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Novel Antifungals Based on 4-Substituted Imidazole: A Combinatorial Chemistry Approach to Lead Discovery and Optimization

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Abstract—A series of 4-substituted imidazole sulfonamides has been prepared by solid-phase chemistry. These compounds were found to have good in vitro antifungal activity and constitute the first examples of C-linked azoles with such activity. The most potent inhibitor (**30**) demonstrated inhibition of key *Candida* strains at an in vitro concentration of <100 nM and compared favorably with in vitro potency of itraconazole. © 2000 Elsevier Science Ltd. All rights reserved.

Efforts to discover new antifungal agents continue to this day, even as breakthrough products such as itraconazole, fluconazole, and recently voriconazole (in phase III clinical trial) have reached significant clinical successes[1] (Fig. 1). This is largely due to the perceived threat of emerging new pathogenic fungi and resistance of many strains to existing therapy.[2] In spite of significant research on alternative mechanisms,[3] the triazole class of drugs remains the mainstay of therapy for systemic life threatening fungal infections. These drugs, often referred as azole antifungals, share a common mode of action in that they inhibit Cyt-P450 dependent sterol 14-α-demethylase.[4] Inhibition of this enzyme results in depletion of ergosterol, an essential sterol for fungal cell membrane synthesis. Nearly all azole antifungals described in the literature are *N*-substituted azoles. In addition, an azolyl phenethyl amine pharmacophore (**A**, Fig. 1) is uniquely shared by all azole antifungals. We have synthesized focused libraries with the 1*H*-imidazole-4-methylaminosulfonamide structure as part of a combinatorial chemistry program and discovered potent new antifungal lead compounds. Synthesis, in vitro activity profile and structure–activity relationship (SAR) data are described.

We employed a solid-phase strategy for the synthesis of our exploratory and lead optimization libraries. 4-Formyl imidazole was chosen as the starting material of choice due to the ease with which the formyl group can be derivatized on solid support. In addition, the imidazole N-1 atom can be readily attached to support prior to synthesis and cleaved after synthesis, thus allowing for traceless synthesis of target compounds. Reaction with 2-chlorotrityl chloride PS resin[5] in the presence of triethyl amine (Scheme 1A) gave the immobilized aldehyde **1**. The extent of loading was assessed from aldehyde recovered after cleavage of measured amounts of dried resin. It was generally found that this corresponded closely to loading of the trityl linker (0.9–1.3 mmols/g) reported for the commercial batches utilized. Reductive amination[6] of resin bound aldehyde **1** with primary amines in the presence of 1% acetic acid went smoothly to give secondary amine products **2** (Scheme 1A). A very large variety of amines could be utilized for this reaction with excellent results. Simple coupling of the amines **2** with sulfonyl chlorides gave sulfonamide products **3**, which upon resin cleavage with trifluoroacetic acid gave the products **6**. 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. Approximately 100 mg of resin or 0.1 mmol was employed in the synthesis of library compounds, which usually led to 7–20 mg of final compound. All compounds submitted for screening were >90% pure by HPLC either after cleavage from resin or after HPLC purification. Characterization was by NMR, HPLC and MS.

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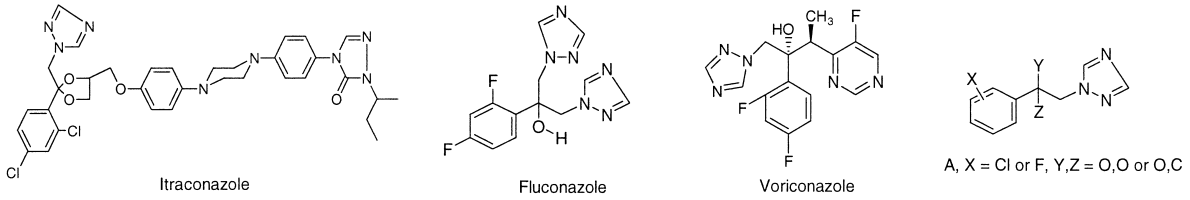
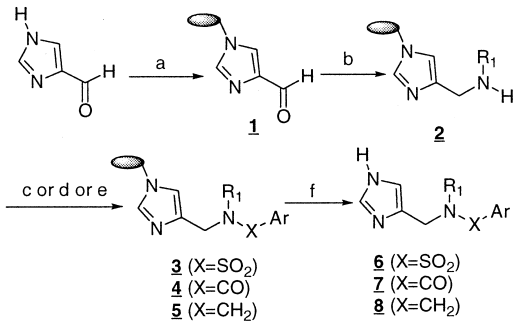
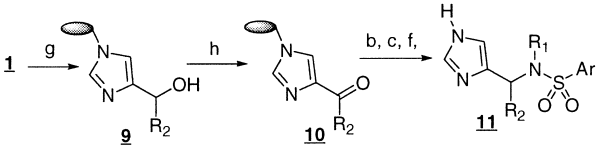


Figure 1. Representative azole antifungals.

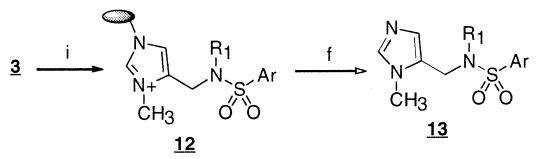


Scheme 1A. Basic reaction scheme for synthesis of 1*H*-imidazole-4-methylaminosulfonamide.

The amines **2** could also be reacted with commercially available acid chlorides to give amide products **7** after cleavage. Reactions with aldehydes under reductive conditions gave tertiary amine products **8** in excellent yield and purity. Grignard reaction with resin bound aldehyde **1** at 60 °C gave the secondary alcohol **9** (Scheme 1B), which was oxidized smoothly to ketone **10** with iodoxy benzoic acid.[7] Reaction sequence of reductive amination and sulfonylation followed by resin cleavage gave the desired α-substituted analogues **11** cleanly. Resin bound sulfonamides **3** could be selectively methylated at the imidazole ring N-3 atom by reaction with methyl triflate followed by cleavage to give products such as **13**.



Scheme 1B. Grignard addition and IBX oxidation towards α-substituted compounds.

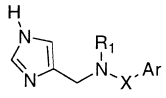


Scheme 1C. Selective N-3 methylation. Reagents and conditions: (a) 2-Cl Trityl resin, DMF:DCE:Et_3N; (b) R_1NH_2, 1% HOAc–DCE, $NaBH(OAc)_3$; (c) $ArSO_2Cl$, iPr_2EtN, CH_2Cl_2; (d) ArCOCl, iPr_2EtN, CH_2Cl_2; (e) ArCHO, 1% HOAc–DCE, $NaBH(OAc)_3$; (f) 10% CF_3CO_2H in CH_2Cl_2, 30 min; (g) R_2MgBr (1 M in THF), THF, 60 °C, 16 h; (h) IBX, DMSO, rt, 24 h; (i) $CH_3OSO_2CF_3$, CH_2Cl_2, rt, 2 h.

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.[8] A value of 7, for example, implied minimum inhibition at a concentration of 0.1 M (MIC). A composite picture was gathered by creating an average of the pLAD values against yeasts ($pLAD_Y$) and molds ($pLAD_M$). A $pLAD_Y$ value of 6, for example, indicates an average MIC of 1 μM. Ergosterol synthesis inhibition data as measured in a *C. albicans* subcellular screen are expressed as IC_{50} values.[9] The results from screening of our initial library of compounds showed that compound **14** had a $pLAD_Y$ of 6.2. The MICs for *Candida albicans* 2, *Candida tropicalis* and *Candida keyfer* were at or below 10–100 nM. The overall spectrum of activity resembled a pattern similar to classical azoles. Indeed, when they were tested for inhibition of ergosterol synthesis in a *C. albicans* subcellular screen, excellent activity was found. Compound **14** inhibited ergosterol syn-thesis in this screen with an IC_{50} of 26 nM. This compared to an IC_{50} of 1.3 μM for mammalian cholesterol synthesis, a 50-fold selectivity for the yeast enzyme. Final evidence that our compounds were acting in the same mode as classical azoles by inhibiting fungal sterol 14-α-demethylase was found with an IC_{50} determination (47 nM) for compound **14** against the isolated enzyme. This compound did not interact with rabbit liver microsomes (generally indicative of Cyt-P-450 mediated degradation; IC_{50} >10 μM). We were unaware of any reports of antifungal activity from 4-substituted 1*H*-imidazoles such as described here. These structures are not N-linked azoles and do not contain a phenethyl amine type pharmacophore, the features universally common to all azole antifungals. With this in mind, we justified a limited synthesis project with the goal of exploring SAR and broadening the spectrum of in vitro activity. Specifically, our aim was to improve the in vitro activity to $pLAD_Y$ >6.5, with evidence of activity against azole-resistant strains such as *C. albicans* 1 and *C. krusei*. An improvement of activity against pathogenic mold strains ($pLAD_M$ >5) with evidence of activity against *Aspergillus*.

Our SAR exploration initially focused on systematic exploration of side chains derived from commercially

Table 1. In vitro antifungal activity of selected compounds



No.	R_1	Ar	X	$pLAD_Y$	$pLAD_M$	Ergosterol (μM)[a]
14	4-Fluorophenethyl	4-*n*-Butyloxy phenyl	SO_2	6.2	4.6	0.026
15	4-Phenyl butyl	4-*n*-Butyloxy phenyl	SO_2	5.4	5.2	0.027
16	*n*-Hexyl	4-*n*-Butyloxy phenyl	SO_2	6.1	5.2	0.076
17	Thiophene-2-ethyl	4-*n*-Butyloxy phenyl	SO_2	6.5	4.4	0.011
18	Isoamyl	4-*n*-Pentyl phenyl	SO_2	6.4	5	0.12
19	2,4-Dimethyl pent-3-yl	4-*n*-Butyloxy phenyl	SO_2	6.7	5	0.073
20	2,4-Dimethyl pent-3-yl	2,5-Dichlorothiophen-3-yl	SO_2	6.3	4.5	0.071
21	4-Bromophenethyl	4-Biphenyl	CH_2	4.9	5.8	1.24

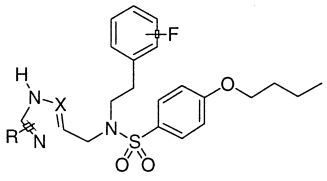
[a]IC_{50} value for inhibition of ergosterol synthesis in a *C. albicans* subcellular screen.[9]

available amines and sulfonyl chlorides. Thus, combinatorial libraries were prepared such that 12–15 aryl and heteroaryl groups were matched with more than 80 miscellaneous R_1 groups to rapidly produce hundreds of single discreet compounds for the moderate throughput antifungal screens. Selected compounds that showed activity improvement are disclosed in the following discussion. Among the arylsulfonamide groups, the 4-*n*-butyloxy phenyl group was consistently better that other related groups. Polar arylsulfonamides such as 4-trifluoromethyl-phenyl, heteroaryl and even 4-methoxy-phenyl were associated with weak to no activity (data not shown). The 4-*n*-pentyl phenyl and the 2,5-dichlorothiophen-3-yl were the only other aryl groups associated with activity. A total of 88 groups were explored to study the SAR for the R_1 groups while retaining the benzene sulfonamide groups more often associated with activity. Benzyl substituents generally had lower activity. Amine side chains with polar groups (e.g., hydroxyl or amine bearing groups), aryl groups as well as heteroaryl groups were inactive. The 4-phenylbutyl (**15**) and *n*-hexyl (**16**) analogues showed improvement of mold activity, but activity against yeasts were lower. Among the arylalkyl groups, only the thiophene-2-ethyl group (**17**) demonstrated a better activity against the yeasts including inhibition of *C. krusei* at 10 μM. Several compounds with cycloalkyl or branched alkyl chains showed improvement of antifungal activity (e.g., **18**, **19**, and **20**). Compound **19** had a $pLAD_Y$ of 6.7 with moderate to strong activity against all *Candida* strains except for *C. albicans* 1. Most of the active compounds inhibited ergosterol synthesis in the *C. albicans* subcellular screen at potencies well below 100 nM (Table 1).

Replacement of the sulfonamide with carboxamide, while the better chains are retained led to complete loss of activity. When the sulfonamide was replaced with a single methylene group, most compounds were inactive except for a few such as compound **21** with good anti-mold activity but far weaker activity against yeasts. The effect of substitution of the imidazole ring with methyl groups at position 5 and 2 (**22** and **23**) was investigated and shown to destroy activity altogether (Table 2). The replacement of the imidazole ring with 1,2,4-triazole, interestingly also led to complete loss of activity (**24**). These results demonstrated that antifungal activity was very specific to the 4-substituted 1*H*-imidazole as core pharmacophore.

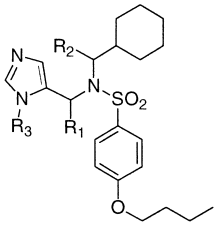
Substitution at the methylene α- to the imidazole ring was explored by introduction of methyl and phenyl groups (Table 3). The methyl substituent was tolerated well, however the phenyl substitution led to loss of

Table 2. Activity of compounds where the heterocycle is modified



No.	R	F	X	$pLAD_Y$	$pLAD_M$
22	5-Me	2-F	CH	< 4	< 4
23	2-Me	4-F	CH	3.95	3.92
24	H	4-F	N	3.95	3.92

Table 3. Effect of substitution with alkyl groups at various positions



No.	R_1	R_2	R_3	$pLAD_Y$	$pLAD_M$
25	H	H	H	6.1	3.6
26	Me	H	H	6.3	4.0
27	Ph	H	H	5.1	4.0
28	H	(*R*)-Me	H	6.125	4.6
29	H	(*S*)-Me	H	5.9	5.0
30	H	(*S*/*R*)-Et	H	>6.8	5.0
31	H	(*S*/*R*)-Cyclohexyl	H	5.0	4.0
32	H	(*R*)-Me	Me	6.6	4.8

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

Compound	MIC (μM) versus												
	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*
Ketoconazole	>10	0.1	10	10	≤0.1	1	0.1	10	1	0.1	10	0.1	10	10
Itraconazole	>10	≤0.1	>10	1	≤0.1	1	≤0.1	0.1	≤0.1	≤0.1	0.1	≤0.1	1	10
Fluconazole	>10	1	1	>10	1	>10	10	>10	10	10	>10	10	>10	>10
14	>10	≤0.1	10	10	≤0.1	>10	1	≤0.1	10	1	>10	1	>10	>10
19	>10	≤0.1	≤0.1	10	≤0.1	10	0.1	0.1	1	1	10	1	>10	>10
30	>10	≤0.1	≤0.1	1	≤0.1	1	≤0.1	1	1	1	10	1	>10	>10
32	>10	≤0.1	10	1	≤0.1	1	0.1	0.1	1	1	10	1	>10	>10

activity (**26**, **27** vs **25**). The α-substituted cyclohexylmethyl groups showed good improvement in activity. The (*R*)- and (*S*)-methyl derivatives (**28** and **29**) showed similar activity, while the (*S*/*R*)-ethyl analogue **30** had the best overall activity with a $pLAD_Y$ value exceeding 6.8. The larger substituent, viz. (*S*/*R*)-cyclohexyl had little activity. The imidazole N-1 methyl analogue **32** showed a slight increase in activity with an attendant improvement in spectrum. The overall spectrum of activity for the best compounds **19**, **30**, and **32**, in this series is compared to reference compounds in Table 4. Examination of this data clearly shows that these compounds had improved spectrum over our original lead (e.g., compound **14**). Although the spectrum of activity against *Candida* strains was superior to itraconazole, the activity against *Mucosporum canis* and *Cryptococcus neoformans* were weak with still no activity against *Aspergillus fumigatus* and *Sporothrix schenckii*.

Conclusion

In conclusion, a new series of antifungal imidazoles was discovered by combinatorial synthesis. Optimization by parallel synthesis led to identification of potent anti-*Candida* compounds, which inhibit the synthesis of fungal ergosterol synthesis with potencies well below 100 nM. Results of further optimization and in vivo experiments will be described in a future publication.

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