Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 16, pp. 2031-2036, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00363-0

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

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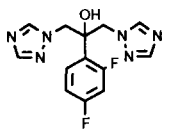
Abstract: Replacement of one triazole ring of fluconazole with 4-pyridinyl leads to an increase in activity against *Aspergillus fumigatus*. Introduction of an α-methyl group has a marked additional beneficial effect. Investigation of pyridinyl and pyrimidinyl analogues resulted in the identification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

Introduction: Azole antifungal agents, which act by inhibition of the cytochrome P450 enzyme lanosterol 14α-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.



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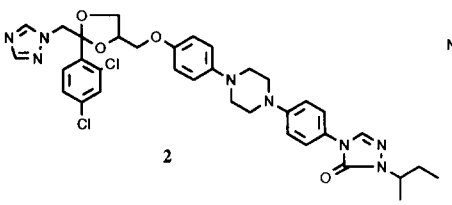

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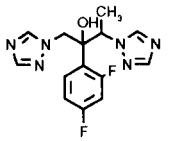


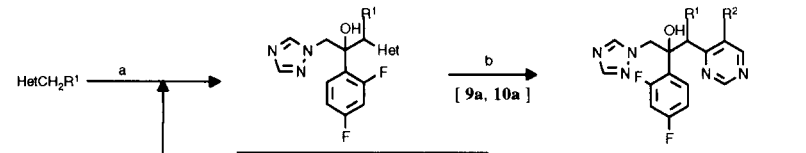
3

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



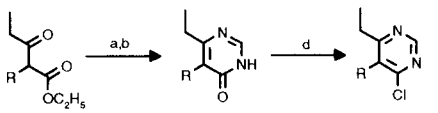
Compound	R^1	Het
5	H	
6	H	
7a, 7b	CH_3	
8a, 8b	CH_3	
9a, 9b	CH_3	
10a, 10b	CH_3	

Compound	R^1	R^2
11	CH_3	Cl
12	CH_3	F

Conditions: (a) LDA/THF; (b) H_2, 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 $POCl_3$ 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 $POCl_3$.

Scheme II



13 R = H
17 R = F

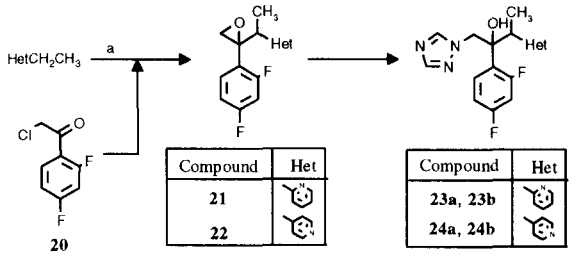
14 R = H
15 R = Cl
18 R = F

16 R = Cl
19 R = F

Conditions: (a) MeONa, MeOH; (b) formamidine acetate; (c) conc. HCl; 30 wt. % H_2O_2; (d) $POCl_3$, 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.

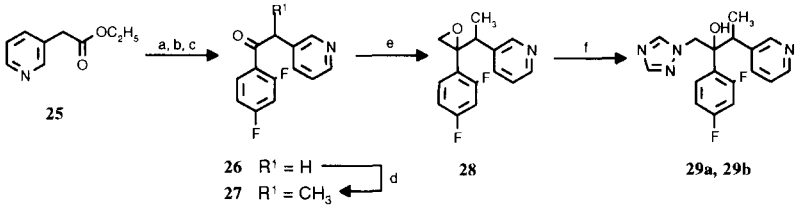
Scheme III



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, $Bu_4N^+HSO_4^-$, $CHCl_3$, H_2O; (e) dimethylsulfoxonium methylide, THF; (f) 1,2,4-Triazole Na salt, DMF, 60°C.

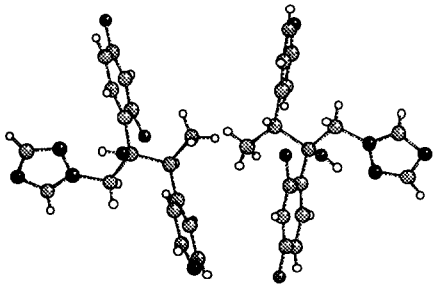
Results and Discussion:

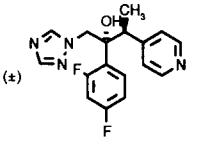
The activity of compounds against *A. fumigatus* is summarised in Table 1.[7] For the compounds existing as diastereomer pairs, one pair was always markedly more potent than the other as shown by comparison of **23a** and **23b**. With the exception of **23a**, data for the less active pair are omitted 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

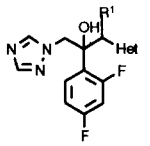




24b

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

Table 1



Cpd.	Het	R	Activity against *A. fumigatus in vitro*		Activity against *A. fumigatus in vivo*[c]	
			MIC (μg/ml)	MFC[a] (μg/ml)	No. of mice surviving	No. of mice cured
1	1*H*-1,2,4-triazol-1-yl	H	>50	>50		
2	-	-	0.14	0.19	4/5[d]	3/5[d]
3	1*H*-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

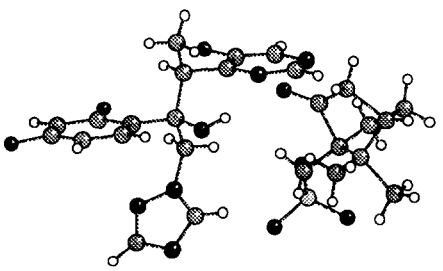
[a] Minimum fungicidal concentration giving at least a 90% reduction in colony forming units compared with the drug-free control after 24 hr. incubation. [b] Not determined. [c] Mice were infected intravenously and given a standard oral dose of 20 mg/kg b.i.d. for 5 days commencing 1hr. post infection. 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.

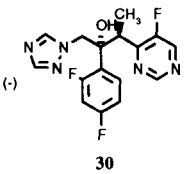
Compounds **5** and **6** demonstrate that a beneficial effect on the MIC against *A. fumigatus* occurs on replacement of one of the triazole rings of **1** with pyridinyl, particularly a 4-pyridinyl group. A marked further enhancement in potency derives from introduction of a methyl group α- to the 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 μg/ml against *A. fumigatus* compared with 50 μg/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





30

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 (μg/ml)				
	Aspergillus fumigatus	*Candida albicans*	*Candida krusei*	*Candida glabrata*	*Cryptococcus neoformans*
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.

Acknowledgements:

We thank K.N. Dack, A.J. Fowler, D.C. Mills, J.A. Morris and K.S. Ruddock for their assistance in preparing the compounds and R.J. Andrews, B.G.H. Lewis, G. Oliver, and G. Pye for the biological data. Thanks are also due to J. Bordner, Pfizer Groton for the X-ray crystal structures.

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(7) For evaluation of compounds *in vitro*, a series of agar plates, or liquid medium in microtiter plates, each having the test compound incorporated at a particular concentration, was inoculated with a standard culture of fungus, e.g. *A. fumigatus*, and each plate was then incubated for 48 hr. at 37°C. The plates were then examined for the presence or absence of growth of the fungus and the appropriate MIC was noted.
(8) Detailed X-ray crystallographic data for **24b** and **30** have been deposited at the Cambridge Crystallographic Data Centre.
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(*Received in Belgium* 3 *June* 1996; *accepted* 26 *July* 1996)