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Novel Antifungals Based on 4-Substituted Imidazole: Solid-Phase Synthesis of Substituted Aryl Sulfonamides Towards Optimization of In Vitro Activity

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Abstract—The in vitro activity of novel 4-substituted imidazole antifungals was optimized by solid-phase chemistry and parallel synthesis. Potent yeast-selective as well as broad-spectrum antifungal compounds (**32** and **20**) were discovered. © 2000 Elsevier Science Ltd. All rights reserved.

Recently there has been a renewed interest in antifungal drug research and development.[1] The currently available therapy suffers from drug-related toxicity, hazardous drug–drug interactions, less than satisfactory pharmacokinetics, and development of resistance. Enzymes in the ergosterol-biosynthetic pathway, specifically, the lanosterol 14-α demethylase is the target for successful marketed antifungal drugs such as itraconazole as well as several promising agents in advanced clinical study such as voriconazole.[2] We have recently disclosed 1*H*-imidazole 4-methaneamine sulfonamides as a novel new class of imidazole-based antifungal agents.[3] These compounds inhibit fungal ergosterol synthesis in much the same manner as the triazole antifungal drugs. The in vitro activity spectrum and overall potency against yeasts compared favorably with itraconazole. We started from an initial screening hit, compound **1** (Fig. 1), which showed an overall MIC against yeasts of approximately 1 μM. Optimization via solid-phase synthesis led to molecules such as **2** with an MIC of ~ 0.1 μM.

A number of factors contributed towards further exploration of these structures. While the in vitro activity against yeasts was adequate, we still needed to enhance activity against molds and gain activity against certain azole resistant yeasts. We were also interested in learning how our molecules may be interacting at the target enzyme viz the demethylase active site. An understanding of such interaction should help in the design of more potent ergosterol synthesis inhibitors. In addition, this work was part of an ongoing combinatorial chemistry effort and benefits were expected from the expansion and development of library synthesis tools. 4-Substituted imidazoles have recently been reported to have activity as farnesyl transferase inhibitors[4] and libraries prepared for this study were therefore of interest in the oncology area.

A molecular modeling study of our lead structures in an active site model built from available coordinates of Cyt-P-450$_{\mathrm{cam}}$ was undertaken.[5,6] This study was complimented with comparative assessments of similar docking of itraconazole. The imidazole N-3 atom was placed in coordinating distance to the heme iron, while the amine was accommodated in a narrow hydrophobic area. Intuitive considerations led to conclusion that the 4-position of the benzene sulfonamide should be investigated further to more fully explore in vitro structure–activity relationships (SARa). A combinatorial chemistry approach was then utilized to evaluate this observation towards the main goal of improved potency and spectrum of antifungal activity. Work towards optimization of the aryl sulfonamide moiety along this thinking is described.

The starting material, 4-formyl imidazole was attached to solid support via 2-chlorotrityl linker.[7] Reaction with the desired amines under reductive conditions[8] gave the secondary amine products **4** (Scheme 1). Three different solid-phase approaches were utilized towards modification of

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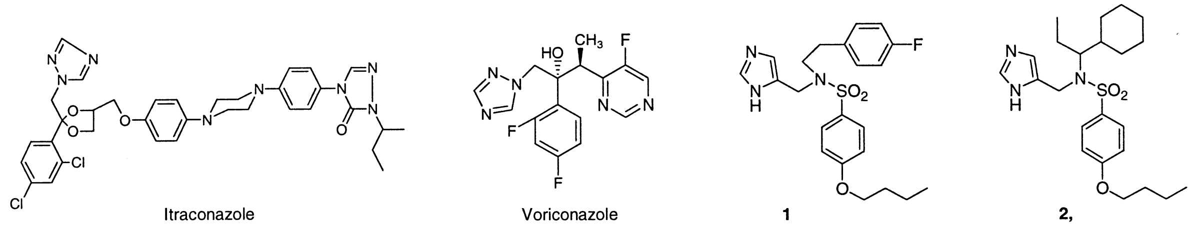
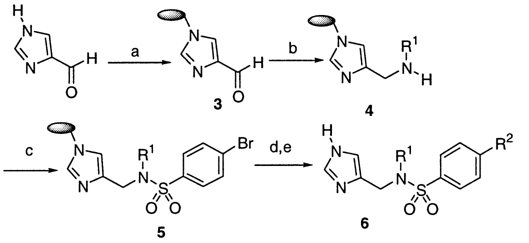


Figure 1.

the aryl group. Sulfonylation with 4-bromo phenyl sulfonyl chloride gave the intermediate bromo phenyl sulfonamides **5**. Suzuki arylation reactions[9] on these intermediates followed by resin cleavage gave the desired biaryl sulfonamide products **6** in excellent yield and purity. The same intermediates were subjected to Pd-catalyzed aryl amination reaction[10] to produce aminoaryl sulfonamides **7** (Scheme 2). A third approach involved reaction of the amine intermediates **4** with 4-bromomethyl benzene sulfonyl chloride to give the sulfonamide intermediate **8**. Subsequent reaction with amines or phenols produced 4-methyleneamino or 4-methyleneoxo modified sulfonamides **9** (Scheme 3). All products were released from resin by treatment with 10% trifluoroacetic acid in methylene chloride and submitted for screening as single pure compounds after any necessary HPLC purification on a Gilson preparative system.[11] All compounds described here were prepared by parallel synthesis in manual mode employing an inexpensive glass/Teflon/polypropylene reaction apparatus employing inverse filtration for resin wash.[12] Approximately 100 mg of resin or 0.1 mmol was employed in the synthesis of library compounds, which usually led to 15–25 mg of the final compound. Characterization was by NMR, HPLC, and MS.

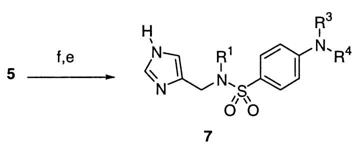
Details on methods for in vitro screening have been described.[13,14] In brief, a composite pLAD (negative logarithm) value was obtained indicative of the concentration at which 65% growth inhibition is achieved over selected sets of yeasts (Y) and pathogenic molds (M). A pLAD$_{Y}$ value of 6 for example implied minimum inhibition concentration of 1 μM (MIC) over the set of selected yeast strains. Inhibition of ergosterol synthesis is measured in a *Candida albicans* subcellular screen and is expressed as IC$_{50}$ values.[15]



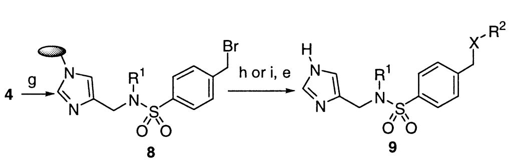
Scheme 1. Reaction scheme for synthesis of biaryl sulfonamides. Reagents and conditions: (a) 2-Cl trityl resin, DMF:DCE:Et$_3$N; (b) R^{1}NH$_2$, 1% HOAc–DCE, NaBH(OAc)$_3$; (c) 4-bromo benzene sulfonyl chloride, *i*Pr$_2$EtN, CH$_2$Cl$_2$; (d) R^{2}B(OH)$_2$, Pd(PPh$_3$)$_4$, 2 M Na$_2$CO$_3$, dioxane 80 °C; (e) 10% CF$_3$CO$_2$H in CH$_2$Cl$_2$, 30 min;

Our earlier study indicated that the non-functionalized alkylamines, arylalkyl amines, and branched alkyl amines were consistently associated with good in vitro activity. For the present study, representative compounds carrying these groups at the R^1 position are described. Screening results from the Suzuki library revealed that biaryl sulfonamides such as **10**, **11**, **12**, and **13** (Table 1) had growth inhibitory activity against yeasts similar to the 4-butoxy benzene sulfonamides such as **1**. Phenyl groups therefore appeared to be excellent substitutes for the 4-butoxy group in our original leads. Inclusion of a polar group (acetamido in the terminal phenyl group, **14**) led to complete loss of activity. The unbranched group *n*-hexyl (**16**) and the cyclohexyl analogue (**15**) showed lower activity relative to the cyclohexylmethyl analogue (**13**). The highly branched alkyl moiety 2,4-dimethyl-pent-3-yl group when combined with biaryl sulfonamides (**17**–**20**) showed distinctly better activity against *Candida* strains than the cyclohexylmethyl analogues. In addition, an increase in the activity against pathogenic molds was observed. For example, biphenyl sulfonamide **19** inhibited yeast and mold strains with pLAD values of 6.6 and 5.2, respectively. The 4-fluoro substitution in the terminal phenyl group (**20**) led to a further increase in potency against the yeasts. Both these compounds demonstrated inhibitory activity against the resistant strain *C. krusei* as well as the therapeutically critical *Aspergillus fumigatus*. They also inhibited ergosterol biosynthesis in our subcellular screen with IC$_{50}$ values near 10 nM.

The screening data from 4-aminoaryl sulfonamides are shown in Table 2. Smaller alkylamines were associated with poor activity (**23** and **28**). Substitutions with more polar groups led to complete loss of activity (**21** and **26**). An exception was the isoamyl analogue **34**, which showed

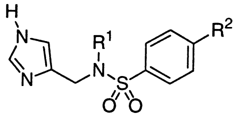


Scheme 2. Synthesis of aminoaryl sulfonamides. Reagents and conditons: (e) 10% CF$_3$CO$_2$H in CH$_2$Cl$_2$, 30 min; (f) Pd$_2$dba$_3$, *rac*-BINAP, R^{3}R^{4}NH, 80°C.



Scheme 3. Synthesis of 4-aminomethyl benzene sulfonamides. Reagents and conditions: (g) 4-BrCH$_2$PhSO$_2$Cl, *i*Pr$_2$EtN, CH$_2$Cl$_2$; (h) R^{2}NH, DMSO, (for X = N); (i) R^{2}OH, aq KOH, DMSO (for X = O).

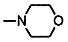
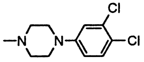
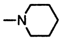
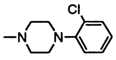
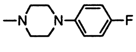
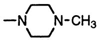
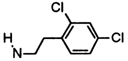
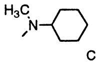
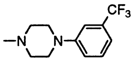
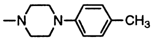
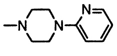
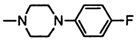
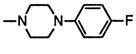
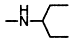
Table 1. In vitro antifungal activity of biaryl sulfonamides



Compd	R^1	R^2	$pLAD_Y$	$pLAD_M$	Ergosterol IC_{50} (μM)[a]
10	Cyclohexylmethyl	3-Thiophene	6.1	4.4	na[b]
11	Cyclohexylmethyl	4-Methoxyphenyl	6.3	4.4	na[b]
12	Cyclohexylmethyl	4-Chlorophenyl	6.3	4.4	na[b]
13	Cyclohexylmethyl	4-Fluorophenyl	6.3	4.4	na[b]
14	Cyclohexylmethyl	3-Acetamidophenyl	4.1	4.0	na[b]
15	Cyclohexyl	4-Fluorophenyl	5.8	4.4	na[b]
16	*n*-Hexyl	4-Fluorophenyl	5.9	4.8	na[b]
17	2,4-Dimethyl pent-3-yl	4-Methoxyphenyl	6.4	5.0	0.0199
18	2,4-Dimethyl pent-3-yl	4-Chlorophenyl	6.4	5.4	0.0167
19	2,4-Dimethyl pent-3-yl	Phenyl	6.6	5.2	0.0133
20	2,4-Dimethyl pent-3-yl	4-Fluorophenyl	6.8	5.2	0.0087

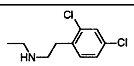
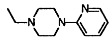
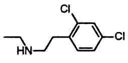
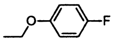
[a] IC_{50} value for inhibition of ergosterol synthesis in a *Candida albicans* subcellular screen.[9]
[b] na = not available.

Table 2. In vitro antifungal activity of aminoaryl sulfonamides

Compd	R^1	R^2	$pLAD_Y$	$pLAD_M$	Ergosterol IC_{50} (μM)[a]
21	2-F-Phenethyl		4.5	4.0	na[b]
22	2-F-Phenethyl		5.8	4.0	na[b]
23	2-F-Phenethyl		5.9	4.2	na[b]
24	2-F-Phenethyl		6.0	4.2	na[b]
25	2-F-Phenethyl		6.8	4.4	0.0034
26	Cyclohexylmethyl		4.6	4.0	na[b]
27	Cyclohexylmethyl		5.4	4.0	na[b]
28	Cyclohexylmethyl		5.5	4.0	na[b]
29	Cyclohexylmethyl		6.3	4.0	0.0394
30	Cyclohexylmethyl		6.3	4.0	na[b]
31	Cyclohexylmethyl		6.4	4.0	0.0178
32	Cyclohexylmethyl		6.9	4.0	0.0169
33	2,4-Dimethyl pent-3-yl		6.4	4.0	0.0248
34	2,4-Dimethyl pent-3-yl		6.5	4.0	0.0107

[a] IC_{50} value for inhibition of ergosterol synthesis in a *Candida albicans* subcellular screen.[9]
[b] na = not available.

Table 3. In vitro antifungal activity of 4-aminomethyl benzene sulfonamides

Compd	R^1	R^2	$pLAD_Y$	$pLAD_M$	Ergosterol IC_{50} (μM)[a]
35	Cyclohexylmethyl		6.5	4.4	0.0153
36	Cyclohexylmethyl		6.4	4.0	0.0167
37	4-F-phenethyl		6.1	4.4	na[b]
38	Cyclohexylmethyl		6.1	4.4	na[b]

[a] IC_{50} value for inhibition of ergosterol synthesis in a *Candida albicans* subcellular screen.[9]
[b] na = not available.

Table 4. Activity spectrum of selected compounds and comparison with reference compounds

Compd	MIC (μM)													
	C. albicans 1	*C. albicans* 2	*C. albicans* 4	*C. glabrata*	*C. kefyr*	*C. krusei*	*C. parapsilosis*	*C. tropicalis*	*M. canis*	*T. rubrum*	*T. mentagrophytes*	*Cr. neoformans*	*A. fumigatus*	*S. schenckii*
Itraconazole	>10	≤0.1	>10	1	≤0.1	1	≤0.1	0.1	≤0.1	≤0.1	0.1	≤0.1	1	10
Voriconazole	>10	< 0.1	na	10	≤0.1	10	≤0.1	1	1	≤0.1	≤0.1	1	1	>10
1	>10	≤0.1	10	10	≤0.1	>10	1	≤0.1	10	1	>10	1	>10	>10
2	>10	≤0.1	≤0.1	1	≤0.1	1	≤0.1	1	1	1	10	1	>10	>10
32	>10	≤0.1	≤0.1	10	≤0.1	10	≤0.1	≤0.1	10	10	>10	1	>10	>10
20	>10	≤0.1	1	1	≤0.1	1	≤0.1	≤0.1	1	1	10	≤0.1	10	>10
35	10	≤0.1	1	10	≤0.1	1	< 0.1	1	10	10	>10	1	>10	>10

a $pLAD_Y$ value of 6.5. The 2,4-dichloro-phenethyl analogue **27** was less active. We incorporated a series of *N*-phenyl piperazines to examine if extending the terminal phenyl group further would have an effect. Indeed, all such analogues demonstrated an enhanced activity against the yeasts. The 4-fluoro-phenyl piperazine analogues **25** and **32** demonstrated extremely potent inhibition of yeast strains with $pLAD_Y$ values over 6.8 and 6.9, respectively. Sterol biosynthesis was inhibited by compound **25** with a remarkable potency of 3 nM (IC_{50}). This compound demonstrated a 163-fold selectivity for inhibition of yeast sterol versus mammalian cholesterol synthesis. These analogues were essentially inactive against mold strains making them uniquely yeast selective.

The representative results from *n*-butoxy replacements obtained via reaction Scheme 3 are shown in Table 3. The 4-fluorophenoxymethyl analogue **38** was the only oxo-based group that retained the yeast activity. The extending of the aryl piperazine ring by one carbon (**36** vs **31**) did not seem to have any appreciable impact on the activity. The 2,4-dichloro-phenethyl analogue **35** interestingly demonstrated activity against the azole resistant strain *C. albicans* 1, while the overall $pLAD_Y$ value improved relative to the lower homologue **27**. It also demonstrated an impressive IC_{50} value of 15 nM for inhibition of sterol biosynthesis.

The overall spectrum of activity for the best compounds (**20**, **32**, and **35**), in this series is compared to screening results for reference compounds in Table 4. Examination of this data clearly shows that these compounds had improved spectrum over our original leads **1** and **2**. The in vitro potency and spectrum against *Candida* strains was comparable or superior to itraconazole and voriconazole. Nitrogen bearing aryl sulfonamides **32** and **35** were not active against molds. The spectrum for compound **20** was the most superior with good activity observed against *Mucosporum canis*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*.

In conclusion, continued optimization of 4-substituted imidazole antifungals by combinatorial chemistry has led to highly yeast-selective as well as potent broad-spectrum antifungal agents. These molecules and their study may provide stimulus for further research towards discovery of clinically successful new antifungal drugs.

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14. In vitro screening was conducted against eight isolates of *Candida* spp., including strains known to be resistant, three isolates of dermatophyte species and one each of *Cryptococcus neoformans, Aspergillus fumigatus,* and *Sporothrix schenckii.* Growth was designated as inhibited when it was reduced below 35% of control and MICs against each test fungus were recorded as the lowest concentration of a compound designated as inhibitory by this definition. Final data were reported as the negative log (pLAD value) of the lowest concentration.

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