The discovery of fluconazole

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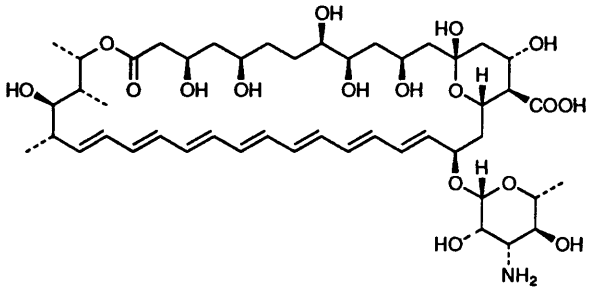
Reviewing the literature up to December 1994

1 Background on the fungal diseases

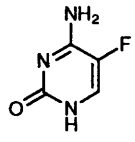
The treatment of fungal infections, particularly in immune-compromised patients, is an increasingly difficult problem facing the medical profession.[1] Fungi are all around us. They are present in the soil, in the air and in most peoples gastrointestinal tracts, but they don't usually cause serious problems because our natural defence system protects us. However, there are a growing number of patients who have deficient immune systems and they are at risk of life threatening infections.[2] The patients particularly at risk are those being treated for leukaemia and certain types of cancer, and those receiving organ transplants. In addition, AIDS patients are particularly susceptible to fungal infections.

In the early 1970s a research programme was set up at Pfizer Central Research in Sandwich with the objective of discovering a drug to treat serious systemic fungal infections. Although the incidence of such infections was quite low at that time, we believed that there would be increasing numbers of susceptible patients in the future. Advances in medical practice, such as organ and bone marrow transplantation, and increasingly aggressive cancer chemotherapy were resulting in a slow but sure increase in the number of serious fungal infections.[3–5]

The very similar biochemistry of fungi and man had made the search for safe and effective antifungal drugs very difficult. The only available drugs at the time were amphotericin B and 5-fluorocytosine. Amphotericin B **1** is a polyene antibiotic which binds to the fungal sterol ergosterol **4**, in the cell membrane, and promotes the loss of vital ions from the cell. Unfortunately, amphotericin also has affinity for the mammalian sterol cholesterol and this lack of selectivity results in a number of side-effects in man, including shaking, chills, fever, nausea and renal failure[6] These side-effects, together with the fact that amphotericin has to be given by intravenous infusion over several hours, made clinicians reluctant to use it and frequently amphotericin was used as a last resort, when it was too late. The only other widely available systemically-effective drug was 5-fluorocytosine **2**, which had the major advantages of good oral bioavailability and low toxicity, but was only active against a limited range of fungi and resistance frequently arose during long-term treatment.[7]



amphotericin B **1**



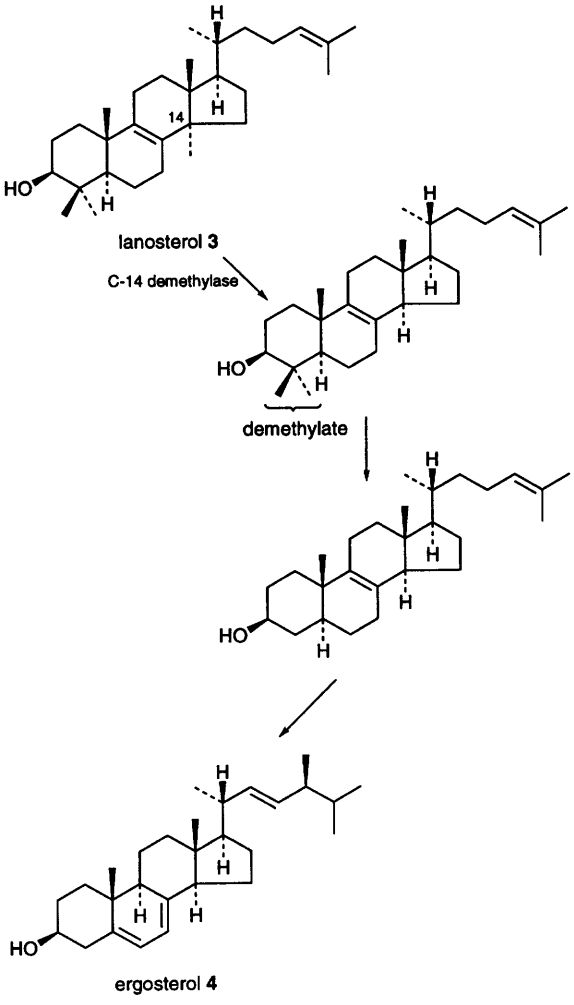
5-fluorocytosine **2**

Clearly, there was a need for a safe, effective agent that could be given on suspicion of a fungal infection and we felt that demand would increase in the future. We believed that the ideal agent would be one that could be administered both orally and intravenously because, although the oral route would be most frequently used, certain cancer patients had difficulty with oral dosing and the availability of an intravenous dosage form would therefore be a considerable advantage. We also wanted our agent to be effective against a wide range of fungal species and it must have a good safety profile. We believed that an agent with these properties would represent a considerable advance in antifungal chemotherapy, and would be used extensively.

2 Lead discovery

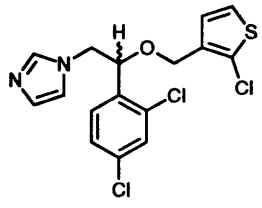
There were a number of potential chemical starting points for our research programme because many

different structural classes had been reported to show antifungal activity. However, most of these chemicals were not selective for fungi and so we chose to work on imidazole compounds because these were known[8] to possess potent and selective *in vitro* activity against a wide range of fungal pathogens. Their antifungal action was known to be due to inhibition of fungal C-14 demethylase (**Scheme 1**), a cytochrome P450-containing enzyme essential for the production of the principal fungal sterol ergosterol **4**.[9] Ergosterol is essential for the fluidity of the fungal membrane, and therefore the viability of fungi.



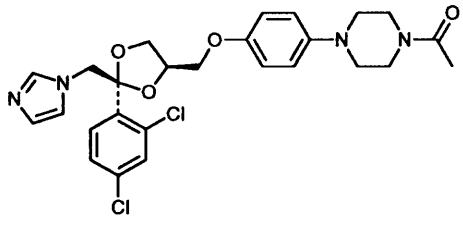
Scheme 1

The imidazole compounds could be remarkably selective for the fungal C-14 demethylase, rather than the closely related mammalian enzyme, which encouraged us to believe that these compounds were inherently safe. Our early work led to tioconazole **5**, which proved to be very effective clinically when administered topically against fungal infection of the skin and the vagina.[8] However, it was poorly effective in animal models of fungal infections when administered by either the oral or intravenous routes. Our studies suggested that these imidazole antifungals were highly susceptible to metabolic inactivation, resulting in low oral bioavailability and low, poorly-sustained plasma levels. In addition, tioconazole was lipophilic ($\log P \sim 5$) and highly bound to plasma proteins resulting in very low circulating levels of the unbound, active form. Therefore, the approach that we decided to adopt was to make the compounds as metabolically stable as possible whilst minimising their overall lipophilicity. We believed that metabolic stability would lead to improved oral bioavailability, while reduced lipophilicity would result in lower protein binding, with the overall effect of producing high sustained plasma levels of unbound drug.



(±)-tioconazole **5**

Our initial modifications of tioconazole included introduction of a range of polar-substituted alkyl, phenyl and heterocyclic groups in place of the chlorothiophene moiety, as an approach to compounds with reduced lipophilicity. Some progress was being made but our plans were modified following the report that orally administered ketoconazole **6** was active in several animal models of fungal infection.[10] Examination of ketoconazole showed that although it was *less* metabolically vulnerable than earlier imidazole antifungal derivatives, resulting in good oral bioavailability, it was still vulnerable to metabolism and very low levels of unchanged drug were excreted in the urine. Ketoconazole was also less lipophilic than earlier imidazole derivatives, resulting in higher blood levels, but it was still highly bound to plasma proteins, with $<1\%$ being in the unbound form. Clearly, ketoconazole was an important advance from the earlier imidazole antifungal derivatives[2] but it left considerable scope for improvement[11] and fell short of the target that we had set ourselves. (Ketoconazole **6** has a *cis* arrangement of the imidazole and substituted phenoxy group and, like tioconazole, is a racemic mixture).



(±)-ketoconazole **6**

Ketoconazole was, however, an important structure–activity step and our plans were adjusted to take account of it. We prepared a range of structural types which were influenced by the structure of ketoconazole, including the dioxolanes, dithiolanes, tetrahydrofurans and hydroxy derivatives shown in **Figure 1**. We investigated a wide range of modifications as we pursued our goal of reduced lipophilicity and increased metabolic stability. The introduction of polar functionality such as carboxamide, sulfone, nitrile and polar heterocycles was usually well tolerated, but ionised groups such as amino and carboxylic acid resulted in a marked loss of both *in vitro* and *in vivo* antifungal activity.

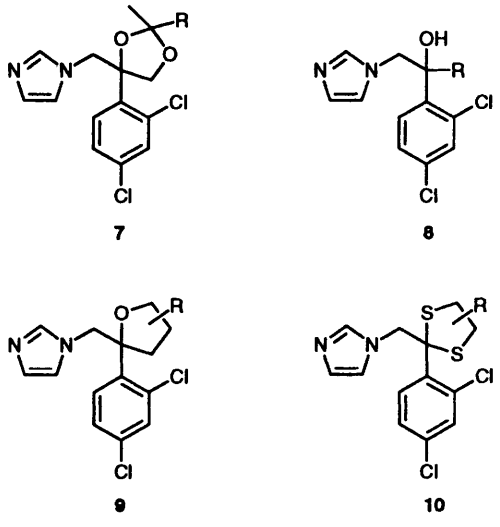


Figure 1

In vitro activity was assessed on agar plates[12] against all of the major fungal pathogens (*Candida, Cryptococcus, Aspergillus* and dermatophytes). *In vivo* efficacy was evaluated initially against a systemic *Candida* infection in mice, where animals had been given a potentially lethal infection and test compounds could be given either orally or by intravenous injection.[13] Efficacy was assessed when all saline-treated animals were dead, and the dose of compound required to protect 50% of the animals was calculated. Ketoconazole was used as a comparative agent, and compounds showing ketoconazole-like levels of activity were progressed to studies in other animal models, including those in immune-suppressed animals. Particularly good compounds were also advanced to pharmacokinetic studies in mice and dogs.

In each of the structural series shown in **Figure 1**, it proved possible to obtain compounds showing *in vivo* efficacy similar to that of ketoconazole, but none of these derivatives showed the clear advantage that we were seeking. In order to increase our rate of progress we decided to concentrate our efforts on one series, the hydroxy series. We chose this series because it was the only non-cyclic series and was therefore most structurally-distinct from ketoconazole, the presence of a hydroxy group lowered the lipophilicity, and this series was readily synthesised and therefore ideas could be rapidly evaluated.

Over the following year, we prepared almost 300 compounds (**Figure 2**) and many showed ketoconazole-like activity, but we were unable to improve upon the *in vivo* activity of ketoconazole. Pharmacokinetic evaluation of 30 of these derivatives in the mouse showed that, despite the wide range of structural variations, they all remained metabolically-vulnerable and had, at best, a ketoconazole-like pharmacokinetic profile, with a half-life of $\sim$1 hour and $<$1% being excreted unchanged in urine.

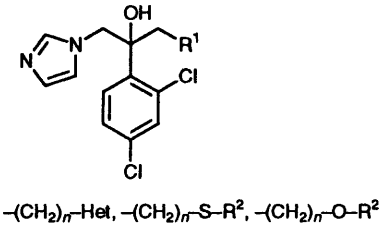


Figure 2

The only consistent structural feature of all the compounds that we had synthesised was the presence of the imidazole moiety and we therefore concluded that it was possible that this group could be one of the reasons for the metabolic vulnerability of these compounds. This view received support from our discovery that in tioconazole the imidazole group was a site for metabolic inactivation, as we later reported.[14] We therefore came to the conclusion that the imidazole had to be replaced and we chose a range of groups (**Figure 3**) which we believed might interact with cytochrome P450, in place of the imidazole unit. We chose this particular series to evaluate potential replacements because compounds were readily synthesised, as shown and the corresponding imidazole derivative demonstrated *in vitro* and *in vivo* activity equivalent to that of ketoconazole. Therefore, we believed that

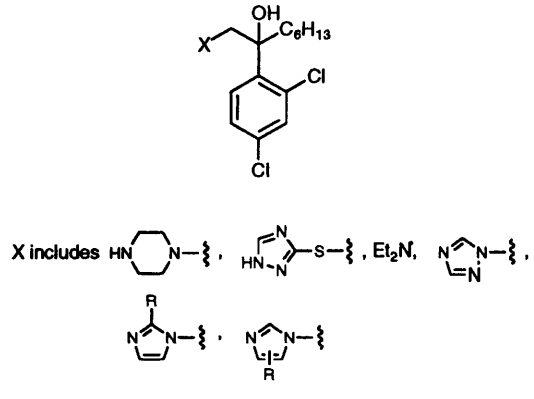
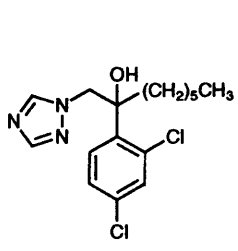


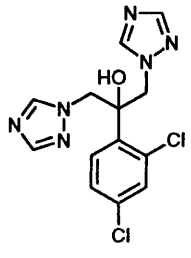
Figure 3

any improvement in metabolic stability would lead to *in vivo* efficacy superior to that of ketoconazole.

Twenty groups were examined as potential replacements, including a range of substituted imidazole derivatives, several other heterocycles and a number of basic groups, but the only group offering encouragement was 1,2,4-triazole when attached by the 1-position. Replacement of imidazole by a 1,2,4-triazol-1-yl unit gave UK-46,245 **11** which was twice as potent in our standard murine systemic candidosis model as the corresponding imidazole analogue, despite being approximately six times less potent against *Candida in vitro*. These data suggested that the triazole group was less susceptible to metabolic inactivation than an imidazole moiety, but yet it retained the ability to interact with the cytochrome P450 unit in the fungal C-14 demethylase enzyme. However, in UK-46,245 there remained a lipophilic, metabolically-vulnerable hexyl group and therefore we considered how we could replace this moiety and achieve our original objective of a compound which would combine good metabolic stability with low lipophilicity. A number of potential replacements were chosen but the first to be examined was a 1,2,4-triazol-1-yl unit to yield the symmetrical bis-triazole compound UK-47,265 **12**.



UK-46,245
11



UK-47,265
12

UK-47,265 was a most remarkable compound! When examined in the mouse systemic candidosis model it was almost 100 times more potent than ketoconazole following administration by either oral or intravenous dosing. This level of activity was completely unprecedented. Further evaluation against vaginal candidosis in mice and rats and against dermatophytosis in mice and guinea pigs showed impressive efficacy, as did examination against systemic candidosis in immune-compromised mice and rats. These findings were even more remarkable when it was shown that UK-47,265 had only modest activity against fungi when examined using standard *in vitro* assay methods.[15] Pharmacokinetic studies in rodents indicated that the *in vivo* activity was not due to the formation of active metabolites since UK-47,265 was extremely stable, showing high and persistent levels of unchanged drug with approximately 30% being excreted intact in the urine, in marked contrast to the $<1\%$ observed with ketoconazole.[16]

This poor correlation between *in vitro* and *in vivo* efficacy was well recognised with the imidazole antifungal compounds, and UK-47,265 appeared to represent a rather extreme example. However, we believed that *in vitro* assessment of antifungal activity would be essential for the development of UK-47,265, since it would be necessary to examine its activity against many clinical isolates and against examples of all important species of fungal pathogens. Therefore, we commenced an investigation of the possible reasons for the very low levels of *in vitro* antifungal activity seen with this compound. The activity of UK-47,265 in over 30 different agar and liquid-based growth media was evaluated and it became clear that complex media, especially those containing peptones, antagonised the activity of UK-47,265 whereas good *in vitro* activity was observed when a tissue culture-based medium similar to SAAMF medium[17] was used.

UK-47,265 showed outstanding activity in a wide range of systemic and superficial infection models. The systemic infections were due to *Candida, Cryptococcus* and *Aspergillus* species, and were in a range of infection sites including the kidney, liver, brain, gut and lung. The animals used were both normal and those which had been immune-compromised with immune suppressants which are used in patients such as cyclophosphamide and steroids. The superficial infections were dermatophytosis in mice and guinea pigs, and vaginal candidosis in mice and rats. Pharmacokinetic evaluation in several species (mouse, rat, guinea pig, rabbit, dog) showed excellent oral bioavailability together with a long plasma half-life and therefore UK-47,265 was progressed into pre-clinical safety evaluation. The results were extremely disappointing since UK-47,265 proved to be hepatotoxic in mice and dogs, and teratogenic in rats. This obviously precluded further progression and we therefore turned our attention to the search for a replacement.

While UK-47,265 was being evaluated in antifungal, pharmacokinetic and safety studies, an intensive follow-up programme had been in progress resulting in the synthesis of over 100 bis-triazole analogues. All of the derivatives were examined in the mouse model of systemic candidosis and a particularly interesting series of compounds resulted from replacement of the dichlorophenyl moiety by a range of aryl and heteroaryl groups (**Table 1**). Many of these derivatives showed very good *in vivo* anti-*Candida* activity and the best of these were progressed to evaluation in mouse models of vaginal candidosis and dermatophytosis (**Table 2**). The best three compounds, the 2,4-difluorophenyl, 2-chloro-4-fluorophenyl and 4-chlorophenyl analogues were progressed to pharmacokinetic evaluation in the mouse (**Table 3**) and the outstanding compound was obviously the 2,4-difluorophenyl analogue. It had a plasma half-life of 5.1 hours, 75% of drug was excreted unchanged in the urine and, in addition, it was water-soluble (8 mg ml^{-1} at room temperature), a property that would greatly facilitate its formulation for intravenous administration. This derivative, UK-49,858, showed outstanding activity

Table 1

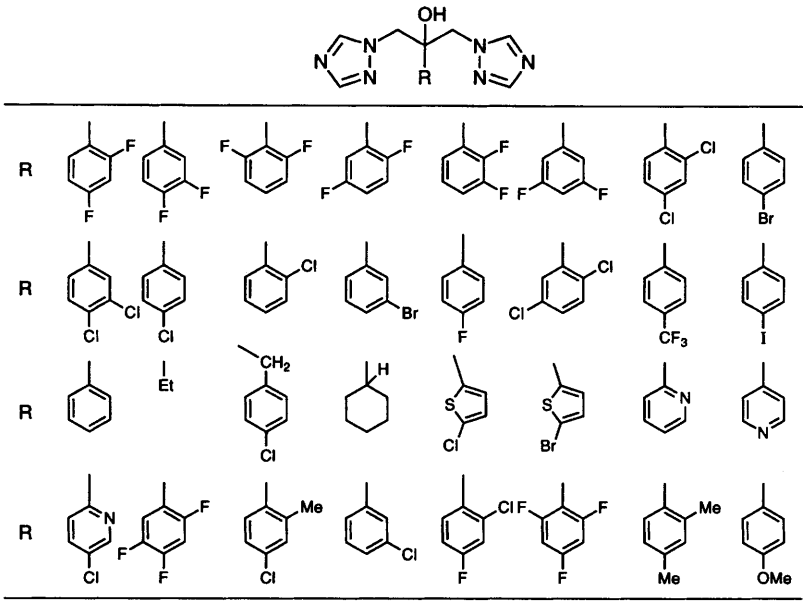


Table 2

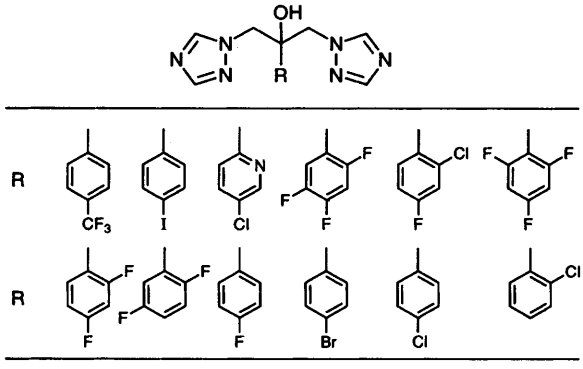
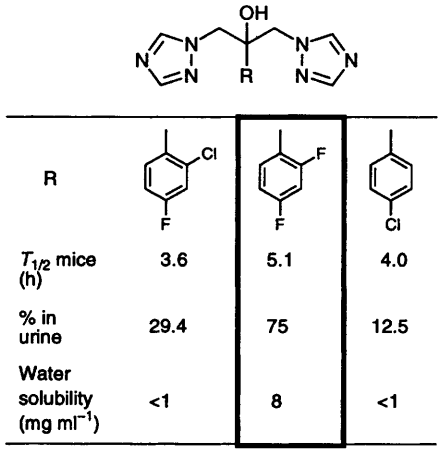
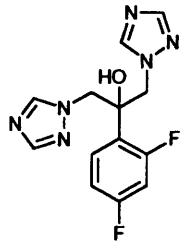


Table 3



R			
$T_{1/2}$ mice (h)	3.6	5.1	4.0
% in urine	29.4	75	12.5
Water solubility (mg ml^{-1})	<1	8	<1

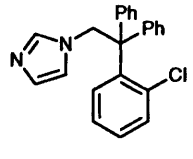
in our full range of fungal infection models in both animals with normal immune function and those with suppressed immune function.[13,18] Safety evaluation showed that UK-49,858 **13**, now known as fluconazole, was not teratogenic or hepatotoxic and it was therefore progressed to studies in man.



fluconazole **13**
(UK-49,858)

In healthy human volunteers, fluconazole showed excellent oral absorption, and food did not affect the bioavailability. The plasma half-life was approximately 30 hours leading to predictable accumulation following daily dosing, with a steady state being reached within 4–5 days.[19] Approximately 90% of orally-administered fluconazole was excreted unchanged in the urine, as predicted from studies in animals, and there were no adverse side-effects. Fluconazole was therefore progressed to evaluation against fungal infections in man.

Initial efficacy studies were carried out in patients with acute vaginal candidosis, with half being given a 150 mg single oral dose of fluconazole and half being dosed intravaginally (200 mg daily for three consecutive days) with commercial Canesten (chlotrimazole **14**) vaginal tablets. Both drugs



chlotrimazole **14**

produced excellent clinical responses (Fluconazole 100%; Canesten 97%) with no side-effects,[20] and therefore fluconazole was progressed to studies in immune-compromised patients. The large majority of these were AIDS patients with oropharyngeal candidosis infections and 'spectacular' clinical efficacy was reported, with a 100% success rate after 5–7 days treatment[21] and there were no clinically significant side-effects. A later study[22] showed that fluconazole was more cost effective than either ketoconazole or ketoconazole **6** followed by fluconazole in HIV-positive patients.

These extremely promising results encouraged progression to studies of potentially life-threatening infections. Cohen[23] reported the treatment of *Candida* infections in five patients who were immune-suppressed because of chemotherapy for cancer or after organ transplantation, and all five patients were cured of their infections after two weeks of therapy. Further studies showed very good efficacy against *Candida* infections at a wide range of body sites in AIDS, cancer, leukaemia and organ transplant patients.[24–27]

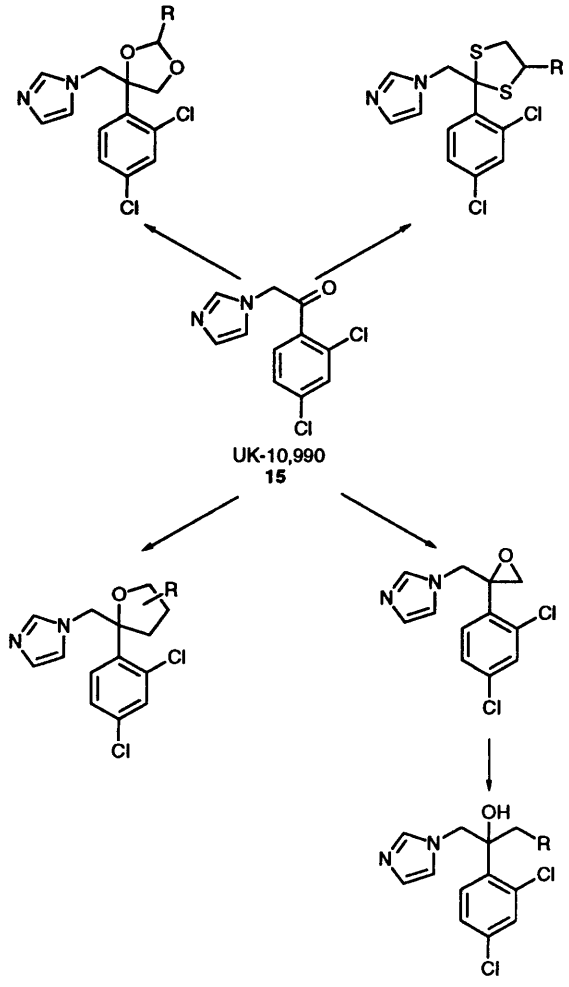
Cryptococcal meningitis is a life-threatening fungal infection in up to 30% of AIDS patients, and Dupont and Drouhet[28] achieved remarkable clinical success with fluconazole, which was confirmed by Dismukes *et al.*[29] A recent study[30] showed that fluconazole combined with 5-fluorocytosine **2** led to improved clinical success compared with either fluconazole alone or amphotericin B **1** alone. Patients with AIDS will require some form of maintenance antifungal therapy to prevent relapse/re-infection and a study[31] showed that prophylaxis with fluconazole was highly effective and fluconazole is now the drug of choice for these patients. Fluconazole (orally) was recently shown to be superior to weekly intravenous amphotericin B as therapy to prevent relapse in AIDS patients with cryptococcal meningitis, after primary treatment with amphotericin B.[32]

3 Synthetic chemistry

Synthesis of the imidazole derivatives went via the key keto-derivative UK-10,990 **15**. This was readily synthesised and easily manipulated to give access to several series of active compounds (**Scheme 2**).

The change to triazole derivatives increased the chemical difficulty because of the opportunity for the triazole to react at both the 1- and 4-positions. In all cases, the product resulting from reaction at the 1-position dominated, but a significant amount of the 4-substituted product was always produced, and had to be removed by either chromatography or crystallisation. The overall yield of the bis-triazole derivatives was approximately 20% in each case.

The bis-triazoles were prepared by several routes, with the usual ones being as shown in **Scheme 3**. The bis-triazoles can be formed in three positional isomers, namely 1,4-, 1,1- and 4,4-bis-triazoles. Only the compounds with both triazoles attached through

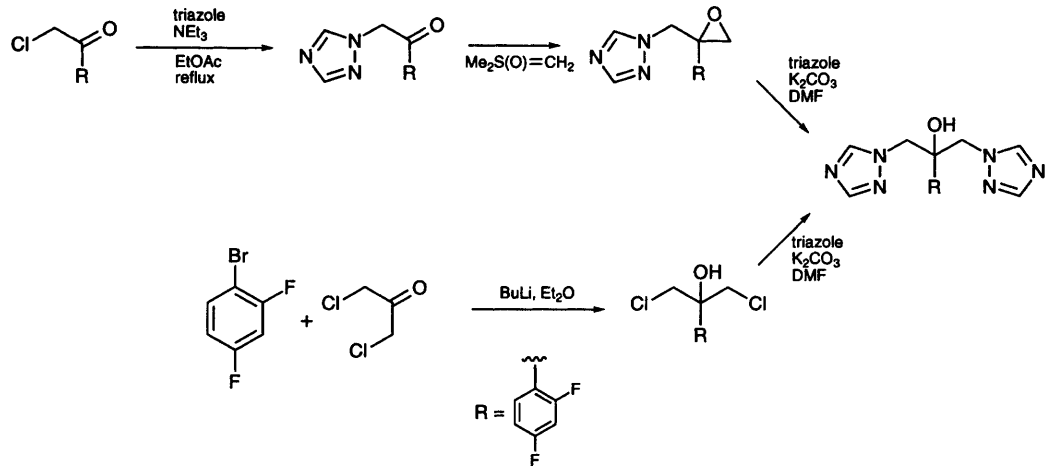


Scheme 2

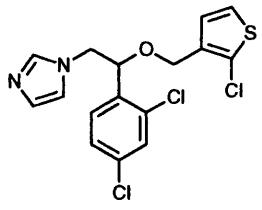
the 1-position showed good *in vivo* antifungal activity.

4 Summary

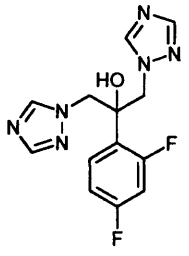
The discovery of fluconazole serves to illustrate several important points. It demonstrates how long the discovery of a drug can take, since it was almost 20 years from the start of the programme until its market launch in 1988. It also shows how one can take a very potent and selective agent like tioconazole **5**, and modify it to overcome its weaknesses of high lipophilicity and metabolic vulnerability. In the process, almost every aspect of tioconazole's structure has been modified, as can be seen by an examination of their chemical structures (below). It also demonstrates that, occasionally in drug discovery one may achieve more than one realised was possible. When we started the research programme that eventually led to fluconazole, we believed that by lowering lipophilicity and decreasing metabolic vulnerability we would obtain a significant improvement in antifungal efficacy. We had not anticipated that such a remarkable



Scheme 3



tioconazole **5**



fluconazole **13**
(UK-49,858)

combination of efficacy, safety and outstanding pharmacokinetic properties would result.

Acknowledgements

The author wishes to acknowledge scientific colleagues and collaborators, too many to mention individually, for their invaluable contributions to the fluconazole programme in all its phases.

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