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Antifungal triazole alcohols: A comparative analysis of structure–activity, structure–teratogenicity and structure–therapeutic index relationships using the Multiple Computer-Automated Structure Evaluation (Multi-CASE) methodology

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SUMMARY

An increase in the opportunistic fungal infections necessitates a design of new more effective and safer antifungal agents. Triazole alcohols are effective antifungals, but have a risk of teratogenicity associated with them. Therefore, successful design of drugs from this class depends on understanding the structure–activity and structure–teratogenicity relationships in conjunction.

To this end, we applied the Multiple Computer-Automated Structure Evaluation (Multi-CASE) methodology to a study of the relationships between the structures of 71 triazole alcohols and their in vitro antifungal activity, teratogenicity, and therapeutic index. For each end point, several relevant structural descriptors were identified.

A comparative analysis of the Multi-CASE results indicates that *cyano, methoxy* groups, and *ortho*-difluorination on the aromatic ring decrease antifungal activity, but not the therapeutic index because of the concomitant negative contribution to teratogenicity. Metabolically deactivating *para*-substitution in the benzene ring is beneficial for the therapeutic index in agreement with the idea of metabolically induced teratogenicity. Fluorinated *para*-alkyl substituents are most preferable. The pattern of *ortho*-substitution in the benzene ring affects both antifungal and teratogenic activity. This suggests that the relative orientation of the benzene ring with respect to the rest of the molecule may play a modulating role.

The Multi-CASE model could correctly predict, a priori, the teratogenicity and antifungal potency of SCH 39304 and ICI 156,066 and be used to optimize the structure and therapeutic index of the latter.

INTRODUCTION

Triazole alcohols constitute a considerable portion of the most recent promising leads in antifungal chemotherapy. These agents are generally well-tolerated, metabolically more stable, and active via both oral and intravenous routes against dermatophytes and systemic fungi [1].

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Fluconazole, a triazole derivative of propanol, was one of the earliest clinically successful antifungals of this class [2] (Fig. 1). The agent was a result of an extensive synthetic effort at Pfizer Central Research (Sandwich, U.K.). The drug proved to have a broad activity spectrum in vivo, a favorable pharmacokinetic profile, and a desired level of aqueous solubility [3].

Shortly after the advent of fluconazole, Schering-Plough and Sumitomo unveiled a new antifungal drug SCH 39304 (Fig. 1), selected by extensive screening of 1-azolyl-alkylsulphonyl-alkanol derivatives. Preliminary animal testing showed SCH 39304 to be orally, parenterally and topically active with potency and metabolic half-life superior to that of fluconazole [4]. SCH 39304 received a 'high priority' status from the AIDS Clinical Drug Development Committee of the US NIH, cleared phase I clinical trials and is progressing through phase II/III studies.

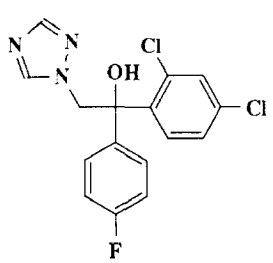
At about the same time Grassberger et al. of Sandoz Pharmaceuticals reported another successful antimycotic triazole alcohol (SDZ 89-485) [5]. Antifungal activity comparable to that of fluconazole was reported for Electrazole (BAY R 3783) [6] – an azole alcohol developed by Bayer.

A synthetic screening effort at ICI Pharmaceuticals in the eighties brought about a novel antimycotic triazole alcohol (ICI 195,739) which proved to be the most potent of all agents tested in animals against fungal infections [7].

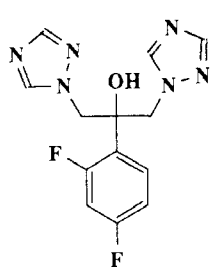
Thus the importance of triazole alcohol derivatives in antifungal chemotherapy has been well established. However, a constant concern haunting those who develop such agents is their teratogenic potential [8,9]. In fact, reproductive side effects stalled one of the earliest orally active triazole alcohol antimycotics – ICI 153,066 (Fig. 1) proposed by ICI Pharmaceuticals in 1982 [10].

Therefore, the successful development of novel antifungal triazole alcohols would benefit from a better understanding of the structural features responsible for both antimycotic activity and teratogenic potential. This would allow a chemist to manipulate chemical structures to enhance antimycotic potency and at the same time reduce the risk of teratogenicity. Such knowledge can be obtained from the derivation of structure–teratogenicity and structure–antifungal activity relationships among already existing agents.

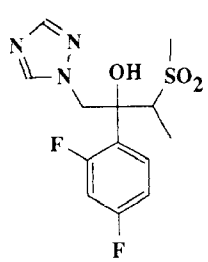
The topic of structure–teratogenicity relationships in antifungal triazole alcohols was addressed in the literature [11]. Except for a few minor trends, no overall meaningful correlations were discovered between structure and either teratogenicity or antifungal activity. Some data were presented to support the hypothesis that teratogenicity of triazole alcohols may be due to the formation of toxic metabolites within the embryonic cells. Bistriazole agents with metabolically inert *para*-substituents (F, CF3, OCF3) were shown to be non-teratogenic in vivo. However, no



ICI 153,066



FLUCONAZOLE



SCH 39304

Fig. 1. Structures of three antifungal triazole alcohols. ICI 153,066 was aborted because of its teratogenicity, whereas fluconazole and SCH 39304 have been successfully pursued in clinical trials.

insight was given into the structure–activity trends within the group of monotriazole derivatives.

We felt that for the benefit of drug design efforts, it is necessary to consider structure–teratogenicity dependencies in triazole alcohols in conjunction with the structure–antifungal activity relationships – an effort never described before. This approach can uncover ways to modify the chemical structure rationally to reduce the risk of teratogenic effects without compromising the antimycotic activity. Conceivably, the antifungal activity of existing leads (e.g. ICI 153,066) can then be enhanced at the expense of their teratogenic potentials.

To this end, we applied the Multiple Computer Automated Structure Evaluation (Multi-CASE) methodology to the analysis of 35 mono- and 36 bistriazole tertiary alcohols with known antifungal activity and teratogenic potentials. Multi-CASE is a computer-automated methodology designed to detect and evaluate the relationships between the molecular structure and biological activity. When presented with a learning set containing both active and inactive molecules of either common or diverse structural types, Multi-CASE can automatically identify the substructures which have a high probability of being relevant or responsible for the observed activity. Novel molecules of interest can then be submitted to the program for a prediction of potential biological activity. Furthermore, the Multi-CASE expert library of structural attributes can be employed in the rational design of new molecules with desired biological properties.

We began with a Multi-CASE analysis of the structure–teratogenicity and structure–antifungal activity relationships as separate endpoints. As a next step, we established correlations between structural features and therapeutic index of triazole alcohols defined as a ratio of antifungal to teratogenic activity. Based on the results of the analysis, the relationships between structure and antifungal activity were discussed in conjunction with structure–teratogenicity correlations and overall therapeutic index.

The Multi-CASE model could correctly predict both the antifungal activity and teratogenicity of SCH 39304 and ICI 153,066 which were not part of the learning set. We also demonstrated the usefulness of the Multi-CASE model for the development of new drugs with increased therapeutic index by deriving a close structural analog of nonteratogenic fluconazole from the structure of the abandoned teratogenic antifungal ICI 153,066 based solely on the Multi-CASE structural correlations.

METHODOLOGY

Multi-CASE and its widely described predecessor CASE (Computer-Automated Structure Evaluation) have been discussed elsewhere [12–14]. For input the program requires molecular structures and their experimental activities. The structures can be entered from a computer terminal with a Klopman Line Notation (KLN) code [15], SMILES code, or graphically. An activity value is chosen which defines a breakpoint between the active and inactive molecules of the learning set. The breakpoint selection is made so as to ensure an approximately equal number of active and inactive molecules in the set.

As a first step, the Multi-CASE program fragments each molecule into all possible chains of 2–10 heavy atoms with attached hydrogens. This pool of structural descriptors is then subjected to a statistical evaluation based on a binomial distribution. Substructures encountered randomly in both active and inactive molecules are regarded as irrelevant to activity. Each fragment with a distribution significantly skewed towards either active or inactive molecules is believed to be

conducive (*biophore*) or disruptive (*biophobe*) to activity, respectively. A fragment is considered significant if its binomial distribution has less than 15% probability of being due to chance.

Initially, the program identifies the most significant substructure which can account for the activity of the largest number of molecules in the database. These molecules are removed from the data set and the remaining compounds are searched for the next most significant structural descriptor. This procedure is repeated either until enough biophores and biophobes have been found to account for the activity of every molecule, or until the remaining data cannot be explained by statistically significant descriptors. On completion, the entire database is reorganized into groups of molecules containing the same biophore (or biophobe). For each group the analysis is performed again to select *modulators* – molecular fragments which are capable of modifying the activity of molecules containing one specific biophore. In addition, within each group the relevance of partition coefficients, water solubilities and molecular weights to biological activity is evaluated.

Once the learning set has been analyzed, new molecules can be submitted to the program for a prediction of activity. Multi-CASE searches the structure for a presence of a biophore. If a biophore is present, the molecule is assigned a certain probability of being active, based on the statistical significance of the biophore(s) found within the molecule. The program also affords a projected activity value based on the average activity of molecules containing the biophore(s) in the learning set and the presence of potential modulators in the query structure. In cases where no known biophores are found in the query structure, the molecule is presumed to be inactive.

It is common for the Multi-CASE analysis to yield a number of single or sparsely represented fragments. Those fragments are not reliable and are sensitive to the choice of cut-off points and cannot withstand cross-validation. We, therefore, regard them as noise and do not discuss them. The only fragments discussed are the ones which are widely represented in the learning set and have >80% probability of relevance to biological activity.

DATABASE

The 71 molecules used in the analysis included 35 monotriazole and 36 bistriazole derivatives. The structures of ICI 153,066 and SCH 39304 were taken out of the training set to be tested at a later stage. All data on teratogenicity and antifungal activity of compounds used in the learning set were obtained from the publications by Flint and Boyle [11]. The antifungal activity was expressed as the concentration in nmol/ml required to reduce the growth of *Candida albicans* to 50% of that in the control (IC_{50c}). Teratogenic potentials are concentrations in nmol/ml inhibiting the differentiation of the rat embryonic limb cells by 50% (IC_{50t}). We also calculated the therapeutic indices as follows:

$$\text{Therapeutic Index (TI)} = IC_{50t}/IC_{50c}$$

For five chemicals the teratogenicity data was not available. Those compounds were, therefore, not included into either teratogenicity or therapeutic index databases. Activity breakpoints between active, marginally active, and inactive compounds were established in each database as illustrated in Table 1. The structures comprising the learning set are shown in Table 2 along with their experimental activities and Multi-CASE activity classifications.

RESULTS AND DISCUSSION

The Multi-CASE analysis identified five *Therapeutic index* biophores along with three *Anti-Candida* and three *Teratogenic* biophores (Figs. 2–4). Five biophobes were selected from the *Therapeutic index* database, five from the *Teratogenicity* set, and four from the *Anti-Candida* database. The overlaps and exclusions between these sets of biophores and biophobes represent a picture of intricate interplay of structural features contributing to desired and undesired effects of triazole alcohol antifungals. Table 3 illustrates the Multi-CASE retrofit of the experimental data.

According to the Multi-CASE results, the therapeutic index of a monotriazole compound will benefit from *para*-substitution in each benzene ring (**Ia**). This result is in agreement with the idea of metabolic activation being responsible for the teratogenicity of the triazole antimycotics: indeed, a *para*-locus of the benzene ring is the most exposed and therefore metabolically unstable position on the ring, most prone to be the target of biotransformation [16]. Therefore, protection of the *para*-position by a substituent will retard the onset of teratogenic metabolites. In fact, a more metabolically vulnerable unsubstituted benzene ring was singled out by the program as a teratogenic biophore regardless of whether it occurs in a bistriazole or a monotriazole derivative (**Ic**).

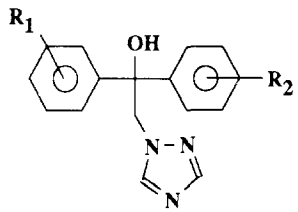
The analysis also indicates that the teratogenic potential of monotriazole compounds will be greater than that of the bistriazoles (**IIc**). This finding again corroborates the concept of the metabolically incurred teratogenicity: replacing a benzene ring with a much more resistant triazole group will slow down the metabolic transformation and decrease the concentration of toxic metabolites.

Ortho-fluorination in the benzene ring increases the therapeutic index (**IVa**) both by diminishing the teratogenic potential (**IIIf**) and enhancing the anticandidal activity (**IIIb**). However, fluorination of both *ortho*-positions in the same ring may cause a drop in antimycotic activity (**IIIe**). In fact, having one of the *ortho*-carbons unsubstituted is beneficial for both anticandidal activity and overall therapeutic index (**Ia** and **Ib**). This observation suggests that the orientation of the aromatic ring with respect to the rest of the molecule may be an important modulator of activity in triazole alcohols. It should also be noticed that the decrease in antifungal activity caused by di-*ortho*-fluorination may not lead to any drastic decline in the therapeutic index because it will also create a negative contribution to teratogenicity (**IIIf**). Likewise, the cyano- and methoxy-groups on the aromatic ring will tend to compromise the anticandidal activity (**Ie** and

TABLE 1
BREAKPOINT VALUES IN THE *ANTI-CANDIDA, TERATOGENICITY* AND *THERAPEUTIC INDEX* DATABASES

Type of activity (I:M:A)	Breakpoints		
	Inactive (I)	Marginal (M)	Active (A)
Anticandidal (22:14:33)	$\geq$ 21.6 nmol/ml	1.8 nmol/ml $\leq IC_{50c} <$ 21.6 nmol/ml	$IC_{50c} <$ 1.8 nmol/ml
Teratogenicity (20:18:26)	$\geq$ 107.8 nmol/ml	13.3 nmol/ml $\leq IC_{50t} <$ 107.8 nmol/ ml	$IC_{50t} <$ 13.3 nmol/ml
Therapeutic index (25:24:15)	$\leq$ 7.3	7.3 < TI $\leq$ 74.2	TI > 74.2

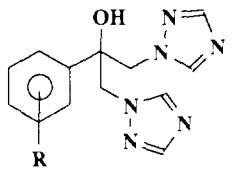
TABLE 2
MONO- AND BIS-TRIAZOLE ALCOHOLS USED IN THE MULTI-CASE ANALYSIS



No.	R_1	R_2	IC_{50c}[a] (nmol/ml)	IC_{50t}[b] (nmol/ml)	TI[c]
1	4-CN	4-CN	14.12 (+)[d]	142.70 (−)	(+)
2	4-NO_2	4-NO_2	10.72 (+)	98.40 (+)	(+)
3	3-NO_2	H	123.03 (−)	1.29 (++)	(−)
4	4-NO_2	H	0.13 (++)	1.61 (++)	(+)
5	H	H	3.02 (+)	2.26 (++)	(−)
6	4-F	H	1.23 (++)	2.82 (++)	(−)
7	4-F	4-F	0.13 (++)	23.23 (+)	(++)
8	2,4-F	4-F	0.08 (++)	6.58 (++)	(++)
9	4-Cl	4-NO_2	0.08 (++)	2.90 (++)	(+)
10	4-CH_3	H	0.21 (++)	1.81 (++)	(+)
11	2-CH_3	H	1.26 (++)	14.32 (+)	(+)
12	2,4-F	2,4-F	1.58 (++)	19.27 (+)	(+)
13	4-Cl	H	0.56 (++)	13.34 (+)	(+)
14	4-F	3-Cl	0.69 (++)	1.26 (++)	(−)
15	2-F	4-Cl	0.23 (++)	12.59 (++)	(+)
16	4-F	4-Cl	0.22 (++)	3.15 (++)	(+)
17	3-CF_3	H	10.23 (+)	0.30 (++)	(−)
18	2-CH_3	4-CH_3	0.89 (++)	7.84 (++)	(+)
19	2,4-F	4-CF_3	0.12 (++)	48.74 (+)	(++)
20	4-CF3	2,4-F	0.24 (++)	67.80 (+)	(++)
21	2-Cl,5-NO_2	H	128.28 (−)	3.77 (++)	(−)
22	4-Cl	4-Cl	0.15 (++)	14.79 (+)	(++)
23	4-F	2-Cl	0.08 (++)	0.005 (++)	(−)
24	2-CF3	4-CF3	0.56 (++)	37.38 (+)	(+)
25	4-F	2-Cl,4-F	0.08 (++)	8.94 (++)	(++)
26	2-Cl	4-Cl	0.01 (++)	0.15 (++)	(+)
27	2,4-Cl	H	0.02 (++)	2.69 (++)	(++)
28	2-F	2,4-Cl	0.03 (++)	0.06 (++)	(−)
29	4-F	2-Br	0.19 (++)	0.17 (++)	(−)
30	2-Cl	2,4-Cl	0.16 (++)	0.22 (++)	(−)
31	4-Cl	2,4-Cl	0.0027 (++)	1.91 (++)	(++)
32	3-Cl	2,4-Cl	0.0026 (++)	3.26 (++)	(++)
33	4-Cl	2-Br	0.03 (++)	5.28 (++)	(++)
34	4-OCF_2CF_2H	H	0.02 (++)	0.16 (++)	(+)

[a] IC_{50c} concentration required to inhibit the growth of *Candida albicans* by 50%.
[b] IC_{50t} concentration required to inhibit the differentiation of embryonic limb cells by 50%.

TABLE 2 (continued)



No.	R	IC_{50c}[a] (nmol/ml)	IC_{50t}[b] (nmol/ml)	TI[c]
35	4-CN	31.49 (−)	>1000 (−)	(+)
36	4-OCH_3	268.00 (−)	199.76 (−)	(−)
37	H	103.58 (−)	166.46 (−)	(−)
38	2-CH_3,4-CN	87.40 (−)	194.20 (−)	(−)
39	4-F	26.01 (−)	173.42 (−)	(−)
40	2,6-F	107.41 (−)	81.62 (+)	(−)
41	2-CN,4-CF_3	78.44 (−)	ND	ND
42	2-CH_3	95.29 (−)	52.94 (+)	(−)
43	2,4,6-F	35.46 (−)	ND	ND
44	2,3,5,6-F	87.64 (−)	759.59 (−)	(+)
45	4-CH_3	13.76 (+)	352.92 (−)	(+)
46	4-SCH_3	90.18 (−)	94.93 (+)	(−)
47	2-CF_3	103.45 (−)	88.67 (+)	(−)
48	3-Cl	541.39 (−)	131.25 (−)	(−)
49	4-Cl	3.02 (+)	114.84 (−)	(+)
50	2-Cl	3.05 (+)	85.31 (+)	(+)
51	2-Cl,4-F	3.25 (+)	61.96 (+)	(+)
52	2-F,4-Cl	1.61 (+)	108.44 (−)	(−)
53	2-CF_3,4-F	60.05 (−)	177.43 (−)	(−)
54	4-CH=CH_2	22.94 (−)	47.24 (+)	(−)
55	2-CH_3,4-F	100.88 (−)	56.23 (+)	(−)
56	4-OCF_3	0.56 (++)	169.33 (−)	(++)
57	4-CF_3	3.25 (+)	413.80 (−)	(++)
58	2-OCH_3,4-CF_3	28.50 (−)	325.77 (−)	(+)
59	2-F,4-CF_3	1.26 (++)	617.42 (−)	(++)
60	3-F,4-CF_3	84.19 (−)	477.10 (−)	(−)
61	4-[4-CNPh]	80.71 (−)	ND	ND
62	4-OCF_2CF_2H	77.85 (−)	7.78 (++)	(−)
63	2,4-Cl	0.74 (++)	17.69 (+)	(+)
64	2-Cl,4-CF_3	0.86 (++)	174.37 (−)	(++)
65	4-Ph	13.28 (+)	ND	ND
66	4-[2,4F-Ph]	18.85 (+)	117.80 (−)	(−)
67	4-OPh	2.88 (+)	ND	ND
68	4-[4-ClPh]	7.19 (+)	131.28 (−)	(+)
69	4-[4-ClPhO]	0.94 (++)	118.83 (−)	(++)

[c] TI (Therapeutic index) = IC_{50t}/IC_{50c}.

[d] Multi-CASE activity classifications: − = inactive, + = marginal, ++ = active.

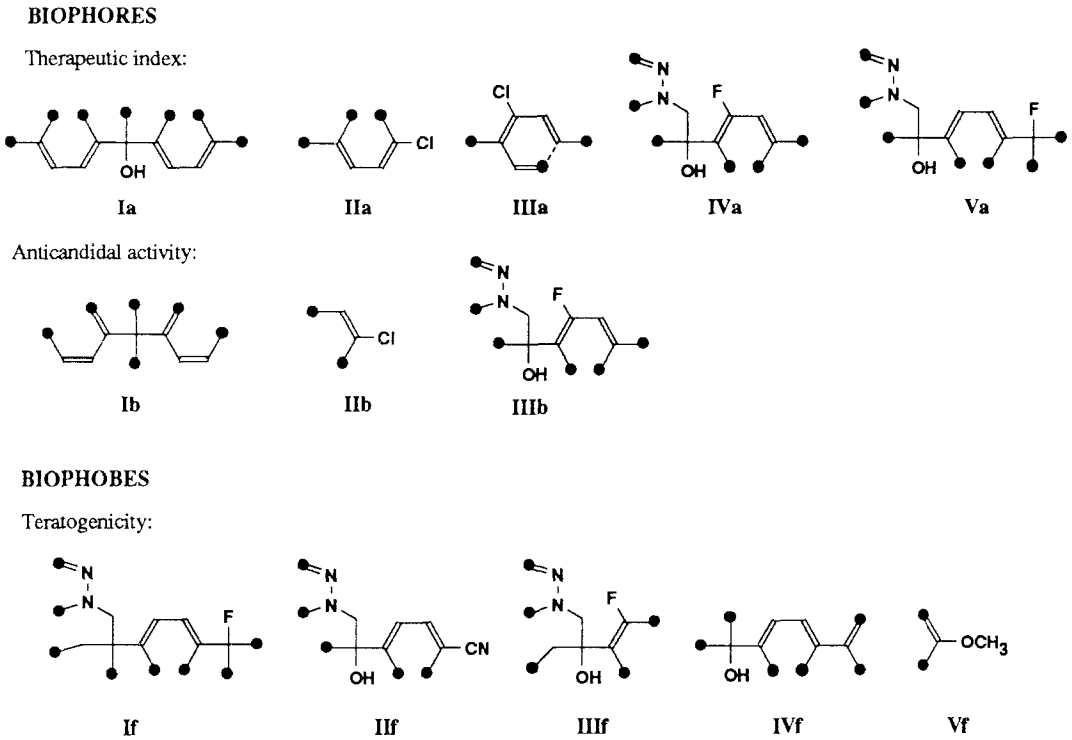


Fig. 2. The *Therapeutic index* and *Anti-Candida* biophores versus *Teratogenic* biophobes. Fragments **IVa**, **IIb**, and **IIIf** represent an overlap between all three sets. *Teratogenic* biophobe **If** is imbedded into the *Therapeutic index* biophore **Va**. The presence of an unspecified *para*-substituent in *Therapeutic index* biophore **Ia** agrees with the idea of a metabolic activation being required for teratogenicity. ● denotes a nonhydrogen substituent.

IVe), but may not be detrimental to the therapeutic index because of the concomitant negative contribution to teratogenicity (**IIIf** and **Vf**).

The fluorinated biophores also mandate the presence of a *para*-substituent on the same ring (**IVa** and **IIIb**). Even though the type of the substituent was not specified by the program, it stands out from other available biophores that trifluoromethyl or other highly fluorinated alkyl substituents would be partners of choice for an *ortho*-fluorinated biophore. Such choice would introduce a therapeutic index biophore with a teratogenic biophobe imbedded hereinto (**Va** and **If**). Moreover, both **Va** and **If** necessitate at least one unsubstituted *ortho*-position within the same ring – a welcome structural feature discussed above.

Although serving well in *para*-position, a fluorinated alkyl substituent in *ortho*-location will detract from the antifungal potency (**Vd**). The same effect may be caused by an *ortho*-methyl group (**IIId**). Once again this brings about the potential importance of the relative orientation of the benzene ring (*ortho-effect*) for the biological activity of the compounds being discussed.

Chlorine substitution will contribute to the anticandidal activity (**IIb**) and to the therapeutic index in general (**IIa** and **IIIa**). One *ortho-effect* exception was found in the case of monotriazole alcohols having at least one *ortho*-substituent on each aromatic ring, one of the substituents being a chlorine atom (**IVd**).

To demonstrate the utility of the Multi-CASE model, we shall consider two triazole alcohols

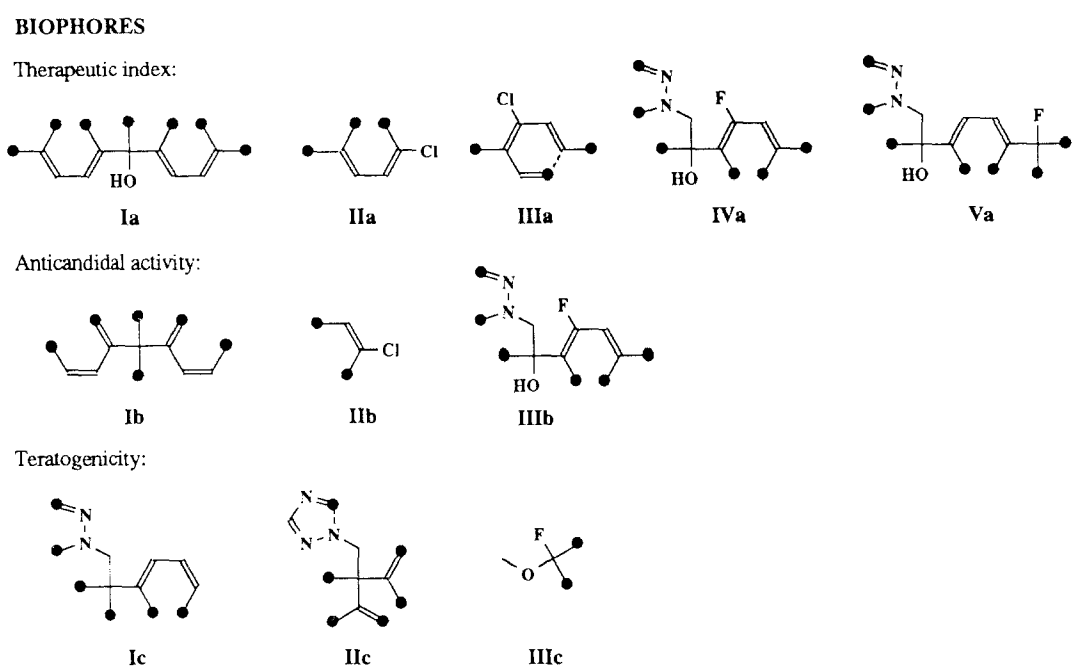


Fig. 3. The *Therapeutic index*, *Anti-Candida*, and *Teratogenic* biophores. There are no explicit overlaps between the latter and the former two. Comparison of **Ib** and **Ic** suggests, however, that *Anti-Candida* biophore **Ib** must be accompanied by a *para*-substituent to avoid creating a *Teratogenic* biophore **Ic**. Also, even though fluorinated alkyl substituents contribute to the therapeutic index (fragment **Va**), introduction of an ether linkage between such substituents and the rest of the molecule may enhance the risk of teratogenicity (fragment **IIIc**). • denotes a nonhydrogen substituent.

– SCH 39304 and ICI 153,066. Both agents have a superior activity against vaginal candidiasis in mice and rats and dermatophyte lesions in mice and guinea pigs following oral administration. However, the teratogenic profiles of the two are drastically different. The development of ICI 153,066 was precluded by its teratogenicity in rats and rabbits, whereas SCH 39304 is not teratogenic in either species and has been moving briskly through the clinical trials.

The Multi-CASE model was able to reproduce the experimental findings with respect to both the antimycotic activity and teratogenicity of these two compounds (Table 4). In accord with the experiment:

(1) both agents are predicted to have >85% probability of antifungal potency;

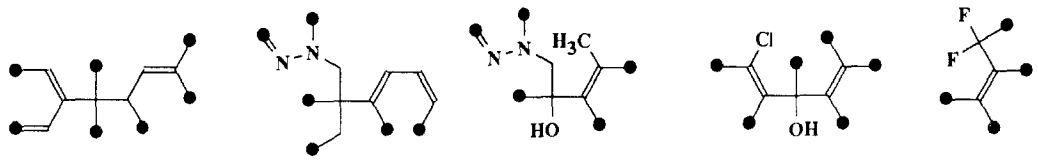
(2) ICI 153,066 has a 92.9% probability of inducing teratogenic effects, whereas SCH 39304 is deprived of any known teratogenic biophore and therefore has no likelihood of reproductive side effects.

Apparently, ICI 153,066 failed mainly because of the choice of a monotriazole skeleton over a bistriazole one. According to the Multi-CASE model, this created a major teratogenic biophore **IIc** in ICI 153,066. Furthermore, the pattern of chlorine substitution chosen exacerbated the problem by being conducive not only to antifungal activity, but also to teratogenicity (Table 4).

Therefore, to develop an alternative antifungal based on ICI 153,066, three steps appear reasonable to start with (Fig. 5): firstly, the *m,p*-chlorinated phenyl ring should be replaced by a triazole ring connected to the rest of the molecule through one methylene unit. This step will

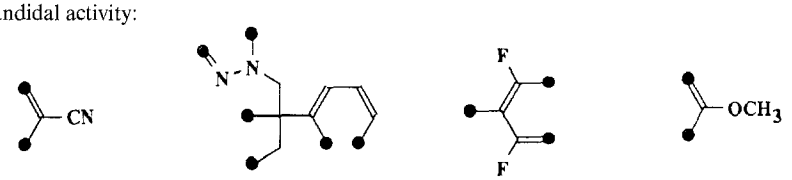
BIOPHOBES

Therapeutic index:



Id **IId** **IIId** **IVd** **Vd**

Anticandidal activity:



Ie **IIe** **IIIe** **IVe**

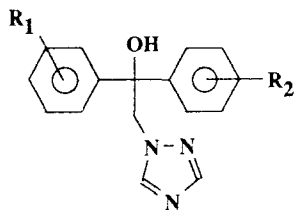
Fig. 4. The *Therapeutic index* and *Anti-Candida* biophobes. The methoxy- and cyano-groups on the aromatic ring are disruptive to the anti-Candida activity (fragments **Ie** and **IVe**), but not to the therapeutic index because of the concomitant negative contribution to teratogenicity (see Fig. 1). However, the *Anti-Candida* biophobe **IIe** will decrease the therapeutic index as well because it will not detract from teratogenicity. • denotes a non-hydrogen substituent.

destroy the teratogenic biophore **IIc** (Fig. 3) along with both of its chlorinated (+)modulators (Table 4). Secondly, one can introduce a fluorine atom in one of the *ortho*-positions on the phenyl ring thus creating a substructure **IVa** (Fig. 3) which is conducive to the anticandidal activity. This same step will also introduce a teratogenic biophobe **IIIf** (Fig. 2) and, consequently, boost the therapeutic index (fragment **IIIb** in Fig. 3). At this stage, one would be tempted to introduce the second *ortho*-fluorine to double the number of substructures **IVa** (Fig. 3), **IIIf** (Fig. 2), and **IIIb** (Fig. 3) in the molecule. This step, however, would most likely result in a decrease in the anticandidal activity due to the formation of the anticandidal biophobe **IIIe** (Fig. 4). Therefore, monofluorination should be preferred.

These simple chemical maneuvers based on the Multi-CASE results will effectively destroy all the teratogenic biophores in ICI 153,066 without creating new ones, and still preserve the antifungal activity, although due to different biophores. In fact, the structural manipulations undertaken will yield a 2-(2,4-difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)-propan-2-ol which is more commonly known as fluconazole – one of the most potent and safe antimycotics in the triazole class. Importantly, neither ICI 153,066, nor fluconazole were included in the training set.

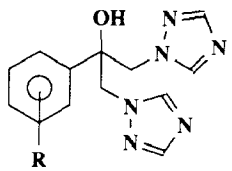
Our journey from ICI 153,066 to fluconazole was a logical and brief one, made such by the derived Multi-CASE model. In this prospective, it is interesting to point out that in 1983 ICI Pharmaceuticals gave up on 153,066, just to let fluconazole emerge from the laboratories of Pfizer Central Research some time later.

TABLE 3
THE RETROFIT OF THE EXPERIMENTAL DATA PROVIDED BY THE MULTI-CASE PROGRAM



No.	R_1	R_2	IC_{50c} (nmol/ml)		IC_{50t} (nmol/ml)		TI	
			Exp.	Calc.	Exp.	Calc.	Exp.	Calc.
1	4-CN	4-CN	+	++	+	+	+	+
2	4-NO_2	4-NO_2	+	++	+	−	+	+
3	3-NO_2	H	−	−	++	++	−	−
4	4-NO_2	H	++	++	++	++	+	−
5	H	H	+	+	++	++	−	−
6	4-F	H	++	++	++	++	−	−
7	4-F	4-F	++	++	+	−	++	+
8	2,4-F	4-F	++	++	++	−	++	+
9	4-Cl	4-NO_2	++	++	++	−	+	+
10	4-CH_3	H	++	++	++	++	+	−
11	2-CH_3	H	++	++	+	++	+	−
12	2,4-F	2,4-F	++	++	+	−	+	+
13	4-Cl	H	++	++	+	+	+	−
14	4-F	3-Cl	++	++	++	++	−	−
15	2-F	4-Cl	++	++	++	++	+	−
16	4-F	4-Cl	++	++	++	−	+	+
17	3-CF_3	H	+	+	++	++	−	−
18	2-CH_3	4-CH_3	++	++	++	++	+	−
19	2,4-F	4-CF_3	++	++	+	−	++	++
20	4-CF_3	2,4-F	++	++	+	−	++	++
21	2-Cl,5-NO_2	H	−	+	++	++	−	−
22	4-Cl	4-Cl	++	++	++	−	++	+
23	4-F	2-Cl	++	++	++	++	−	−
24	2-CF3	4-CF3	++	++	+	−	+	++
25	4-F	2-Cl,4-F	++	++	++	−	++	+
26	2-Cl	4-Cl	++	++	++	++	+	−
27	2,4-Cl	H	++	++	++	++	++	++
28	2-F	2,4-Cl	++	++	++	++	−	−
29	4-F	2-Br	++	++	++	++	−	−
30	2-Cl	2,4-Cl	++	++	++	++	−	−
31	4-Cl	2,4-Cl	++	++	++	−	++	++
32	3-Cl	2,4-Cl	++	++	++	++	++	++
33	4-Cl	2-Br	++	++	++	++	++	+
34	4-OCF_2CF_2H	H	++	++	++	++	+	−

TABLE 3 (continued)

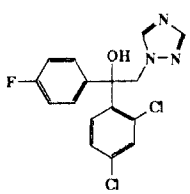
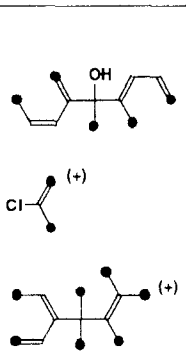
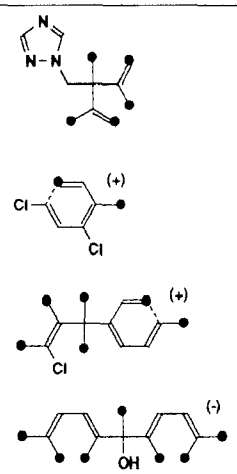
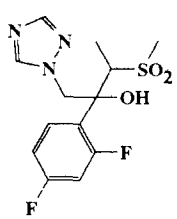
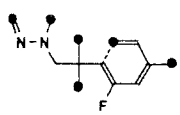


No.	R	IC_{50c} (nmol/ml)		IC_{50t} (nmol/ml)		TI	
		Exp.	Calc.	Exp.	Calc.	Exp.	Calc.
35	4-CN	–	–	–	–	+	–
36	4-OCH_3	–	–	–	–	–	–
37	H	–	–	–	–	–	–
38	2-CH_3,4-CN	–	–	–	–	–	–
39	4-F	–	–	–	–	–	–
40	2,6-F	–	–	+	–	–	–
41	2-CN,4-CF_3	–	–	ND			ND
42	2-CH_3	–	–	+	–	–	–
43	2,4,6-F	–	–	ND			ND
44	2,3,5,6-F	–	–	–	–	+	–
45	4-CH_3	+	–	–	–	+	–
46	4-SCH_3	–	–	+	–	–	–
47	2-CF_3	–	+	+	–	–	–
48	3-Cl	–	+	–	–	–	–
49	4-Cl	+	+	–	–	+	–
50	2-Cl	+	–	+	–	+	–
51	2-Cl,4-F	+	–	+	–	+	–
52	2-F,4-Cl	+	–	–	–	+	++
53	2-CF_3,4-F	–	–	–	–	–	–
54	4-$CH=CH_2$	–	–	+	–	–	–
55	2-CH_3,4-F	–	–	+	–	–	–
56	4-OCF_3	++	++	–	–	++	–
57	4-CF_3	+	–	–	–	++	+
58	2-OCH_3,4-CF_3	–	–	–	–	+	++
59	2-F,4-CF_3	++	+	–	–	++	++
60	3-F,4-CF_3	–	+	–	–	–	–
61	4-[4-CNPh]	–	–	ND		ND	
62	4-OCF_2CF_2H	–	+	++	++	–	–
63	2,4-Cl	++	+	+	–	+	–
64	2-Cl,4-CF_3	++	++	–	–	++	++
65	4-Ph	+	–	ND		ND	
66	4-[2,4F-Ph]	+	–	–	–	–	–
67	4-OPh	+	–	ND		ND	
68	4-[4-ClPh]	+	–	–	–	+	–
69	4-[4-ClPhO]	++	–	–	–	++	+

CONCLUSION

The results of the Multi-CASE analysis could be successfully used for the mechanistic elucidations of structural requirements for antifungal and teratogenic activity of triazole alcohols. Overall, the results agree with the idea of metabolically induced teratogenicity of triazole alcohols and suggest that the therapeutic utility of these agents is to benefit from the minimization of the number of aryl rings in the molecule, as well as from the mono-*ortho*-fluorination and *para*-fluoroalkylation of the aryl rings present. Chlorination of aryl rings may be beneficial for the antifungal activity, but may contribute to the teratogenic effects as we showed in the case of ICI 153,066. The program could successfully predict both the teratogenic and antifungal potentials of ICI 153,066 and SCH 39304 which were not part of the learning set.

TABLE 4
MULTI-CASE CORRECTLY PREDICTED > 90% PROBABILITY OF ANTIFUNGAL EFFECT FOR BOTH ICI 153,066 AND SCH 39304

No.	Compound	Antifungal biophores	Probability of being an antifungal	Teratogenic biophores	Probability of being a teratogen
1	ICI 153,066 		97.1 %		92.9 %
2	SCH 39304 		87.5 %	NONE	0 %

In agreement with experimental findings, the program also projected ICI 153,066 as a teratogen while predicting no reproductive side-effects for SCH 39304.
(+): an activating modulator; (−): a deactivating modulator. ● denotes a nonhydrogen substituent.

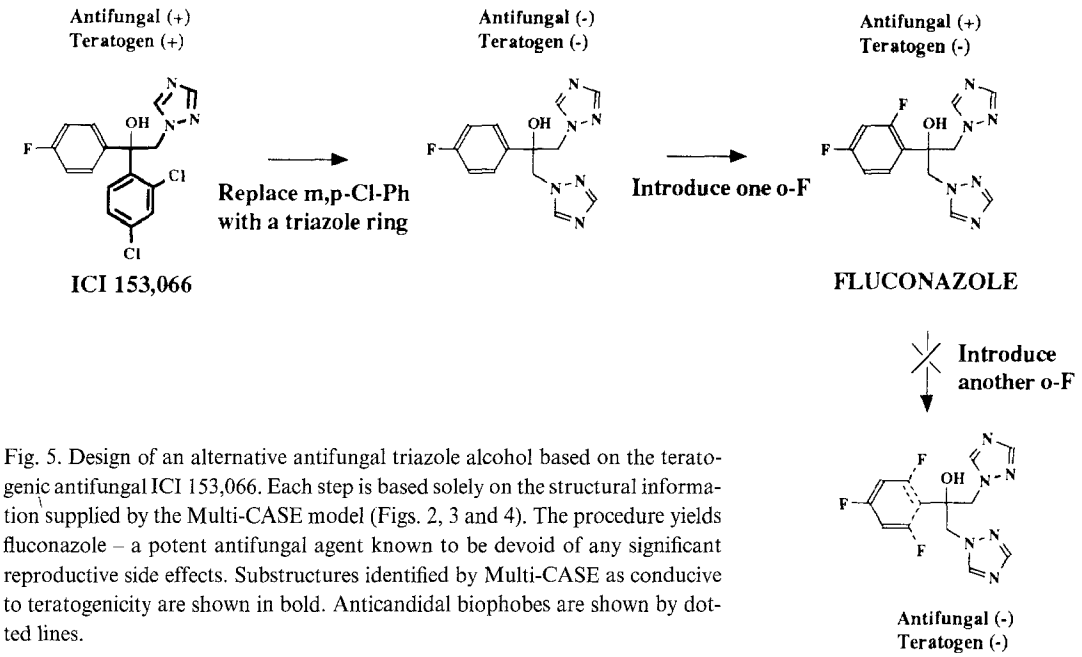


Fig. 5. Design of an alternative antifungal triazole alcohol based on the teratogenic antifungal ICI 153,066. Each step is based solely on the structural information supplied by the Multi-CASE model (Figs. 2, 3 and 4). The procedure yields fluconazole – a potent antifungal agent known to be devoid of any significant reproductive side effects. Substructures identified by Multi-CASE as conducive to teratogenicity are shown in bold. Anticandidal biophobes are shown by dotted lines.

Multi-CASE results allowed rational selection of molecular features which may be conducive only to antifungal activity without encouraging the reproductive side effects. We showed how this information can be used for the optimization of the therapeutic index of already existing leads. Naturally, it can also be employed for the de novo design of drugs with a desired therapeutic profile.

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