



NOVEL ANTIFUNGAL 2-ARYL-1-(1H-1,2,4-TRIAZOL-1-YL)BUTAN-2-OL DERIVATIVES WITH HIGH ACTIVITY AGAINST *ASPERGILLUS FUMIGATUS*.

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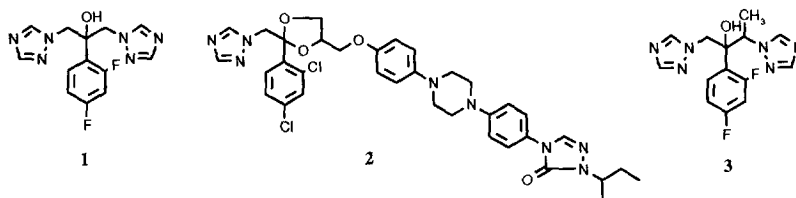
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Abstract: Replacement of one triazole ring of fluconazole with 4-pyridinyl leads to an increase in activity against *Aspergillus fumigatus*. Introduction of an α -methyl group has a marked additional beneficial effect. Investigation of pyridinyl and pyrimidinyl analogues resulted in the identification of **30** (UK-109,496, voriconazole) which has excellent potency against a broad range of fungal pathogens including *A. fumigatus* and *Candida krusei*.

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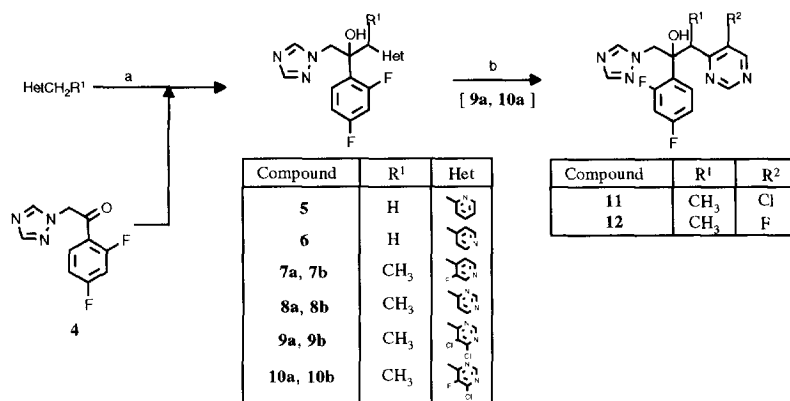
Introduction: Azole antifungal agents, which act by inhibition of the cytochrome P450 enzyme lanosterol 14 α -demethylase, thereby preventing conversion of lanosterol to ergosterol, now play a leading role in the treatment of a variety of fungal infections.^{1,2} Fluconazole (**1**) is the agent of choice for the treatment of infections due to *Candida albicans* and *Cryptococcus neoformans* due to its potency, excellent safety profile, good aqueous solubility and favourable pharmacokinetic characteristics.^{2,3} However, it is poorly effective against *Aspergillus* infections compared with itraconazole (**2**).^{1,2} We were therefore interested in the design of an agent which would combine the favourable features of fluconazole with a broader spectrum of action, including efficacy in aspergillosis.



As part of our follow-up programme to fluconazole, it was shown that introduction of a methyl group α - to one of the triazole rings of **1** to give **3**⁴ resulted in increased potency against *A. fumigatus*.⁵ In this communication, we report that replacement of one of the triazole rings in **1** with a 6-membered heterocycle such as pyridine also leads to an increase in potency against *A. fumigatus*, and introduction of a methyl group α - to the 6-membered heterocycle, gives a marked additional increase.

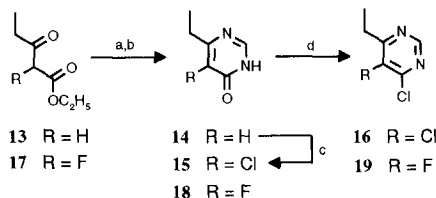
Chemistry:

In general, compounds were prepared by reaction of the anion derived from an alkyl-substituted heterocycle derivative with a suitable (2,4-difluorophenyl) ketone. Thus, treatment of the ketone **4**⁶ with an alkylheterocycle anion gave the products shown in Scheme I. In the cases where an ethyl-substituted heterocycle were used, the products were obtained as mixtures of diastereomer pairs which were separated by chromatography on silica gel into a less polar pair (labelled **a**) and a more polar pair (labelled **b**). Hydrogenolysis of the chloro-substituted pyrimidines **9a** and **10a** gave the halopyrimidine derivatives **11** and **12**. The stereochemistry of the products is discussed in the Results and Discussion section below.

Scheme I

Conditions: (a) LDA/THF; (b) H₂, Pd/C, AcONa, EtOH, 20°C.

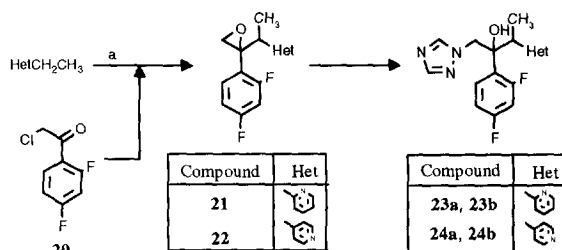
The novel 4-ethyl-3-fluoropyridine precursor to **7a** and **7b** was prepared by treatment of 3-fluoropyridine with LDA in THF at -70°C, followed by iodoethane (-70°C to room temperature). The halopyrimidine intermediates were prepared as summarised in Scheme II. Reaction of the keto ester **13** with formamidine gave the pyrimidinone **14** which was chlorinated to give **15**. Treatment of **15** with POCl₃ gave the dichloropyrimidine **16**. Use of the fluorinated keto ester **17** as starting material gave the pyrimidinone **18** which was converted to **19** by similar treatment with POCl₃.

Scheme II

Conditions: (a) MeONa, MeOH; (b) formamidine acetate; (c) conc. HCl; 30 wt. % H₂O₂; (d) POCl₃, reflux.

Other products were prepared by successive treatment of 2- or 4-ethylpyridine with LDA/THF followed by the chloroketone **20** to give the epoxides **21** and **22** respectively (Scheme III). Reaction of **21** and **22** with 1,2,4-triazole Na salt in DMF gave the products **23a** and **b**, and **24a** and **b**, respectively.

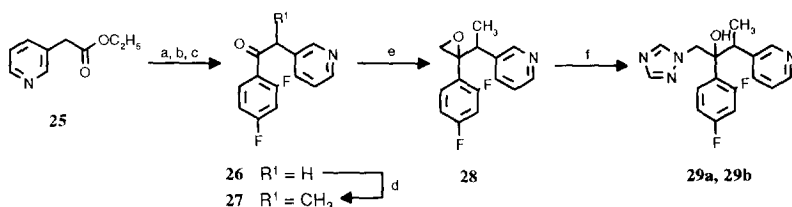
Scheme III



Conditions: (a) LDA/THF; (b) 1,2,4-triazole Na salt, DMF, 60°C.

The 3-pyridinyl isomers **29a** and **29b** were prepared as summarised in Scheme IV. Treatment of the ester **25** with LDA/THF and methyl (2,4-difluorobenzoate) gave, following hydrolysis and decarboxylation of the resulting keto ester, the ketone **26**. This was methylated under phase transfer conditions to give **27**, which was converted to the epoxide **28** using dimethylsulfoxonium methylide. Treatment of **28** with 1,2,4-triazole Na salt as before gave the product as a pair of diastereomers **29a** and **29b**.

Scheme IV



Conditions: (a) LDA/THF; (b) methyl (2,4-difluorobenzoate); (c) conc. HCl, reflux; (d) MeI, NaOH, $\text{Bu}_4\text{N}^+\text{HSO}_4^-$, CHCl_3 , H_2O ; (e) dimethylsulfoxonium methylide, THF; (f) 1,2,4-Triazole Na salt, DMF, 60°C.

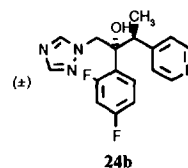
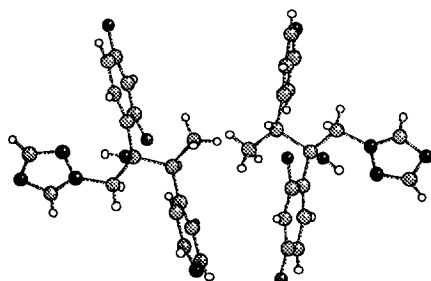
Results and Discussion:

The activity of compounds against *A. fumigatus* is summarised in Table 1.⁷ For the compounds existing as diastereomer pairs, one pair was always markedly more potent than the other as shown by comparison of **23a** and **23b**. With the exception of **23a**, data for the less active pair are omitted for the sake of brevity. For compounds **7,8, 23, 24** and **29**, the more polar pair had the higher activity, but in the case of the halogenated pyrimidine analogues **9** and **10**, the order was reversed.

X-ray crystallography of **24b** showed it to be a racemate with the relative stereochemistry (2*R*,3*S*/2*S*,3*R*) as indicated in Figure 1.⁸ The chemical shift of the α -methyl group (δ 1.11, CDCl_3) in **24b** was at higher field than

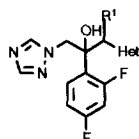
the corresponding signal in **24a** (δ 1.56). For all the more potent diastereomer pairs the chemical shifts of the α -methyl groups were in the range 1.03-1.16, compared with 1.52-1.60 for the less potent pairs. On this basis, it is concluded that the more potent pairs probably have the same relative stereochemistry as **24b**. The more potent diastereomers in a series of analogues of **3** also showed characteristic chemical shifts for the α -methyl groups, and all were shown to belong to the same stereochemical series.⁹

Figure 1



X-Ray crystal structure and relative stereochemistry of **24b**

Table 1



Cpd.	Het	R	Activity against <i>A. fumigatus</i> in vitro		Activity against <i>A. fumigatus</i> in vivo ^c	
			MIC (μ g/ml)	MFC ^a (μ g/ml)	No. of mice surviving	No. of mice cured
1	1 <i>H</i> -1,2,4-triazol-1-yl	H	>50	>50		
2	-	-	0.14	0.19	4/5 ^d	3/5 ^d
3	1 <i>H</i> -1,2,4-triazol-1-yl	CH ₃	12.5	25.0		
5	2-pyridinyl	H	25	ND ^b		
6	4-pyridinyl	H	3.1	ND		
23a	2-pyridinyl	CH ₃	12.5	100		
23b	2-pyridinyl	CH ₃	0.39	1.56		
24b	4-pyridinyl	CH ₃	0.05	0.09	4/5	0/5
29b	3-pyridinyl	CH ₃	0.39	ND		
7b	3-fluoro-4-pyridinyl	CH ₃	0.098	0.19	5/5	5/5
8b	4-pyrimidinyl	CH ₃	0.39	0.39	3/5	0/5
9a	5,6-dichloro-4-pyrimidinyl	CH ₃	3.1	12.5		
10a	5-fluoro-6-chloro-4-pyrimidinyl	CH ₃	3.1	3.1		
11	5-chloro-4-pyrimidinyl	CH ₃	0.39	1.56		
12	5-fluoro-4-pyrimidinyl	CH ₃	0.39	0.78	5/5	4/5

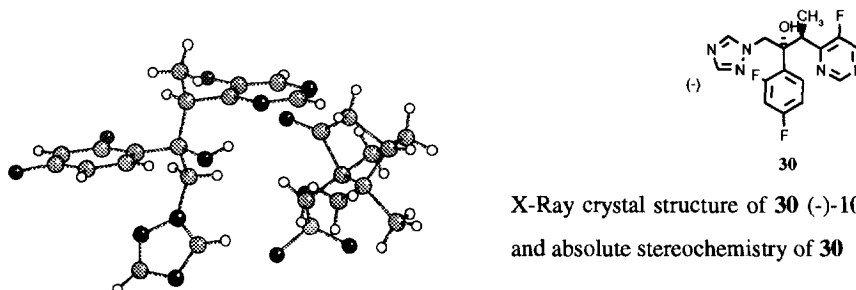
^a Minimum fungicidal concentration giving at least a 90% reduction in colony forming units compared with the drug-free control after 24 hr. incubation. ^b Not determined. ^c Mice were infected intravenously and given a standard oral dose of 20 mg/kg b.i.d. for 5 days commencing 1hr. post infection. Compound efficacy was assessed by means of animal survivors and reduction in kidney fungal burden after 10 days. Kidneys were judged to be cured of *Aspergillus* when no viable organisms could be recovered from tissue homogenates placed on Sabouraud's dextrose agar. ^d Dosed at 50 mg/kg b.i.d.

Compounds **5** and **6** demonstrate that a beneficial effect on the MIC against *A. fumigatus* occurs on replacement of one of the triazole rings of **1** with pyridinyl, particularly a 4-pyridinyl group. A marked further enhancement in potency derives from introduction of a methyl group α - to the pyridine ring (**23b** and **24b**), with the 4-isomer again being the more potent. The 3-pyridinyl and 4-pyrimidinyl analogues **29b** and **8b** were equipotent with **23b**. Introduction of a 3-fluoro substituent into the 4-pyridinyl ring caused a slight reduction in potency *in vitro* (compare **7b** and **24b**). The di-halogenated pyrimidine analogues **9a** and **10a** were also less potent than the unsubstituted compound **8b**, but activity was restored by removal of the 6-chloro substituents to give **11** and **12**, respectively. Several of the compounds were also found to be cidal against *A. fumigatus* at concentrations close to their MICs.

The most promising compounds were examined for their ability to protect mice against *A. fumigatus* infections at a standard dose of 20 mg/kg b.i.d. for 5 days. The 4-pyridinyl and the 4-pyrimidinyl analogues **8b** and **24b** each protected against death for 10 days, although no actual cures were observed. However, introduction of a fluoro substituent had a beneficial effect as shown by the high cure rates achieved with **7b** and **12**. The improved performance of the fluoro analogues *in vivo* is believed to be a consequence of a reduction in susceptibility to metabolic oxidation in the pyridine and pyrimidine rings, leading to a lower rate of clearance.¹⁰

Based on its potency *in vitro* and *in vivo*, together with its more favourable solubility profile, **12** was selected for further evaluation. Resolution of **12** was accomplished by crystallisation of the (-)-10-camphorsulfonic acid salt from methanol, followed by regeneration of the free bases of the individual enantiomers. The activity was found to reside almost entirely in the (-)- enantiomer **30**¹¹ (MIC 0.09 $\mu\text{g/ml}$ against *A. fumigatus* compared with 50 $\mu\text{g/ml}$ for the (+)-enantiomer). X-ray crystallography of the (-)-10-camphorsulfonate salt showed the absolute configuration of **30** to be (2*R*,3*S*) as shown in Figure 2.⁸

Figure 2



X-Ray crystal structure of **30** (-)-10-camphorsulfonate and absolute stereochemistry of **30**

The results in Table 2 show that **30** is more potent than **1** against a range of fungi, and compares favourably with the spectrum of **2**. The increased potency against *C. krusei* is particularly significant since **1** has low potency

against this organism,^{12,13} and there have been reports of increased *C. krusei* infections among immune-compromised patients undergoing prophylactic therapy with **1**.¹⁴⁻¹⁷

Table 2

Compound	MIC ($\mu\text{g/ml}$)				
	<i>Aspergillus fumigatus</i>	<i>Candida albicans</i>	<i>Candida krusei</i>	<i>Candida glabrata</i>	<i>Cryptococcus neoformans</i>
1	>50	1.00	>25	1.90	9.6
2	0.39	0.12	0.05	0.19	0.39
30	0.09	0.03	0.24	0.19	0.39

In summary, we have demonstrated that the combined effect of replacement of one triazole ring in fluconazole (**1**) with a pyridine or pyrimidine ring, and introduction of an α -methyl group leads to a dramatic increase in potency against *A. fumigatus*. Fluoro-substituted pyridine and pyrimidine analogues are particularly effective in curing *A. fumigatus* infections in mice. Based on its increased potency against a range of fungal pathogens, particularly *A. fumigatus* and *C. krusei*, **30** (UK-109,496, voriconazole) has been selected for further development, and is currently undergoing Phase III clinical evaluation.

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- (8) Detailed X-ray crystallographic data for **24b** and **30** have been deposited at the Cambridge Crystallographic Data Centre.
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