, Me	H, H	H, H	0.005	<0.03 (2)	<0.03	0.11	7	-2.5 (25) -0.2 (12.5) -0.9 (6.25)
8m	iPr, Me	H, H	H, Me	0.004	0.125 (8)	<0.03	0.06	5	-2.6 (25) -1.6 (12.5) -0.6 (6.25)
8n	iPr, Me	H, H	Me, Me	0.009	0.25 (8)	<0.03	0.30	16	-2.4 (25) -1.3 (12.5) -1.0 (6.25)
8o	iPr, Me	H, H	H, Et	0.008	0.25 (16)	<0.03	0.10	13	-2.8 (25) -1.4 (12.5) -0.8 (6.25)

8p	iPr, Me	H, H	H, Pr	0.007	0.5 (16)	<0.03	0.26	33	-1.0 (12.5) -1.1 (6.25)
8q	iPr, Me	H, H	H, iPr	0.010	0.25 (16)	<0.03	0.28	10	-2.3 (25) -1.8 (12.5) -0.4 (6.25)
8r	iPr, Me	H, H	H, cPr	0.005	0.25 (16)	<0.03	0.44	30	-2.1 (25) -0.9 (6.25) -0.8 (6.25)
8s	iPr, Me	H, Me	H, H	0.005	0.25 (8)	<0.03	0.33	5	-1.2 (25) -0.5 (6.25) -0.4 (6.25)
8t	iPr, Me	Me, Me	H, H	0.015	0.5 (16)	<0.03	1.35	21	-1.8 (25) -1.4 (12.5) -0.3 (6.25)
8u	iPr, Me	H, Et	H, H	0.011	0.25 (16)	<0.03	0.64	17	-1.8 (25) -1.1 (12.5) -1.1 (6.25)
8v	iPr, Me	H, Pr	H, H	0.015	0.5 (32)	<0.03	0.93	19	-1.9 (25) -0.8 (12.5) -0.7 (6.25)
8w	t-Bu, H	H, H	H, H	0.008	0.25 (8)	<0.03	0.22	10	-2.6 (25) -1.7 (12.5) -1.9 (6.25)
8x	t-Bu, Me	H, H	H, H	0.003	0.06 (2)	<0.03	0.29	12	-2.2 (25) -2.5 (12.5) -1.5 (6.25)
8y	Et, Et	H, H	H, H	0.007	0.125 (16)	<0.03	0.11	3	-1.9 (25) -0.6 (12.5) -0.2 (6.25)
8z		H, H	H, H	0.010	0.06 (16)	<0.03	-	-	-1.8 (25) -1.8 (12.5) -0.5 (6.25)
8aa		H, H	H, H	0.008	0.125 (2)	<0.03	-	-	-

[a]Change in colony forming units/g kidney relative to sham treated control animals.



Reagents and conditions: (a) PTAB, Chloramine-T, ACN, rt; (b) Ts-Cl, DMAP, TEA, DCM, rt.

Scheme 4. Synthesis of aziridines.

and 12.5 mpk. Spirotetrahydropyran analog **8aa** showed similar potency to **8h**, but was not obtained in sufficient quantity to allow further testing.

Table 4
Extended antifungal activity, PK, and efficacy evaluation of **8x**

Antifungal activity of 8x verses panel of Candida species				
Species			**MIC + (ser) µg/mL**	
C. *albicans* (MY1055)			0.06 (2)	
C. *glabrata* (MY1381)			0.125	
C. *krusei* (ATCC6258)			1	
C. *tropicalis* (MY1012)			0.5	
C. *lusitaniae* (MY1396)			0.125	
C. *parapsilosis* (ATTC22109)			0.06	
Pharmacokinetics of 8x across species				
	Mouse	**Rat**	**Dog**	**Rhesus**
Clearance (mL/min/kg)	11	6	2	4
$t_{1/2}$ (h)	5.1	5.5	12.3	8.5
nAUCoral (µM·hr·kg/mg)	0.29	0.72	4.18	0.79
F_{oral}	12%	17%	35%	12%
Efficacy of 8x in 7D TOKA				
Dose			**Δ log CFUs (% survival)**	
25 mpk PO			-3.57 (100)	
12.5 mpk PO			-3.41 (100)	
6.25 mpk PO			-2.09 (80)	
12.5 mpk IP			-3.89 (100)[a]	
ED_{99} mpk			6.4	
Efficacy of 8x in Aspergillosis Survival Model				
Dose		**% survival**	**Mean Survival (days)**	
12.5 mpk IP		60	14.4	
6.25 mpk IP		40	12.5	
3.125 mpk IP		10	7.3	
1.56 mpk IP		0	6.5	

[a] Kidneys from 3/5 animals were below the limit of detection (60% clearance)

8x was selected for further evaluation (Table 4) due to its superior TOKA efficacy at 12.5 mpk. It demonstrated excellent broad spectrum Candida antifungal activity versus a panel of clinically relevant species[6-7] with MICs of ≤ 1 µg/ml against challenging organisms including *Candida glabrata* and *Candida krusei*. In general, the PK profile improved moving from rodents to higher species, particularly dog. The clearance rate dropped roughly 2X between each species moving from mouse to rat to rhesus to dog. A concurrent 1.5-2X increase in half life was observed moving from rodents to rhesus and dog. Oral exposure improved about 2.5X moving from mouse to rat and rhesus and 14X to dog. Oral bioavailability for dog was 2-3X improved over the other species. The efficacy of **8x** was examined in the more stringent 7 day TOKA model where 80% and 40% survival rates were observed for PO and IP sham treated control groups.[26] Over a 3.4 log reduction in CFUs were observed for both the 25 and 12.5 mpk PO doses, equating to an almost complete eradication of the infection. The 6.25 mpk PO dose produced a robust 2 log reduction in CFUs, leading to an ED_{99} (estimated dose to reduce fungal burden by 99%) of 6.4 mpk. Impressively, the 12.5 mpk IP dose completely cleared the infection in 60% of the animals. The in vivo efficacy of **8x** was further evaluated in a murine survival model of disseminated Aspergillosis employing *Aspergillus*

observed in the sham treated control group with a mean survival of 3.9 days. **8x** produced dose dependent survival out to 21 days with a reasonable 60% survival rate at 12.5 mpk before dropping to 40% at 6.25 mpk, 10% at 3.125 mpk, and 0% at 1.56 mpk. A dose dependent trend of prolonged mean survival time at the higher doses was observed, but even the less effective lower doses, 3.125 and 1.56 mpk, increased the mean survival time by several days relative to the sham control group.

In conclusion, the 3-carboxamide-2-[1,2,4-triazole] of **8h** was identified as a suitable replacement for the C2 aminotetrazole of the previous lead **2** for maintaining potent GS and antifungal activity.[18] Alkylation of either the C2 3-carboxamidetriazole or the C3 aminoether of **8h** improved oral exposure, but a concurrent loss of antifungal activity produced no improvement in TOKA efficacy. Increasing the lipophilicity of the substituent alpha to the amine, by moving from the (*R*) isopropyl, methyl of **8h** to the (*R*) t-butyl, methyl of **8x**, improved oral exposure while maintaining potent antifungal activity and resulted in improved TOKA efficacy. These structural changes from **2** to **8x** lowered the ED_{99} in the 7D TOKA from 9 mpk to 6.4 mpk and removed the complication of active metabolites from the dealkylation of **2**.[18] **8x** was selected as a development compound (MK-5204) because of its broad spectrum Candida antifungal profile and its superior PK and efficacy in the 7D TOKA and Aspergillosis survival models. Development of MK-5204 was later discontinued in favor of ibrexafungerp (MK-3118, SCY-078), which is currently in phase III clinical trials. The final optimization from MK-5204 to ibrexafungerp will be the subject of a forth coming publication.

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Appendix A – Supplemental Data

Supplementary data for this article can be found online at…

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27. DBA/2N mice were treated bid IP for 7 days after being challenged with *A. fumigatus* (MF5668) at 6.7 x 10^5 CFU/mouse via IV delivery. The first treatment was delivered 15-30 minutes following the challenge and survival was monitored out to 21 days.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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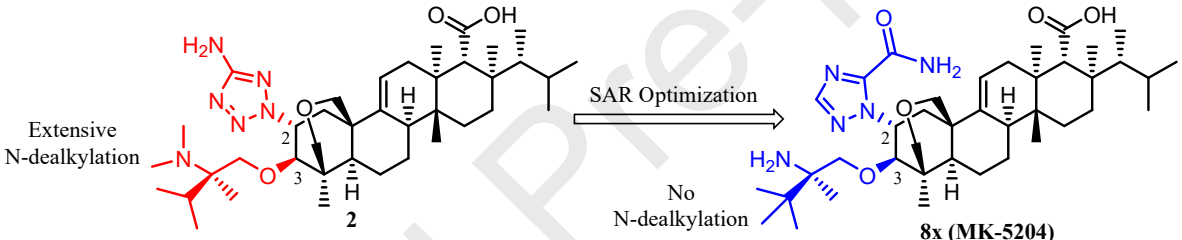

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ED_{99} 9 mpk vs. C. *albicans*

8x (MK-5204)
ED_{99} 6.4 mpk vs. C. *albicans*