Journal Pre-proofs

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

James M. Apgar, Robert R. Wilkening, Dann L. Parker Jr., Dongfang Meng, Kenneth J. Wildonger, Donald Sperbeck, Mark L. Greenlee, James M. Balkovec, Amy M. Flattery, George K. Abruzzo, Andrew M. Galgoci, Robert A. Giacobbe, Charles J. Gill, Ming-Jo Hsu, Paul Liberator, Andrew S. Misura, Mary Motyl, Jennifer Nielsen Kahn, Maryann Powles, Fred Racine, Jasminka Dragovic, Weiming Fan, Robin Kirwan, Shu Lee, Hao Liu, Ahmed Mamai, Kingsley Nelson, Michael Peel

PII: S0960-894X(20)30772-1
DOI: https://doi.org/10.1016/j.bmcl.2020.127661
Reference: BMCL 127661

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 31 August 2020
Revised Date: 26 October 2020
Accepted Date: 28 October 2020

Please cite this article as: Apgar, J.M., Wilkening, R.R., Parker, D.L. Jr., Meng, D., Wildonger, K.J., Sperbeck, D., Greenlee, M.L., Balkovec, J.M., Flattery, A.M., Abruzzo, G.K., Galgoci, A.M., Giacobbe, R.A., Gill, C.J., Hsu, M-J., Liberator, P., Misura, A.S., Motyl, M., Nielsen Kahn, J., Powles, M., Racine, F., Dragovic, J., Fan, W., Kirwan, R., Lee, S., Liu, H., Mamai, A., Nelson, K., Peel, M., Ibrexafungerp: an orally active β-1,3-glucan synthesis inhibitor, *Bioorganic & Medicinal Chemistry Letters* (2020), doi: https://doi.org/10.1016/j.bmcl.2020.127661

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier Ltd.

Journal Pre-proofs

ELSEVIER

Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

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

James M. Apgar [a,*], Robert R. Wilkening [a], Dann L. Parker, Jr. [a], Dongfang Meng [a], Kenneth J. Wildonger [a], Donald Sperbeck [a], Mark L. Greenlee [a], James M. Balkovec [a], Amy M. Flattery [a], George K. Abruzzo [a], Andrew M. Galgoci [a], Robert A. Giacobbe [a], Charles J. Gill [a], Ming-Jo Hsu [a], Paul Liberator [a], Andrew S. Misura [a], Mary Motyl [a], Jennifer Nielsen Kahn [a], Maryann Powles [a], Fred Racine [a], Jasminka Dragovic [a], Weiming Fan [b], Robin Kirwan [b], Shu Lee [b], Hao Liu [b], Ahmed Mamai [b], Kingsley Nelson [b], Michael Peel [b]

[a]*Merck & Co. Inc., 2000 Galloping Hill Road, Kenilworth, NJ 07033, USA*
[b]*Scynexis Inc., 1 Evertrust Plaza, Jersey City, NJ 07302, USA*

ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
Ibrexafungerp
SCY-078
MK-3118
β-1,3-glucan synthesis inhibitor
Enfumafungin

ABSTRACT

We previously reported medicinal chemistry efforts that identified MK-5204, an orally efficacious β-1,3-glucan synthesis inhibitor derived from the natural product enfumafungin. Further extensive optimization of the C2 triazole substituent identified 4-pyridyl as the preferred replacement for the carboxamide of MK-5204, leading to improvements in antifungal activity in the presence of serum, and increased oral exposure. Reoptimizing the aminoether at C3 in the presence of this newly discovered C2 substituent, confirmed that the (*R*) t-butyl, methyl aminoether of MK-5204 provided the best balance of these two key parameters, culminating in the discovery of ibrexafungerp, which is currently in phase III clinical trials. Ibrexafungerp displayed significantly improved oral efficacy in murine infection models, making it a superior candidate for clinical development as an oral treatment for *Candida* and *Aspergillus* infections.

2009 Elsevier Ltd. All rights reserved.

* Corresponding author. Tel.: +1-908-740-5122; fax: +1-908-740-6136; e-mail: james_apgar@merck.com

Public

Journal Pre-proofs

echinocandins), high mortality rates are observed for invasive candidiasis (IC) and aspergillosis, which comprise the majority of systemic, opportunistic fungal infections.[1-6] Immunocompromised and intensive care unit (ICU) patients are at highest risk for developing IC, with candidemia being cited as one of the most common bloodstream infections in ICUs, particularly among patients with central venous catheters.[1-5] The emergence of multidrug resistant organisms, including *Candida auris* with some isolates showing resistance to all three antifungal classes, highlights the urgent need for the development of novel antifungal treatments.[4-5,7-11] Reports of azole resistant isolates of *Aspergillus fumigatus* arising from the widespread agricultural use of azole antifungals is also concerning.[9-10]

The three classes of antifungals available to treat systemic fungal infections have distinct advantages and limitations. Polyenes (Amphotericin B), the oldest class of antifungals, disturb fungal cell membranes by binding to ergosterol, resulting in the leakage of cell contents. They have very broad spectrum antifungal activity, but their clinical use is often limited by nephrotoxicity caused by non-specific binding to mammalian sterols.[3,6,12] Azoles (fluconazole, voriconazole, disrupt fungal cell membranes by inhibiting the cytochrome P450 enzyme (Cyp51p) responsible for a key step in the biosynthesis of ergosterol. They are better tolerated than the polyenes and have the advantage of being orally bioavailable; however, inhibition of host P450 enzymes result in a plethora of drug-drug interactions (DDIs) that present challenges for patients taking multiple medications.[3,6,12] Echinocandins (caspofungin, micafungin, anidulafungin), the most recent class of antifungals, compromise the integrity of the fungal cell wall, eventually leading to cell lysis under osmotic stress, through the inhibition of β-1,3-glucan synthase (GS), which produces β-1,3-glucan, a critical component of the cell wall.[6,13] Due to the specificity of their target for fungal cells, the echinocandins have demonstrated improved tolerability and fewer DDIs compared to the polyenes and azoles.[3,6,12] The echinocandins are now recommended as first line therapy for IC, as a result of these advantages and because of their broad spectrum of activity for *Candida* species.[1,3-4,8,14] Restriction to parenteral administration due to poor oral bioavailability constitutes a significant limitation for echinocandin therapy.[12-15] A novel class of GS inhibitor, combining oral bioavailability with the improved safety profile of the echinocandins, would have the potential to

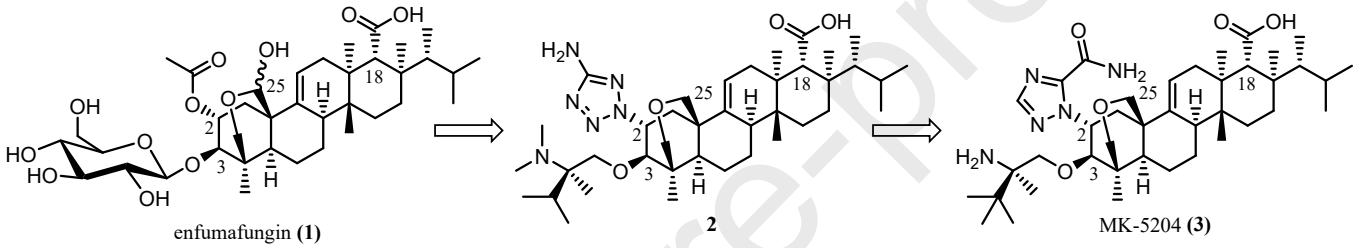
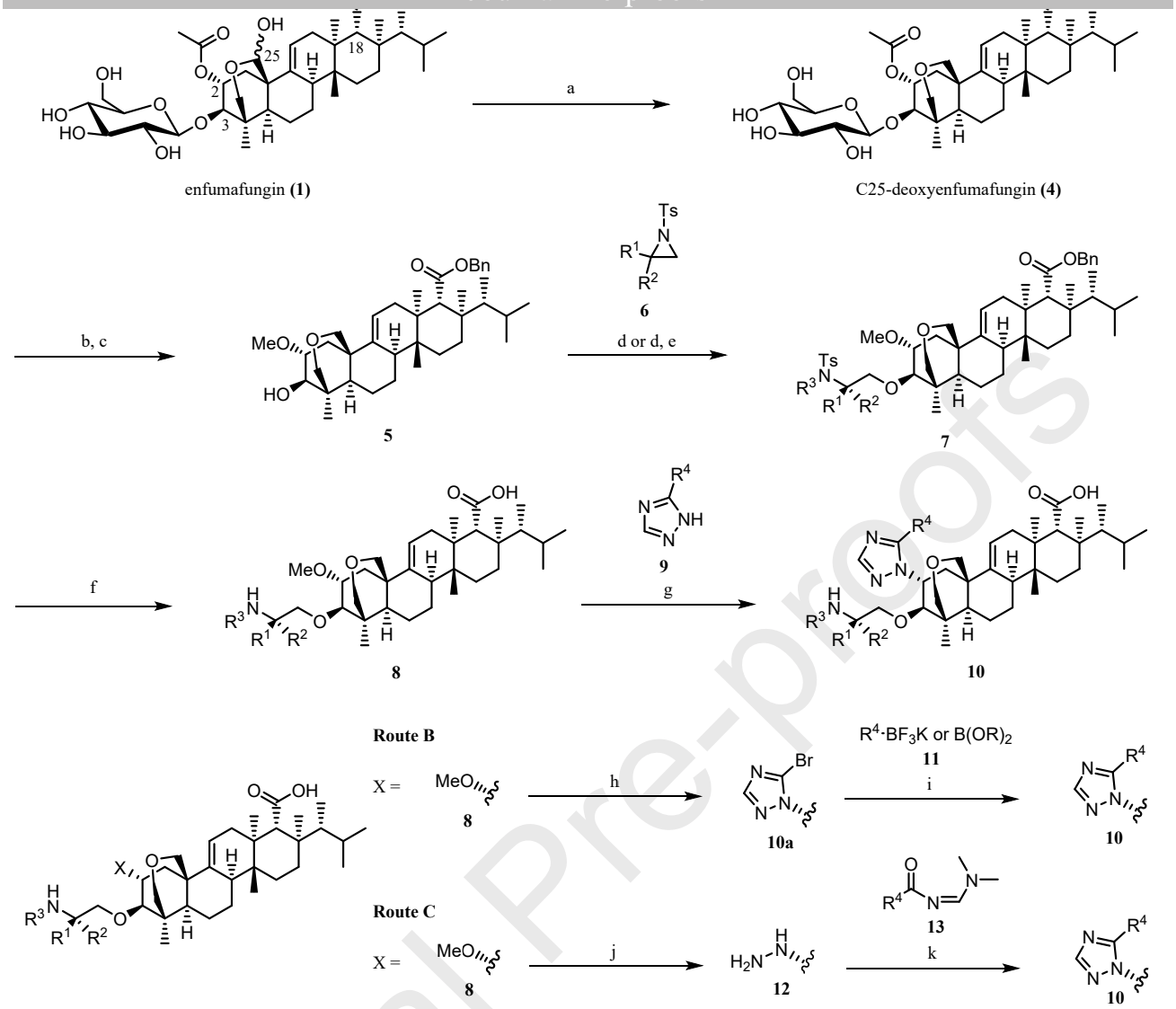


Figure 1. Progression from enfumafungin to MK-5204

provide benefit over existing treatment options for patients with resistant infections or requiring step-down treatment from a parenteral antifungal.

The most recent report from our group concerning synthetic modification of enfumafungin,[16] a naturally occurring GS inhibitor,[17-18] focused on replacing the C2 acetoxy group with a carboxamidetriazole moiety, the C3 glycoside with an aminoether, and the C25 bridging hemiacetal with an ether (**Figure 1**).[19] These efforts eventually led to the identification of MK-5204 **(3)**, which demonstrated improved oral efficacy in a 7-day murine model of disseminated candidiasis (6.4 mpk ED_{99} – effective dose to reduce fungal burden by 99%) compared to an earlier C2 aminotetrazole lead[20] **(2)** (9 mpk ED_{99}) while also removing concerns about N-dealkylation metabolism from the C3 aminoether substituent.[19] Herein we present efforts to further optimize the C2 and C3 substituents to produce additional improvement in oral efficacy. Initially, we focused on expanding our scan of C2 triazole substituents from previously reported polar and/or electron withdrawing groups to alkyl and aryl moieties in conjunction with an (*R*)-isopropyl, methyl amino ether at C3 using

Public

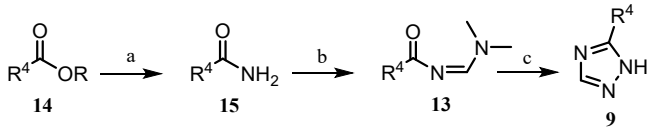


Reagents and conditions: (a) Et_3SiH, TFA, DCM, rt; (b) H_2SO_4, MeOH, 65 °C; (c) BnBr, $NaHCO_3$, DMF, 65 °C, 76% (3 steps); (d) 1) K t-pentylate, 18-crown-6, DMA, 0 °C, or 2) KH, 18-crown-6, DME, 0 °C, 17-100%; (e) NaH, MeI, DMF, 50 °C, 69-98%; (f) Na, NH_3, THF, -35 °C, 70-100%; (g) BF_3•OEt_2, DCE, 50 °C, 4-60%; (h) 3-Br-1,2,4-triazole, BF_3•OEt_2, DCE, 50 °C, 30-49%; (i) 1) $Pd(OAc)_2$ or $PdCl_2(PPh_3)_2$, XPHOS, Cs_2CO_3, EtOH, H_2O, 80 °C, or $Pd(OAc)_2$. XPHOS, Cs_2CO_3, DMA or DME, 100 °C, 6-35%; (j) hydrazine or benzyl carbazate, BF_3•OEt_2, DCE, 50 °C, 38-59%; (k) AcOH, 90 °C, 42-70%.

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

the general synthetic route shown in **Scheme 1**.

C25-Deoxyenfumafungin **(4)** was obtained *via* reduction of the bridging hemiacetal of enfumafungin **(1)** with triethylsilane.[20-23] Sulfuric acid in methanol was employed to hydrolyze the C3 glycoside of **4** to the alcohol with concurrent displacement of the C2 acetoxy moiety to furnish the methyl ether.[20] Benzyl ester protection of the C18 carboxylic acid afforded **5** in 76% overall yield from **1**. Alkylation of the C3 alcohol with tosyl protected aziridine **6** provided access to **7**, which was treated with iodomethane to achieve mono-methyl alkylation of the protected amine.[19,22-23] Intermediate **8** was obtained *via* Birch reduction, which simultaneously removed the benzyl ester from C18 and the tosyl protecting group from the C3 aminoether. Displacement of the C2 methoxy, which occurred with retention of stereochemistry, by substituted 1,2,4-triazoles was promoted by borontrifluoride diethyletherate as previously described (Route A – **Scheme 1**).[20] The triazoles **(9a-b)** used in the synthesis of **10n** and **10v** were prepared according to **Scheme 2**. Condensation of commercial aryl-amides **(15)** with N,N-dimethylformamide diethyl acetal provided acyl-amidines **(13)**, which were cyclized with hydrazine hydrate to give 3-aryl-1,2,4-triazoles **(9)**.[24]



Reagents and conditions: (a) NH_4OH, 77%-quant.; (b) DMF diethyl acetal, 120 °C, 81%-quant.; (c) hydrazine hydrate, AcOH, 90 °C, 27-65%.

Scheme 2. Synthesis of acyl-amidines and 3-aryl-1,2,4-triazoles.

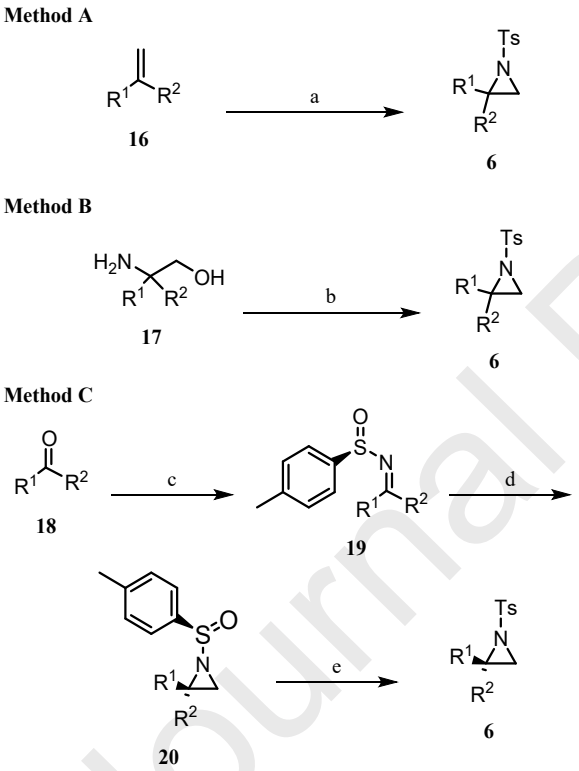
The identity of the 3-substitutent on the triazole determined the distribution of regioisomeric products (1-[1,2,4-triazole], the desired 2-[1,2,4-triazole] **(10)**, 4-[1,2,4-triazole]) from the displacement reaction in **Scheme 1**.[19] Reverse phase HPLC was employed to separate the three regioisomers.[19] In the displacement reaction, 3-alkyl-1,2,4-triazoles exemplified by 3-methyl-1,2,4-triazole, failed to produce enough of the desired 2-[1,2,4-triazole] isomer to isolate. 3-aryl-1,2,4-triazoles only performed marginally better with yields of 5% or less for the desired isomer **(10g**, **10n**, **10v)**. These limitations forced us to develop alternative synthetic strategies to access desired chemical space and enable scale-up of analogs of interest. Fortunately, the displacement reaction with 3-bromo-1,2,4-triazole, which was synthesized

Public

Journal Pre-proofs

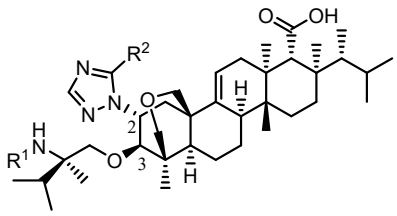
acc
with yields ranging from 30-49% (Route B – **Scheme 1**). Suzuki cross-coupling with **10a** afforded aryl substituted analogs **(10g-10q)** in low to moderate yield (6-35%). This route was also employed to deliver the ethyl and propyl substituted triazole analogs **(10d-10e)** *via* hydrogenation of olefin Suzuki products.

To further expand access to chemical space, we chose to construct the C2 triazole substituent stepwise on the modified enfumafungin scaffold **(8)**. Performing the displacement reaction with anhydrous hydrazine or benzyl carbazate (deprotection occurred *in situ* under the reaction conditions) provided the C2 hydrazine **(12)** in yields ranging from 38-59% (Route C – **Scheme 1**). Cyclization of **12** with acyl-amidines **(13)** proceeded smoothly with exclusive formation of the desired 2-[1,2,4-triazole] isomer to provide 3-alkyl and aryl triazole analogs **(10c, 10f, 10q-10u, 10w-10y)** in 42-70% yield.[24] Acyl-amidines **(13)** were generated *via* condensation of N,N-dimethylformamide diethyl acetal with amides[24] **(15)** that were obtained commercially or from treatment of commercial esters **(14)** with ammonium hydroxide as shown in **Scheme 2**, except for the amide used in the synthesis of **10r**, which was prepared from the carboxylic acid using literature procedures.[26] This third and final route to install the C2 triazole



Reagents and conditions: (a) PTAB, Chloramine-T, ACN, rt, 5-54%; (b) Ts-Cl, DMAP, TEA, DCM, rt, 14-60%; (c) (*R*)-p-toluenesulfinamide, $Ti(OEt)_4$, DCE, 70 °C, 7-82%; (d) trimethylsulfoxonium chloride, n-BuLi, THF, 0 °C-rt, 46-100%; (e) mCPBA, 1 M $NaHCO_3$, EtOAc / hexanes or DCM, 0 °C, 50-99%.

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



provided the broadest substrate scope and was employed to scale-up key analogs.

The tosyl-aziridines **(6)** used to synthesize the analogs displayed in **Tables 1-2** were prepared *via* the three methods shown in **Scheme 3**. The racemic/achiral tosyl-aziridines **(6)** employed in the preparation of **10q**, **10z-10ad**, and **10ag** were obtained either by treatment of olefins **(16)** with chloramine-T (Method A - **Scheme 3**) or cyclization of amino-alcohols with tosyl chloride (Method B – **Scheme 3**).[19,22-23] C3 alkylation with the racemic isopropyl, methyl and t-butyl, methyl aziridines prepared according to Method A, necessitated a difficult resolution of diastereomeric products *via* normal phase chromatography. Desiring broad, straightforward access to (*R*)-alpha-disubstituted aziridines, we successfully developed an enantioselective method (Method C – **Scheme 3**). Condensation of ketones **(18)** with (*R*)-p-toluenesulfinamide afforded **19**, which was cyclized *via* treatment with trimethylsulfoxonium chloride and n-butyllithium to give chiral toluenesulfinyl aziridines **(20)**.[27-28] Oxidation of **20** with mCPBA furnished the tosyl protected (*R*)-alpha-disubstituted aziridines **(6)** used in the synthesis of **10ac-10af** and **10ah**. Greater stereochemical control was achieved starting from acyclic ketone substrates with cyclization producing high diastereomeric ratios (dr > 10:1) for (*R*) t-butyl, methyl and methylcyclopropyl, methyl toluenesulfinyl aziridines **(20)**. In contrast, the cyclic ketone substrate used to prepare the spirotetrahydropyran aziridine employed in the synthesis of **10ah** only attained a 2:1 diastereomeric ratio upon cyclization.

Synthesized compounds were evaluated for GS inhibition (IC_{50}, **Table 1**) in *Candida albicans* (MY1055) microsomal membrane fragments using a previously reported method.[17] Onishi et al established CLSI protocols in RPMI medium that were employed to measure in vitro antifungal

All concentrations in µg/mL	Mouse PK and PO Efficacy

Public

Journal Pre-proofs

Compound	Route	R[1]	R[2]	IC_{50} GS	MIC[a] (+ ser) C. *albicans*	MEC[b] A. *fumigatus*	$nAUC_{oral}$ ± S.D.[c] μM·hr·kg/mg	F_{oral}[d] (%)	Δlog CFUs[e] ± S.D.[c] (dose mpk)
Caspofungin	-	-	-	0.001	0.06 (0.25)	<0.03	-	-	-4.2 ± 0.7 (0.5)[f,g,***] -4.2 ± 0.7 (0.125)[f,h,***] -2.3 ± 1.1 (0.03)[f,***]
10b	A	H	H	0.007	0.25 (4)	<0.03	-	-	-
10c	C	H	Me	0.005	0.25 (4)	0.03	-	-	-
10d	B	H	Et	0.010	0.25 (8)	<0.03	-	-	-
10e	B	H	Pr	0.003	8 (16)	<0.03	-	-	-
10f	C	H	cPr	0.006	0.125 (16)	0.008	-	-	-
10g	A and B	H	Phenyl	0.003	0.25 (4)	<0.03	0.50 ± 0.13	28	-2.4 ± 0.7 (25)[***] -1.7 ± 1.2 (12.5)[**] -0.2 ± 1.1 (6.25)
10h	B	H	2-F Phenyl	-	0.5 (8)	<0.03	-	-	-
10i	B	H	3-F Phenyl	0.011	0.25 (8)	<0.03	-	-	-
10j	B	H	4-F Phenyl	0.011	0.25 (8)	<0.03	-	-	-
10k	B	H	3-OMe Phenyl	0.011	0.25 (32)	<0.03	-	-	-
10l	B	H	4-OMe Phenyl	0.015	0.5 (8)	<0.03	-	-	-
10m	B	Me	2-Pyridyl	0.003	0.25 (16)	<0.03	-	-	-
10n	A and B	Me	3-Pyridyl	0.005	0.25 (1)	<0.03	0.59 ± 0.13	21	-3.0 ± 0.4 (25)[***] -2.4 ± 0.9 (12.5)[*] -1.1 ± 0.5 (6.25)[*]
10o	B	Me	4-Pyridyl	0.001	0.06 (0.5)	<0.03	0.83 ± 0.34	29	-3.7 ± 0.7 (25)[***] -3.1 ± 0.4 (12.5)[***] -0.9 ± 0.6 (6.25)[*]
10p	B	H	3-Pyridyl	0.004	0.06 (1)	<0.03	0.17 ± 0.03	11	-3.1 ± 0.3 (25)[***] -2.9 ± 0.5 (12.5)[***] -1.4 ± 0.8 (6.25)[*]
10q	B and C	H	4-Pyridyl	0.002	<0.03 (0.5)	<0.03	0.42 ± 0.14	31	-4.5 ± 0.6 (25)[i,***] -2.9 ± 0.3 (12.5)[***] -2.4 ± 1.0 (6.25)[*]
10r	C	H	2-F, 4-Pyridyl	0.001	0.015 (1)	0.008	0.34 ± 0.10	26	-4.2 ± 0.3 (25)[i,***] -2.5 ± 0.6 (12.5)[***] -2.6 ± 0.5 (6.25)[***]
10s	C	H	3-OMe, 4-Pyridyl	0.008	0.25 (4)	<0.03	-	-	-
10t	C	H	2,3-Pyridazine	0.005	<0.03 (2)	<0.03	-	-	-
10u	C	H	2,4-Pyrimidine	0.005	<0.03 (4)	<0.03	2.38 ± 1.20	43	-
10v	A	H	2,5-Pyrazine	0.004	0.5 (4)	<0.03	-	-	-
10w	C	H	2,6-Pyrimidine	0.004	0.25 (8)	<0.03	-	-	-
10x	C	H	3,4-Pyridazine	0.002	0.125 (1)	<0.03	0.23 ± 0.06	29	-2.6 ± 0.8 (25)[**] -1.2 ± 0.4 (12.5)[**] -1.2 ± 2.2 (6.25)
10y	C	H	3,5-Pyrimidine	0.006	0.06 (1)	<0.03	0.33 ± 0.10	26	-3.0 ± 0.5 (25)[***] -3.0 ± 1.1 (12.5)[**] -2.1 ± 0.3 (6.25)[j,***]

[a]Minimum inhibitory concentration.
[b]Minimum effective concentration.
[c]Standard deviation.
[d]Oral bioavailability = oral area under the concentration time curve normalized to intravenous (IV) dose / IV area under the concentration time curve.
[e]Change in colony forming units/g kidney relative to sham treated control animals.
[f]Dosed intraperitoneally (IP).
[g]Kidneys from 100% of animals were below the limit of detection (100% clearance).
[h]Kidneys from 82% of animals were below the limit of detection (82% clearance).
[i]Kidneys from 75% of animals were below the limit of detection (75% clearance).
[j]25% mortality (1 of 4 animals).
[*]$p < 0.05$ for CFUs/g kidney versus sham treated control animals (two tail t test).
[**]$p < 0.01$ for CFUs/g kidney versus sham treated control animals (two tail t test).
[***]$p < 0.001$ for CFUs/g kidney versus sham treated control animals (two tail t test).

susceptibilities of clinically relevant fungal species (minimum inhibitory concentration for *Candida albicans* (MY1055) and minimum effective concentration for *Aspergillus fumigatus* (MF5668), **Table 1**).[17] Mouse pharmacokinetics (PK) and oral antifungal efficacy in a murine model of disseminated candidiasis (Target Organ Kidney Assay – TOKA) using C. *albicans* (MY1055) as the pathogen were utilized to further profile analogs of interest.[29-30] Caspofungin, an echinocandin GS inhibitor, was employed as the positive control for *in vivo* efficacy studies.

All of the 3-alkyl and aryl 2-[1,2,4-triazole]

Public

Journal Pre-proofs

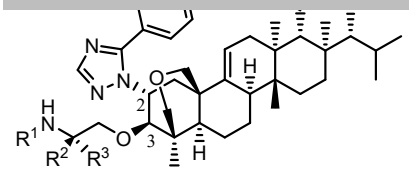
anal wer inhibition (**Table 1**), but significant trends were identified for whole cell antifungal activity in the presence and absence of 50% mouse serum. With the exception of cyclopropyl **(10f)**, alkyl substituents **(10c-10f)** did not confer improvement to the inherent antifungal activity against C. *albicans* relative to the unsubstituted triazole **(10b).** Furthermore, in the presence of serum, a clear trend was observed whereby increasing lipophilicity of the alkyl substituent produced a 2-fold loss in potency for each additional methylene moving from **10c** to **10f**. In contrast, the increased lipophilicity of phenyl analog **(10g)** did not diminish antifungal activity relative to **10b**, even in the presence of serum, and contributed reasonable oral exposure, translating into moderate TOKA efficacy at 25 mpk. Installing a fluoro or methoxy substituent **(10h-10l)** at various positions on the phenyl produced little impact as **10h-10l** were all within 2-fold of the antifungal potency of the unsubstituted phenyl **(10g)**, except the 3-methoxy **(10k)**, which was 8-fold less potent in the presence of serum. Incorporation of a nitrogen into the aryl ring was explored in analogs containing a N-methyl aminoether **(10m-10o)** with profound effects observed on antifungal potency and efficacy based on the regioisomeric position of the nitrogen. While the 2 and 3-pyridyl analogs **(10m-10n)** were equipotent with phenyl **(10g)**, the 4-pyridyl **(10o)** showed a 4-fold improvement. The importance of the nitrogen position was pronounced in the presence of serum, with a drastic 32-fold shift in potency observed as the nitrogen moved around the ring from the 2 to the 4 position. The 3-pyridyl analog **(10n)** maintained similar oral exposure to the phenyl **(10g)**, but the 4-pyridyl analog **(10o)** exhibited a roughly 1.5X improvement over either analog. Compounds **10n** and **10o** both demonstrated excellent efficacy in TOKA with over a 3 log reduction in fungal CFUs at 25 mpk. Analog **10o** still provided a robust 3 log reduction in fungal burden at 12.5 mpk, but both analogs failed to retain efficacy at 6.25 mpk. Compounds **10p** and **10q** showed a 2-4 fold improvement in antifungal potency over their N-methyl aminoether counterparts **(10n-10o)**, though potency in the presence of serum remained unchanged. Despite a 5X lower oral exposure versus **10n**, **10p** demonstrated similar TOKA efficacy at 25 mpk and had a half-log improvement in fungal CFU reduction at 12.5 mpk. Compound 1**0q** only produced a 2X loss in oral exposure compared to **10o** and maintained similar exposure to the parent phenyl analog **(10g)**. The reasonable oral exposure of **10q** coupled with outstanding antifungal potency in the presence of serum resulted in exceptional oral efficacy in TOKA at 25 mpk with a 4.5 log reduction in fungal CFUs and clearance of the infection in 75% of the animals. Compound **10q** maintained robust efficacy at 12.5 mpk and demonstrated reasonable efficacy at 6.25 mpk with over a 99% reduction in fungal burden. With **10q** as the new benchmark, substituted 4-pyridyl and an array of 6-membered dinitrogen heterocycles were evaluated. Combining 2-fluoro substitution with the 4-pyridyl **(10r)** drastically improved antifungal activity versus the 2-fluorophenyl analog **(10h)**, and was within 2-fold of the antifungal potency of the 4-pyridyl analog **(10q)** in the presence of serum. Compound **10r** had slightly lower oral exposure than **10q**, but displayed a similarly excellent efficacy profile in TOKA with 75% clearance and over a 4 log reduction in

Table 2
C3 Aminoether Derivatives

end of the dose titration. Unlike the 2-fluoro, combining the 3-methoxy substituent with the 4-pyridyl **(10s)** only modestly improved antifungal potency in the presence of serum relative to the 3-methoxy phenyl **(10k)**, leaving **10s** significantly less potent than the 4-pyridyl analog **(10q)**. Di-aza aryl analogs **(10t-10y)** displayed similar trends to those observed for the pyridyl analogs **(10m-10q)** with distal positioning of the nitrogen favored over substitution proximal to the neighboring triazole for maintaining antifungal activity in the presence of serum. The 3,4-pyridazine **(10x)** and 3,5-pyrimidine **(10y)** analogs, displayed antifungal activity in the presence of serum that warranted evaluation in TOKA, but both analogs produced diminished efficacy relative to **10q** due to decreased oral exposure.

Having identified aryl-triazole substituents with improved oral efficacy, we turned our attention to reoptimizing the combination of the C2 triazole and the C3 aminoether. With the exceptional antifungal potency of **10q**, especially in the presence of serum, and its outstanding oral efficacy in TOKA, the 4-pyridyl substitution was selected for further exploration over the 2-fluoro-4-pyridyl of **10r**, which exhibited a similar profile. Holding the 4-pyridyl-triazole constant at C2, various alkyl substitutions alpha to the amine on the C3 aminoether (**Table 2**) were prepared according to Route C of **Scheme 1** with the aim of increasing oral exposure while maintaining antifungal activity in the presence of serum to maximize oral efficacy. Potent GS activity was maintained across the series, but differences in antifungal activity and oral exposure produced profound effects on TOKA efficacy. The gem-dimethyl analog **(10z)** retained equivalent antifungal potency relative to the isopropyl, methyl **(10q)** compound. Compound **10z** only produced a 1 log reduction in fungal burden after oral dosing at 12.5 mpk in TOKA, but produced over a 4 log reduction when dosed intraperitoneally at 12.5 mpk, indicating that the gem-dimethyl substitution failed to produce sufficient oral exposure to achieve the TOKA activity observed for **10q**. The increased lipophilicity of the gem-diethyl compound **(10aa**) resulted in improved oral TOKA efficacy compared to **10z**, but a 2-4 fold loss in antifungal activity prevented **10aa** from equaling the TOKA efficacy of **10q**. The t-butyl analog **(10ab)** lost 4-8 fold in antifungal activity relative to **10q**, resulting in marginal efficacy at 25 and 12.5 mpk. Conversely, the t-butyl, methyl analog **(10ac)**, maintained antifungal activity compared to the isopropyl, methyl analog **(10q)**, while achieving a slight increase in oral exposure. Compound **10ac** rivaled the exceptional dose-dependent oral TOKA efficacy observed for **10q**, producing more than a 99% reduction in fungal burden even at 6.25 mpk, with 50% clearance of the infection at 25 mpk. N-methylation of the aminoether of **10ac (10ad)** and cyclizing two methyl groups from the t-butyl of **10ac** to a methylcyclopropyl **(10ae)**, both resulted in similar antifungal potency and oral exposure relative to **10ac**. Compounds **10ad-10ae** demonstrated excellent TOKA efficacy with over a 4 log reduction in fungal burden at 25 mpk with 75% **(10ad)** and 100% **(10ae)** clearance. Analog **10ad** maintained superior

Public



				All concentrations in µg/mL			Mouse PK and PO Efficacy		
Compound	**Method**	**R¹**	**R², R³**	**IC_{50} GS**	**MIC (+ ser) C. *albicans***	**MEC A. *fumigatus***	**$nAUC_{oral}$ ± S.D. µM·hr·kg/mg**	**F_{oral}[a] (%)**	**TOKA Δlog CFUs[b] ± S.D. (dose mpk)**
Caspofungin	-	-	-	0.001	0.06 (0.25)	<0.03	-	-	-4.2 ± 0.7 (0.5)[c,d,***] -4.2 ± 0.7 (0.125)[c,e,***] -2.3 ± 1.1 (0.03)[c,***]
10q	A	H	iPr, Me	0.002	<0.03 (0.5)	<0.03	0.42 ± 0.14	31	-4.5 ± 0.6 (25)[f,***] -2.9 ± 0.3 (12.5)[***] -2.4 ± 1.0 (6.25)[*]
10z	A	H	Me, Me	0.001	0.015 (0.5)	<0.03	-	-	-4.2 ± 0.5 (12.5)[c,***] -1.1 ± 0.8 (12.5)[*]
10aa	A	H	Et, Et	0.001	0.06 (1)	<0.03	-	-	-3.3 ± 0.5 (25)[***] -2.3 ± 0.3 (12.5)[***] -1.3 ± 1.1 (6.25)
10ab	B[g]	H	t-Bu, H	0.007	0.25 (2)	<0.03	-	-	-2.6 ± 0.6 (25)[***] -2.4 ± 1.4 (12.5)[*] -1.0 ± 0.7 (6.25)[*]
10ac	A and C	H	t-Bu, Me	0.001	0.06 (0.5)	<0.03	0.59 ± 0.19	34	-4.6 ± 0.4 (25)[h,***] -3.2 ± 0.4 (12.5)[***] -2.2 ± 0.8 (6.25)[**]
10ad	A and C	Me	t-Bu, Me	0.002	0.03 (1)	0.008	0.62 ± 0.03	42	-4.2 ± 0.5 (25)[f,***] -3.2 ± 0.9 (12.5)[i,***] -1.7 ± 1.7 (6.25)
10ae	C	H		0.002	<0.03 (0.5)	<0.03	0.65 ± 0.23	36	-4.3 ± 0.5 (25)[d,***] -2.6 ± 1.1 (12.5)[**] -1.5 ± 0.8 (6.25)[*]
10af	C	H	Phenyl, Me	0.006	0.25 (4)	0.125	-	-	-2.6 ± 0.6 (25)[***] -1.5 ± 0.9 (12.5)[*] -0.5 ± 0.6 (6.25)
10ag	B	H		0.002	<0.03 (0.25)	<0.03	0.18 ± 0.08	13	-3.9 ± 0.8 (25)[***] -3.1 ± 0.4 (12.5)[***] -1.4 ± 0.8 (6.25)[*]
10ah	C	H		0.004	0.06 (1)	0.004	0.35 ± 0.16	25	-3.2 ± 1.0 (25)[**] -2.2 ± 0.9 (12.5)[**] -0.9 ± 0.5 (6.25)[*]

[a]Oral bioavailability = oral area under the concentration time curve normalized to intravenous (IV) dose / IV area under the concentration time curve.
[b]Change in colony forming units/g kidney relative to sham treated control animals.
[c]Dosed intraperitoneally (IP).
[d]Kidneys from 100% of the animals were below the limit of detection (100% clearance).
[e]Kidneys from 82% of animals were below the limit of detection (82% clearance).
[f]Kidneys from 75% of the animals were below the limit of detection (75% clearance).
[g]Chiral amino alcohol starting material used.[31]
[h]Kidneys from 50% of the animals were below the limit of detection (50% clearance).
[i]Kidneys from 25% of the animals were below the limit of detection (25% clearance).
[*]$p < 0.05$ for CFUs/g kidney versus sham treated control animals (two tail t test).
[**]$p < 0.01$ for CFUs/g kidney versus sham treated control animals (two tail t test).
[***]$p < 0.001$ for CFUs/g kidney versus sham treated control animals (two tail t test).

efficacy at 12.5 mpk relative to **10ae** with over a 3 log reduction in fungal burden and 25% clearance; however, both analogs diminished in efficacy more rapidly between 12.5 mpk and 6.25 mpk compared to **10ac**. Replacing the t-butyl of **10ac** with a phenyl **(10af)** produced a 4-8 fold loss of antifungal activity, resulting in only marginal TOKA efficacy at 25 mpk. The spirotetrahydropyran analog **(10ag)** possessed exquisite antifungal activity with a 2-fold potency improvement in the presence of serum compared to **10ac**; however, a 3X drop in oral exposure accompanied **10ag**. As a result of these opposing trends, **10ag** produced robust TOKA efficacy at 25 and 12.5 mpk, but abruptly declined at 6.25 mpk. Increasing the lipophilicity of the spirotetrahydropyran through the incorporation of gem-dimethyl substitution alpha to the quaternary center **(10ah)**, resulted in a 4-fold loss of antifungal activity in the presence of serum relative to **10ag**. Compound **10ah** produced a 2X increase in oral exposure compared to **10ag**, but demonstrated diminished TOKA efficacy, indicating that the modest gains in oral exposure were insufficient to compensate for the loss of antifungal potency in the presence of serum.

The isopropyl, methyl **(10q)** and the t-butyl, methyl **(10ac)** analogs were selected for further evaluation as a result of displaying exceptional TOKA efficacy with partial clearance observed at 25 mpk as well as maintenance of efficacy through the low end of the dose titration at 6.25 mpk. The spirotetrahydropyran analog **(10ag)** was also chosen for additional

Table 3
PK evaluation of **10q**, **10ac**, and **10ag**

Public

Journal Pre-proofs

Ph				
10q	**Mouse**	**Rat**	**Dog**	**Rhesus**
Clearance ± S.D. (mL/min/kg)	17 ± 1	12 ± 1	6 ± 2	14 ± 1
$t_{1/2}$ ± S.D. (h)	4.9 ± 0.4	3.9 ± 1.3	9.0 ± 1.4	6.1 ± 0.1
nAUCoral ± S.D. (μM·hr·kg/mg)	0.42 ± 0.14	0.56 ± 0.17	0.90 ± 0.27	0.08 ± 0.06
F_{oral}[a]	30%	28%	21%	5%
10ac	**Mouse**	**Rat**	**Dog**	**Rhesus**
Clearance ± S.D. (mL/min/kg)	14 ± 2	8 ± 2	4 ± 1	11 ± 2
$t_{1/2}$ ± S.D. (h)	4.4 ± 1.8	8.3 ± 3.8	11.3 ± 1.2	5.7 ± 0.7
nAUCoral ± S.D. (μM·hr·kg/mg)	0.59 ± 0.19	0.69 ± 0.27	1.07 ± 1.31	0.33 ± 0.05
F_{oral}[a]	34%	23%	18%	16%
10ag	**Mouse**	**Rat**	**Dog**	**Rhesus**
Clearance ± S.D. (mL/min/kg)	18 ± 6	20 ± 2	-	-
$t_{1/2}$ ± S.D. (h)	2.1 ± 0.3	1.9 ± 0.1	-	-
nAUCoral ± S.D. (μM·hr·kg/mg)	0.18 ± 0.08	0.34 ± 0.16	-	-
F_{oral}[a]	13%	30%	-	-

[a]Oral bioavailability = oral area under the concentration time curve normalized to intravenous (IV) dose / IV area under the concentration time curve.

study to see, if its exquisite antifungal activity in the presence of serum would translate to improved efficacy upon extended dosing. Dog displayed the best overall profile in a PK assessment across preclinical species (**Table 3**). Clearance dropped while half-life and oral exposure increased moving from mouse to dog for **10q** and **10ac**, but all three parameters eroded moving from dog to rhesus. The oral bioavailability of both analogs slowly diminished moving from mouse to rhesus. Oral bioavailability and exposure increased for **10ag** moving from mouse to rat, while clearance and half-life remained flat. Compounds **10q** and **10ac** demonstrated superior PK profiles in rodents compared to **10ag** with improvements in clearance (rat), half-life (mouse/rat), oral exposure (mouse/rat), and oral bioavailability (mouse). In general, **10q** and **10ac** produced similar PK profiles across preclinical species with **10ac** holding advantages in rat half-life (2X), rhesus oral exposure (4X), and rhesus oral bioavailability (3X).

An extended 7-day survival version of TOKA was employed to further evaluate the efficacy of **10q**, **10ac**, and **10ag** versus *Candida albicans* (**Table 4**).[32] Sham treated IP and PO control groups had survival rates of 40% and 80%, respectively. Impressively, all three analogs produced 100% clearance of the infection in study animals when dosed at 12.5 mpk IP. In addition, all compounds reduced fungal burden by over 4.5 logs with 100% (**10q** and **10ac**) or 80% (**10ag**) clearance at 25 mpk PO. **10ac** maintained 100% clearance at 12.5 mpk PO with **10q** and **10ag** dropping to 40% and 20%, respectively. Nevertheless, **10q** and **10ag** lowered the fungal burden at this dose by over 3.9 logs, indicating that the infection was nearly eradicated. Moving to the 6.25 mpk PO dose, **10q** and **10ac** achieved robust 3.5 log reductions in fungal burden, while **10ag** fell below a 3 log reduction. These results led to a 3-4X improvement in ED_{99} (effective dose to reduced fungal burden by 99%) for **10q** and **10ac** over **10ag**. A 21-day murine survival model of disseminated aspergillosis utilizing *Aspergillus fumigatus* (MF5668) as the pathogen was employed to further evaluate the efficacy of **10q**, **10ac**, and **10ag** (**Table 4**).[15,33] The sham treated control group had a mean survival of 3.9 days with 100% mortality. Compounds **10q** and **10ag** produced dose-dependent survival with 60% and 40%

Table 4
Extended efficacy evaluation of **10q**, **10ac**, and **10ag**

Efficacy in 7-day TOKA				
Dose		**Δ log CFUs[a] (% clearance)**		
	Caspofungin	**10q**	**10ac**	**10ag**
25 mpk PO	-	-4.57 ± 0.85 (100)***	-4.62 ± 0.85 (100)***	-4.62 ± 0.85 (80)***
12.5 mpk PO	-	-4.37 ± 0.93 (40)***	-4.62 ± 0.85 (100)***	-3.92 ± 0.98 (20)***
6.25 mpk PO	-	-3.50 ± 1.10 (0)***	-3.62 ± 1.03 (0)***	-2.89 ± 1.40 (0)**
12.5 mpk IP	-	-4.02 ± 0.53 (100)***	-3.99 ± 0.53 (100)***	-4.00 ± 0.53 (100)***
0.5 mpk IP	-3.96 ± 0.53 (100)***	-	-	-
0.125 mpk IP	-4.00 ± 0.53 (100)***	-	-	-
0.03 mpk IP	-3.28 ± 0.93 (20)***	-	-	-
0.0078 mpk IP	-3.62 ± 0.75 (60)***	-	-	-
ED_{99} mpk (95% C.I.)[b]	<0.0001	1.2 (0.22 – 6.3)	0.84 (0.11 – 6.2)	3.8 (2.1 – 7.0)
Efficacy in Aspergillosis Survival Model				
Dose		**% Survival (Mean Survival Time)**		
	Caspofungin	**10q**	**10ac**	**10ag**
12.5 mpk IP	-	60 (14.7 days)	90 (19.4 days)	60 (14.6 days)
6.25 mpk IP	-	40 (12.4 days)	90 (19.2 days)	40 (12.5 days)
3.125 mpk IP	-	30 (10.7 days)	20 (12.2 days)	10 (7.3 days)
2 mpk IP	90 (19.8 days)	-	-	-
1.56 mpk IP	-	0 (4.2 days)	0 (5.1 days)	0 (4.1 days)
0.5 mpk IP	90 (19.4 days)	-	-	-
0.125 mpk IP	60 (14.7 days)	-	-	-
0.03 mpk IP	50 (13.1 days)	-	-	-
ED_{50} mpk (95% C.I.)[b]	0.07 (0.03 – 0.16)	8.2 (4.9 – 14)	4.5 (3.4 – 6.0)	11 (6.5 – 17)
ED_{90} mpk (95% C.I.)[b]	0.83 (0.22 – 3.1)	31 (10 – 96)	8.9 (5.8 – 14)	26 (10 – 66)

[a]Change in colony forming units/g kidney relative to sham treated control animals.
[b]95% confidence interval.
*$p < 0.05$ for CFUs/g kidney versus sham treated control animals (two tail t test).
**$p < 0.01$ for CFUs/g kidney versus sham treated control animals (two tail t test).
***$p < 0.001$ for CFUs/g kidney versus sham treated control animals (two tail t test).

Public

Journal Pre-proofs

surv ... its at these doses was nearly identical for **10q** and **10ag**, but at 3.125 mpk, **10q** achieved 30% survival with a mean survival time of 10.7 days versus 10% and 7.3 days for **10ag**. Compound **10ac** demonstrated exceptional efficacy with 90% survival at both 12.5 and 6.25 mpk with mean survival of over 19 days. The efficacy of **10ac** fell precipitously with only 20% survival at 3.125 mpk, though the mean survival time (12.2 days) was longer than **10q** or **10ag**. At the 1.56 mpk dose, 100% mortality was observed for all three analogs with only a 1-2 day extension in mean survival time. The higher survival rates observed for **10ac** translated into 2-3X improvements in ED_{50} (effective dose for 50% survival) and ED_{90} (effective dose for 90% survival) relative to **10q** and **10ag**.

Due to its superior efficacy in the aspergillosis survival model, antifungal susceptibility testing against additional fungal species was performed with **10ac** to determine its spectrum of activity (**Table 5**). Compound **10ac** demonstrated antifungal activity against the clinically relevant yeast and mold species displayed in **Table 5**. Higher concentrations of **10ac** were required to achieve complete inhibition of growth (MIC-100) for *Candida krusei* and *Candida tropicalis*, though prominent inhibition (MIC-50) was observed at 2-8 fold lower concentrations. Testing against expanded panels of *Candida* (113 isolates) and *Aspergillus* (71 isolates) species further established **10ac** as a potent, broad spectrum antifungal.[34-35] Additional studies demonstrated that **10ac** is active against a majority of echinocandin resistant *Candida* and *Aspergillus* isolates as well as the recently emerging multi-drug resistant *Candida auris*.[36-38]

Table 5
Extended antifungal activity spectrum of **10ac**

Antifungal activity of 10ac verses Candida and Aspergillus species		
Species	**MIC-100[a] µg/mL**	**MIC-50[b] µg/mL**
C. *albicans* (MY1055)	0.06	<0.03
(+50% mouse serum)	0.5	0.25
C. *glabrata* (MY1381)	1	1
C. *krusei* (ATCC6258)	4	2
C. *tropicalis* (MY1012)	8	1
C. *lusitaniae* (MY1396)	2	1
C. *parapsilosis* (ATTC22109)	0.5	<0.03
Species	**MEC µg/mL**	
A. *fumigatus* (MF5668)	<0.03	
(+50% human serum)	0.25	

[a]Lowest concentration of compound to completely inhibit fungal growth.
[b]Lowest concentration of compound to restrict fungal growth by 50%.

In conclusion, a scan of 3-alkyl and aryl-2-[1,2,4-triazole] substituents identified 3-(4-pyridyl)-2-[1,2,4-triazole] as the optimal replacement for the 3-carboxamide-2-[1,2,4-triazole] substituent of MK-5204 **(3)**.[19] The 4-pyridyl substituent of **10q** ((*R*) isopropyl, methyl aminoether) produced a 4-fold improvement in antifungal activity in the presence of serum relative to **3** in conjunction with a 1.5X increase in oral exposure. Re-optimization of the alkyl substituents alpha to the amine of the C3 aminoether in the presence of the 3-(4-pyridyl)-2-[1,2,4-triazole] substituent at C2, established (*R*) t-butyl, methyl **(10ac)** as the preferred C3 aminoether with a 2X increase in oral exposure over **3**, while maintaining the 4-fold improvement in antifungal activity in the presence of serum observed for **10q**. The concurrent improvements in these two parameters, which had previously correlated with oral efficacy, resulted in a drastic improvement in the 7-day TOKA ED_{99} for **10ac** (0.84 mpk) relative to MK-5204 **(3)** (6.4 mpk).[19] Compound **10q** demonstrated similar efficacy to **10ac** in the 7-day TOKA, but **10ac** displayed superior efficacy in the aspergillosis survival model and exhibited a favorable rhesus PK profile compared to **10q**. As a result of these advantages and its broad spectrum of antifungal activity for *Candida* and *Aspergillus* species, **10ac** was selected as the development improved efficacy profile in the 7-day TOKA and aspergillosis survival model, the clinical development of ibrexafungerp was prioritized over MK-5204. Having progressed to phase III clinical trials, ibrexafungerp has the potential to become an important antifungal therapy with benefits over existing options due to its oral efficacy and broad spectrum antifungal activity, which includes echinocandin resistant isolates and *Candida auris*.

Acknowledgements

The authors wish to thank Dr. Ashok Arasappan, Dr. Jason Cox, and Ms. Helen Chen for their helpful suggestions in the preparation of this manuscript.

Appendix A – Supplemental Data

Supplementary data for this article can be found online at…

References and notes

1. Kullberg, B. J.; Arendrup, M. C. *N. Engl. J. Med.* **2015**, *373*, 1445.
2. Kauffman C. A. *Proc. Am. Thorac. Soc.* **2006**, *3*, 35.
3. Tragiannidis A.; Tsoulas, C.; Kerl, K.; Groll, A. H. *Expert Opin. Pharmacother.* **2013**, *14*, 1515.
4. Moriyama, B.; Gordon, L. A.; McCarthy, M.; Henning, S. A.; Walsh, T. J.; Penzak, S. R. *Mycoses* **2014**, *57*, 718.
5. Pfaller, M. A.; Diekema, D. J. *Clin. Microbiol. Rev.* **2007**, *20*, 133.
6. Denning, D. W.; Hope, W. W. *Trends Microbiol.* **2010**, *18*, 195.
7. McCarthy, M. W.; Walsh, T. J. *Expert Opin. Invest. Drugs* **2017**, *26*, 825.
8. Arendrup, M. C.; Perlin, D. S. *Curr. Opin. Infect. Dis.* **2014**, *27*, 484.
9. Pfaller, M. A. *Am. J.Med.* **2012**, *125*, S3.
10. Arikan-Akdagli, S.; Ghannoum, M.; Meis, J. F. *J. Fungi* **2018**, *4*, 129.
11. Lockhart, S. R. *Fungal Genet. Biol.* **2019**, *131*, 103243.
12. Odds, F. C.; Brown, A. J.P.; Gow, N. A.R. *Trends Microbiol.* **2003**, *11*, 272.
13. Wiederhold, N. P.; Lewis, R. E. *Expert Opin. Investig. Drugs* **2003**, *12*, 1313.
14. Perlin, D. S. *Future Microbiol.* **2011**, *6*, 441.
15. Abruzzo, G. K.; Flattery, A. M.; Gill, C. J.; Kong, L.; Smith, J. G.; Pikounis, V. B.; Balkovec, J. M.; Bouffard, A. F.; Dropinski, J. F.; Rosen, H.; Kropp, H.; Bartizal, K. *Antimicrob. Agents Chemother.* **1997**, *41*, 2333.
16. Schwartz, R. E.; Smith, S. K.; Onishi, J. C.; Meinz, M.; Kurtz, M.; Giacobbe, R. A.; Wilson, K. E.; Liesch, J.; Zink, D.; Horn, W.; Morris, S.; Cabello, A.; Vicente, F. *J. Am. Chem. Soc.* **2000**, *122*, 4882.
17. Onishi, J; Meinz, M.; Thompson, J.; Curotto, J.; Dreikorn, S.; Rosenbach, M.; Douglas, C.; Abruzzo, G.; Flattery, A.; Kong, L.; Cabello, A.; Vicente, F.; Peláez, F.; Díez, M. T.; Martin, I.; Bills, G.; Giacobbe, R.; Dombrowski, A.; Schwartz, R.; Morris, S.; Harris, G.; Tsipouras, A.; Wilson, K.; Kurtz, M. B. *Antimicrob. Agents Chemother.* **2000**, *44*, 368.
18. Peláez, F.; Cabello, A.; Platas, G.; Díez, M. T.; González del Val, A.; Basilio, A.; Martán, I.; Vicente, F.; Bills, G. F.; Giacobbe, R. A.; Schwartz, R. E.; Onishi, J. C.; Meinz, M. S.; Abruzzo, G. K.; Flattery, A. M.; Kong, L.; Kurtz, M. B. *System. Appl. Microbiol.* **2000**, *23*, 333.
19. Apgar, J. M.; Wilkening, R. R.; Parker Jr., D. L.; Meng, D.; Wildonger, K. J.; Sperbeck, D.; Greenlee, M. L.; Balkovec, J. M.; Flattery, A. M.; Abruzzo, G. K.; Galgoci, A. M.; Giacobbe, R. A.; Gill, C. J.; Hsu, M. J.; Liberator, P.; Misura, A. S.; Motyl, M.; Kahn, J. N.; Powles, M.; Racine, F.; Dragovic, J.; Fan, W.; Kirwan, R.; Lee, S.; Liu, H.; Mamai, A.; Nelson, K.; Peel, M. *Bioorg. Med. Chem. Lett.* **2020**, *30*, 127357.
20. Apgar, J. M.; Wilkening, R. R.; Greenlee, M. L.; Balkovec, J. M., Flattery, A. M.; Abruzzo, G. K.; Galgoci, A. M.; Giacobbe, R. A.; Gill, C. J.; Hsu, M. J.; Liberator, P.; Misura, A. S.; Motyl, M.; Kahn, J. N.; Powles, M.; Racine, F.; Dragovic, J.; Habulihaz, B.; Fan, W.; Kirwan, R.; Lee, S.; Liu, H.; Mamai, A.; Nelson, K.; Peel, M. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 5813.
21. Balkovec, J. M., Bouffard, F. A., Tse, B., Dropinski, J., Meng, D., Greenlee, M. L., Peel, M. R., Fan, W., Mamai, A., Liu, H., Li, K. PCT Intl. Patent Appl., WO 2,007,127,012 A1, 2007.
22. Greenlee, M. L., Wilkening, R., Apgar, J., Wildonger, K. J., Meng, D., Parker, D. L. PCT Intl. Patent Appl. WO 2,010,019,203 A1, 2010.
23. Greenlee, M. L., Wilkening, R., Apgar, J., Sperbeck, D., Wildonger, K. J., Meng, D., Parker, D. L., Pacofsky, G. J., Heasley, B. H., Mamai, A., Nelson, K. PCT Intl. Patent Appl. WO 2,010,019,204 A1, 2010.

Public

24. [illegible] **1979**, *44*, 4160.
25. Roppe, J.; Smith, N. D.; Huang, D.; Tehrani, L.; Wang, B.; Anderson, J.; Brodkin, J.; Chung, J.; Jiang, X.; King, C.; Munoz, B.; Varney, M. A.; Prasit, P.; Cosford, N. D. P. *J. Med. Chem.* **2004**, *47*, 4645.
26. Lecomte, L., Ndzi, B., Queguiner, G., Turck, A. FR 2686340 A1, 1993.
27. Davis, F. A.; Zhou, P,; Liang, C.-H.; Reddy, R. E. *Tetrahedron Asymmetry* **1995**, *6*, 1511.
28. Morton, D.; Pearson, D.; Field, R. A.; Stockman, R. A. *Chem. Commun.* **2006**, *17*, 1833.
29. DBA/2 mice were treated orally (PO) or intraperitoneally (IP) with test compounds twice daily (bid) for two days after being challenged with *C. albicans* (MY1055). Four days after challenge, the kidneys from euthanized mice were removed and analyzed for colony forming units (CFUs) as described in reference 30. The CFUs/g of kidney from the groups of treated animals were compared to the CFUs/g of kidney from sham treated control animals.
30. Bartizal, K.; Abruzzo, G.; Trainor, C.; Krupa, D.; Nollstadt, K.; Schmatz, D.; Schwartz, R.; Hammond, M.; Balkovec, J.; Vanmiddlesworth, F. *Antimicrob. Agents Chemother.* **1992**, *36*, 1648.
31. Tang, T. P., Volkman, S. K., Ellman, J. A. *J. Org. Chem.* **2001**, *66*, 8772.
32. DBA/2N mice were treated bid PO or IP for 7 days after being challenged with *C. albicans* (MY1055) at 2.44 x 10^4 CFU/mouse. Seven days after challenge, kidneys were aseptically collected and analyzed as described in reference 30.
33. 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.
34. Pfaller, M. A.; Messer, S. A.; Motyl, M. R.; Jones, R. N.; Castanheira, M. *J. Antimicrob. Chemother.* **2013**, *68*, 858.
35. Pfaller, M. A.; Messer, S. A.; Motyl, M. R.; Jones, R. N.; Castanheira, M. *Antimicrob. Agents Chemother.* **2013**, *57*, 1065.
36. Jiménez-Ortigosa, C.; Paderu, P.; Motyl, M. R.; Perlin, D. S. *Antimicrob. Agents Chemother.* **2014**, *58*, 1248.
37. Larkin, E.; Hager, C.; Chandra, J.; Mukherjee, P. K.; Retuerto, M.; Salem, I.; Long, L.; Isham, N.; Kovanda, L.; Borroto-Esoda, K.; Wring, S.; Angulo, D.; Ghannoum, M. *Antimicrob. Agents Chemother.* **2017**, *61*, e02396-16.
38. Berkow, E. L.; Angulo, D.; Lockhart, S. R. *Antimbicrob. Agents Chemother.* **2017**, *61*, e00435-17.

Journal Pre-proofs

Public