Isobenzofurans as Conformationally Constrained Miconazole Analogues with Improved Antifungal Potency[1]

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A series of halogen-substituted isobenzofuran analogues was synthesized, which represented conformationally constrained analogues of miconazole (1). In vitro and in vivo topical antifungal activity against both dermatophytes and *Candida* species varied widely, but **13c** proved to be significantly superior to both 1 and clotrimazole against a vaginal *Candida* infection in hamsters, while **13b** was significantly more active than 1 against a a topical *Trichophyton* infection in guinea pigs. None of the compounds were orally active. When the most direct analogue of 1 proved to be among the least active, a molecular modeling study was done using 1, the two active analogues **13b** and **13c**, and the inactive analogue **13a**. All four compounds possessed skeletally similar conformations either at or energetically readily accessible from the global minimum energy conformations. This common conformation of the inactive analogue **13a**, however, occupies unique molecular volume space associated with two chlorine atoms, which must also present unique electrostatic properties at the receptor. The conformation–activity relationships discussed may contribute toward deduction of additional structural requirements for pharmacophore optimization and more efficacious antifungal drugs.

Miconazole (1) has become a well-established drug for the treatment of many mycotic infections since it was first described.[2] It acts by competitive inhibition[3,4] through direct interaction[5-7] of the cytochrome P-450 enzyme which catalyzes the 14-α-demethylation of 24-methylene-24,25-dihydrolanosterol (P-450$_{14\alpha DM}$). This inhibition of 14-α-demethylation of 24-methylene-24,25-dihydrolanosterol results in a lethal disruption in the normal sterol biosynthesis chain in fungi, but is of minimal consequence to mammals. Miconazole was the first commercially significant example of what has become known as a broad class of α-arylazolylethanol derivatives, but its use has been limited by a lack of oral absorption, limited spectrum of activity, and occasional undesirable side effects observed when administered at therapeutic doses intravenously.[8,9] Despite intensive research efforts over the years, azole antifungals still remain a viable lead structure in pursuit of a more efficacious orally active, broad spectrum, systemic fungicidal drug. Moreover, it is one of the few classes of compounds offering a clear identity of the target enzyme and adequate specificity for fungal organisms.

Miconazole is a flexible molecule containing six rotatable bonds (Figure 1). Examination of the gas-phase conformational hyperspace available to 1 starting from, and including, its initial conformation, and defined about the rotatable bonds described in Figure 1, suggests that 1 can adopt a great number of energetically accessible conformations. The total number of valid conformations available to 1, Σ = 827 valid conformations, also includes its bioactive conformation, i.e., that conformation of 1 which is responsible for expression of its biological activity at the P-450$_{14\alpha DM}$ binding site.

It is conceivable that a conformationally constrained analogue that mimics the bioactive conformation might exhibit a higher level of intrinsic antifungal potency such that the analogue, or further structural modification of the analogue, could produce a candidate from this series for preclinical progression. Furthermore, it is also possible that a conformationally constrained analogue of 1 might not be as suitable a substrate for mammalian aromatase enzymes. Thus, by virtue of introducing this conformational constraint, a potential liability of this series might be eliminated.

This report describes a series of azolylisobenzofurans that are skeletal analogues of 1, but whose conformationally constrained structure allows them to adopt only a limited subset of conformations relative to 1, possibly including the bioactive conformation.

Chemistry

Substituted 2-(azolylmethyl)isobenzofurans **13** were prepared by one of two methods shown in Schemes I and II.[10] Method A was based upon acid-catalyzed dehydrative

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(1) Presented in part at the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy, Houston, TX, September 1989, Abstract 729.

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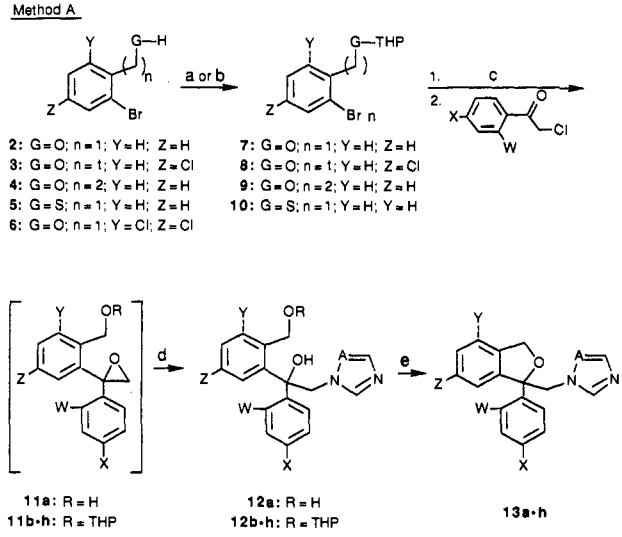
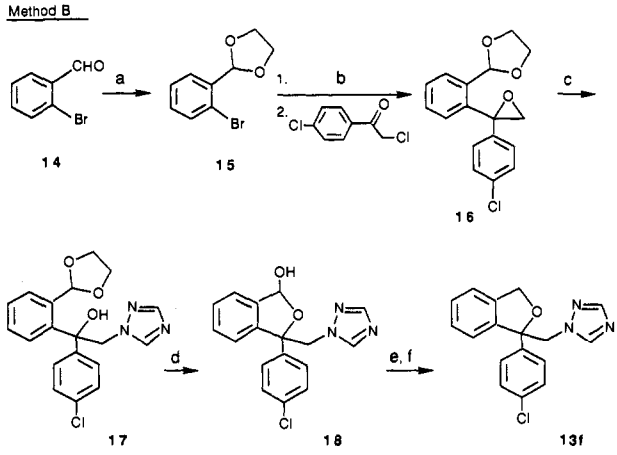
Scheme I [a]



[a] (a) Dihydropyran, TsOH, CH_2Cl_2 (2–5); (b) *n*-BuLi, THF, −70 °C (6); (c) Mg, THF, 50 °C; (d) 1-sodium 1,2,4-triazole or 1-sodium imidazole, DMF, 80 °C; (e) 6 N HCl, reflux.

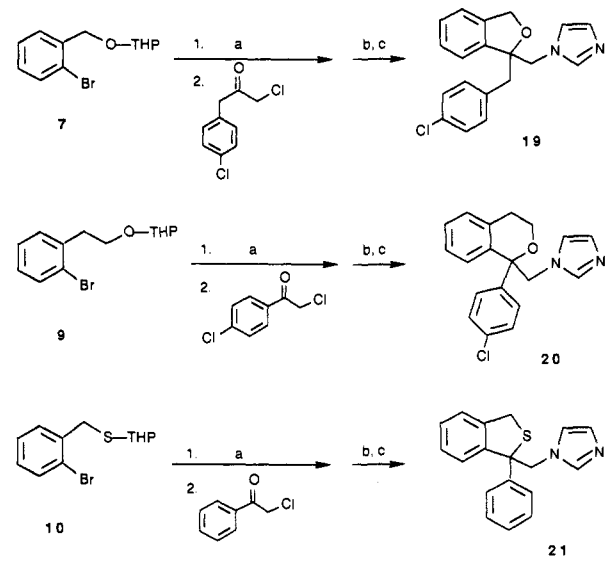
Scheme II [a]



[a] (a) Ethylene glycol, TsOH, CH_2Cl_2; (b) Mg, THF, 50 °C; (c) 1-sodium 1,2,4-triazole, DMF, 80 °C; (d) oxalic acid, aqueous dioxane, reflux; (e) $NaBH_4$, aqueous EtOH; (f) 6 N HCl, reflux.

cyclization of *o*-bis(hydroxymethyl)benzenes as adapted from Martin.[11] Substituted *o*-bromobenzyl alcohols **2** and **3** were protected as THP ethers **7** and **8** and treated with magnesium in THF to form a Grignard reagent, which required heating at near reflux temperature to both initiate and maintain the reaction. The Grignard reagents were reacted with a substituted phenacyl chloride to form chlorohydrins, which cyclized in situ to epoxides **11** under the reaction conditions used.[12] The epoxides were then treated with the freshly prepared sodium derivative of an azole to afford the azolyl ethanols **12**. Heating **12** at reflux in aqueous HCl removed the THP protecting group when present and affected cyclization of the intermediate diols to the desired isobenzofurans **13**. The intermediate diols could be obtained by conducting the hydrolysis in an aqueous oxalic acid–dioxane mixture.

Scheme III [a]



[a] (a) Mg, THF, 50 °C; (b) 1-sodium imidazole, DMF, 80 °C; (c) 6 N HCl, reflux.

An exception to the general procedure of method A was required when the Grignard reagent of the THP ether of **6** failed to form under all forcing conditions tried. However, by treating **6** with 2 equiv of *n*-butyllithium at below −65 °C, a marginally stable *o*-lithioaryl intermediate was formed. When alkylated immediately with 2,4-dichlorophenacyl chloride, **11a** could be obtained in modest yield and subsequently reacted as for its THP ether-protected analogues.

Method B (Scheme II) entailed much the same chemistry except that the starting material was a benzaldehyde rather than benzylic alcohol and was protected as a dioxolane. Following metallation, alkylation, and azole incorporation, the product of acid-catalyzed deprotection/cyclization of, e.g., **17** was the lactol **18**. The crude product of the reduction of **18** by $NaBH_4$, predominantly the diol analagous to **12a**, was directly cyclized in aqueous HCl at reflux to the isobenzofuran **13f**. Unless the lactol was desired, reduction of benzaldehydes to benzyl alcohols for use via method A proved experimentally more convenient. Compounds **13b** and **13f**, and isobenzofurans in general, have also been prepared by other methods.[13]

Method A was used to prepare the structural variants **19–21** (Scheme III). When 3-chloro-1-(4-chlorophenyl)-propanone[14] was reacted with the Grignard reagent of **7**, the chlorobenzyl analogue **19** was obtained. When 2-(2-bromophenyl)ethanol[15] (**4**) was used to prepare the Grignard reagent of **9**, the benzopyran **20** was obtained. Similarly, the Grignard reagent of **10** provided the isobenzothiophene analogue **21**.

Most of the alcohols used as starting materials for **2–5** were commercially available. Alcohol **6**, not previously known, was prepared by electrophilic bromination of 2,4-dichlorotoluene, followed by peroxide-catalyzed benzylic

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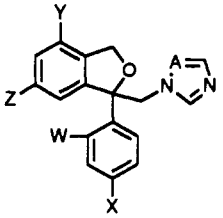
Table I. Physical Data for Substituted azolylisobenzofurans and analogues



13a–h

no.	A	W	X	Y	Z	mp, °C	formula	anal.
13a	CH	Cl	Cl	Cl	Cl	200–203	$C_{18}H_{12}Cl_4N_2O\cdot HCl\cdot 2H_2O$	H,N;C[a]
13b	CH	H	Cl	H	H	192–193	$C_{18}H_{15}ClN_2O\cdot HCl$	C,H,N
13c	CH	Cl	Cl	H	H	220–222	$C_{18}H_{14}Cl_2N_2O\cdot HCl$	C,H,N
13d	CH	Cl	Cl	H	Cl	195–198	$C_{18}H_{13}Cl_3N_2O\cdot HCl$	C,H,N
13e	CH	F	F	H	H	143–146	$C_{18}H_{14}F_2N_2O\cdot HCl\cdot {}^1/_2CH_3CN$[b]	C,H,Cl,F;N[c]
13f	N	H	Cl	H	H	188–196	$C_{17}H_{14}ClN_3O\cdot HCl$	C,H,N
13g	CH	H	Ph	H	H	221–224	$C_{24}H_{20}N_2O\cdot HCl$	C,H,N
13h	CH	H	H	H	H	209–212	$C_{18}H_{16}N_2O\cdot HCl$	C,H,N
18						215–217	$C_{17}H_{14}ClN_3O_2$	C,H,N
19						118–124 dec	$C_{19}H_{17}ClN_2O\cdot HCl$	C,H,N
20						154–158	$C_{19}H_{17}ClN_2O\cdot HCl\cdot {}^1/_2H_2O$	C,H,N
21						219–222	$C_{18}H_{18}N_2S\cdot HCl$	H,N;C[d]

[a] C: calcd, 44.89; found, 44.46. [b] Recrystallization solvent and observed in NMR spectrum. [c] N: calcd, 61.98; found, 61.55. [d] C: calcd, 65.74; found, 66.23.

Table II. Antifungal Minimum Inhibitory Concentration Ranges (μg/mL)

	SDB medium, pH 5.7										EMEM			
	organisms[a]								geometric mean		organisms[a]			geometric
no.	*C.a.*	*C.t.*	*C.s.*	*C.p.*	*T.m.*	*T.r.*	*M.g.*	*M.c.*	*E.f.*	*Candida*	dermatophytes	*C.a.*	*C.t.*	*C.s.*	mean
1	2–8	4	8	<0.031	<0.031	<0.031	1	<0.031	<0.031	≤1.8	≤0.03	0.125–0.25	0.125	0.125	0.18
13a	2–8	8	4	4	8	8	16	8		4.9	9.5	2	2	2	2.0
13b	4–16	16	32	0.25	2	2	4	2	<0.031	7.2	≤0.86	<0.031	0.125	<0.031	≤0.03
13c	4–32	32	32	<0.031	<0.031	<0.031	0.5	<0.031	0.063	7.2	≤0.04	≤0.031	<0.031	<0.031	≤0.02
13d	64	8	64	<0.031	0.5	8		0.5		≤15	1.3	<0.031	1	<0.031	≤0.03
13e	>64	64	>64	0.25	0.5	16		2		48	2.5	<0.031	<0.031	<0.031	≤0.02
13f	8–64	64	32	1	1	1	4	1	<0.031	16	≤0.57	<0.031	<0.031	<0.031	≤0.02
13g	16–32	32	16	<0.031	<0.031	<0.031	8	1	<0.031	7.2	≤0.12	0.125–0.25	0.063	0.125	0.16
13h	64	64	64	8	16	16	64	16	<0.031	48	≤5.2	0.125–0.25	0.125	0.25	0.20
18	>64	>64	>64	32	>64	64	>64	>64	8	≥105	≥54	NT[b]	NT	NT	NT
19	>64	>64	>64	4	8	8	32	4	0.25	≥78	4.6	0.25	0.25	0.25	0.25
20	32–64	64	64	2	0.125	0.25	1	1	≤0.031	51	≤0.22	≤0.031	<0.031	<0.031	≤0.02
21	64	64	64	16	2	2	16	4	0.063	53	1.7	0.125–0.25	0.125	0.25	0.19

[a] Abbreviations: *C.a.*, *Candida albicans* (four strains); *C.t.*, *C. tropicalis*; *C.s.*, *C. stellatoidea*; *C.p.*, *C. parapsilosis*; *T.m.*, *Trichophyton mentagrophytes*; *T.r.*, *T. rubrum*; *M.g.*, *Microsporum gypseum*; *M.c.*, *M. canis*; *E.f.*, *Epidermophyton floccosum*. [b] Not tested.

bromination using NBS.[16] The bromide was displaced with acetoxy anion, followed by alkaline hydrolysis of the acetate. When alcohols were not directly available, the corresponding carboxylic acids were reduced to the requisite alcohols by diborane[17] in THF. Physicochemical data for 2-(azolylmethyl)isobenzofurans **13**, **18**, and **19** and analogues **20** and **21** is summarized in Table I.

Biological Results

Minimum inhibitory concentrations (MICs) for compounds were determined using standard dilution methods in both Sabouraud dextrose broth (SDB) and Eagles minimum essential medium (EMEM).[18] Most azoles are much more active in EMEM, and EMEM appears to be a more predictive indicator of in vivo topical activity against *Candida* species for the azole class of antifungals. The MICs of racemic compounds **13a–h**, **18**, and **19–21**, particularly in EMEM, showed that desirable activity levels were limited to the subset of compounds **13b–h** (Table II).

To facilitate overall evaluations of compounds, geometric mean MICs[19] were calculated for *Candida* species separately from dermatophytes. The hydroxyl-substituted compound **18** was compared to **13f**, the conformationally more mobile benzylic analogue **19** was compared to **13b**, and the unsubstituted analogue **13h** was compared to **13b**. In each case significant reduction in activity was observed. Similarly, reduction of activity was seen for the sulfur substitution of **21** compared to **13h**, as well as for the expansion of the furan ring to a pyran ring in **20** compared to **13b**. Most importantly, **13a**, the direct analogue of **1**, was by far the least active compound among **13a–h**, both against dermatophytes and *Candida*. The dichloro-

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(19) For our purposes, the geometric mean of a group of MICs provided a statistically more useful summary number than weighted averages or alternative methods.

Table III. Inhibition of a Topical *Trichophyton*[a] Infection in Guinea Pigs[b]

no.	% cultures negative: during administration	% cultures negative: post administration	lesion score: (no. of animals)–days to become negative
1	20	60	(4)–14.2 (1)–≥16
13b	90	87	(3)–3.7 (2)–≥16
clotrimazole	75	87	(5)–8.2
vehicle[c]	5	33	(5)–≥16

[a] D-24 strain. [b] All compounds administered topically at 0.25% (w/v) concentration. [c] 10:45:45 (v/v/v) EtOH–PEG 400–glycerol.

Table IV. Inhibition of a Vaginal *Candida* Infection in Hamsters

no.	% area under curve: 0.05% concn[a]: C-43 strain, od × 8 D	C-60 strain: od × 8 D	C-60 strain: od × 4 D	0.01% concn: C-60 strain, od × 4 D
1	69	63	63	33
13a	<30			
13b	89	88	88	30
13c			94	78
13d			64	26
13e			88	69
clotrimazole		34		

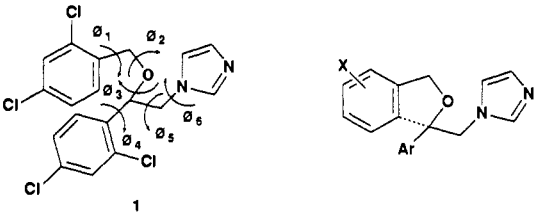
[a] Except miconazole (1) at 0.25%.

substituted analogue **13c** was the most potent against dermatophytes, while **13b–f** were similar in activity against *Candida*.

Compound **13b** was examined in vivo in guinea pigs against a topical *Trichophyton* infection. The infected area was cultured during and after administration of compound, and the results are shown in Table III. Compound **13b** was more potent than **1**, both in the percent of animals that were mycologically negative and in significantly shorter times to cure the lesions in those animals which had negative cultures. In this test, **13b** was similar to clotrimazole.

Compounds **13a–e** were also tested in a hamster vaginal *C. albicans* infection model and compared to **1** and clotrimazole. Results are shown in Table IV. In order to differentiate among the compounds, it was necessary to use two *C. albicans* strains, two dose regimens, and two concentrations. Strain C-43, at 0.05% concentration, administered once daily for 8 days showed that **13a**, the direct analogue of **1**, was inactive in vivo as predicted by MICs. At the same time, **13b** appeared to be somewhat more potent than **1**. Using strain C-60, again in an 8-day regimen, showed **13b** to be more potent than **1**, and both were superior to clotrimazole. Reducing once daily administration to only 4 days showed no loss of potency for **13b** or **1** from the 8-day regimen, but showed that the dichloro analogue **13c** and the difluoro analogue **13e** were comparable in potency to **13b**, while the trichloro analogue **13d** was less potent and comparable to **1**. Maintaining the once daily administration for 4 days but reducing the concentration to 0.01% showed that the dihalo analogues **13c** and **13e** were about twice as potent as **13b**, **13d**, and **1**.

Compounds **13b** and **13c** were administered orally at 50 mpk to mice with systemic *C. albicans* infection and compared with ketoconazole. Using a dosage regimen which produced 100% survival and residual kidney infections of 10^4 organisms/kidney for ketoconazole, both resulted in >50% mortality and residual kidney infections of $>10^6$. They are essentially inactive orally.



1

Figure 1. Miconazole with rotatable bond designations and the isobenzofuran skeleton formed by the dashed bond formation.

Molecular Modeling

A molecular modeling study was conducted on selected compounds, **1** and **13a–c**, to determine if the antifungal activity might be related to common conformational attributes shared by active analogues and if the lack of antifungal activity of **13a** was consistent with such a determination. The *R* isomer of **1** was created by using molecular fragments obtained from the crystallographic fragment library available within SYBYL 5.32. The *R* isomer was chosen since for those racemic azoles where it has been determined, it is the *R* isomer that exhibits maximal antifungal potency. The resulting structure was minimized in MacroModel V3.1 using the MM2 force field and applying the truncated Newton–Raphson conjugate gradient algorithm. The energy is expressed in kilojoule/mole and the gradient first derivative root mean square (RMS) convergence criteria for the total energy was ≤0.05 kJ/mol per Å (0.01 kcal/mol).

Using the minimized structure, a search of the conformational hyperspace of **1** about all rotatable bonds described in Figure 1 was conducted using the conformational search routine with SYBYL. The incremental angle of rotation about the rotatable bonds was 30°, and the associated ranges of rotation were 0–179° for attached substituted-phenyl groups, and 0–359° for all other rotatable bonds. The van der Waals scaling factors used were general VDW interaction/1,4-interaction/hydrogen-bonding interaction = 0.965/0.87/0.65, respectively. Conformations were examined for van der Waals contacts, and conformations that allowed two atoms to be closer to one another than the sum of their van der Waals radii were discarded. Each of the valid conformations derived from the conformational search of **1** was individually minimized using the MacroModel batch minimizer (BatchMin) using the MM2 force field and applying the truncated Newton–Raphson conjugate gradient algorithm. The global minimum energy conformation and those conformations within 20 kJ/mol (~5 kcal/mol) of the minimum-energy conformation were stored. The energy is expressed in kJ/mol and the gradient first derivative root mean square (RMS) convergence criteria for the total energy was ≤0.05 kJ/mol per Å (0.01 kcal/mol).

Using the procedure described above, conformational analysis of the *S* isomer of **1** produced a conformation of identical energy relative to the *R* isomer. This conformation was identified as the enantiomer of the global minimum energy conformation of the *R* isomer of **1**, as expected.

The most active conformationally constrained isobenzofuran, **13c**, was also created using the crystallographic fragment library in SYBYL 5.32 and minimized as described above. The *R* isomer of **13c** was chosen again

for geometric consistency relative to 1. Using the minimized structure, a conformational search about all rotatable bonds and the ring-closure bond was conducted. Once again, the incremental angle of rotation about the rotatable bonds was 30°, and the associated ranges of rotation were 0–179° for the attached substituted-phenyl group and 0–359° for all other rotatable bonds. The permitted variation in the distance between the ring closure atoms was modified to 0.25 Å, while the permitted variation in the valence angles about the ring closure atoms was relaxed to 15°. All other ring closure and search criteria were the default values within SYBYL. The valid conformations derived from this search were individually minimized, and the global minimum energy conformation, and those conformations within 20 kJ/mol (~5 kcal/mol) of the minimum-energy conformation were stored. The energy is expressed in kJ/mol and the gradient first derivative root mean square (RMS) convergence criteria for the total energy was ≤0.05 kJ/mol per Å (0.02 kcal/mol).

Compound **13c** was then used as a template to create compounds **13a** and **13b**.[20] Following the same procedure described above, conformational analysis of each identified their global minimum energy conformations and those conformations within 20 kJ/mol (~5 kcal/mol) of their minimum-energy conformations, respectively.

The geometry of the global minimum energy conformation of the *R* isomer of 1 does not accurately mimic the geometry of the global minimum energy conformation of the most active, conformationally constrained isobenzofuran, **13c**. However, a conformational search of 1 using the distance defined by the position between the centroids of the imidazole and the two different substituted-phenyl rings, as well as the distance defined by the position between the centroids of the two different substituted-phenyl rings in **13c**, as distance constraints, identified a subset of conformations that were reduced relative to the unconstrained conformational search of 1. Minimization of each of these conformations identified a slightly higher energy conformation of 1 that is energetically accessible from the global minimum energy conformation of 1 and that more closely mimics the global minimum energy conformation of **13c**.

Discussion

In the initial stages of this work, Dreiding models of 1 were examined for possible conformations which brought disconnected carbon atoms in close proximity to one another such that a bond between those atoms could have produced an analogue that was a conformationally constrained conformer of 1. One possibility was an isobenzofuran structure shown generically in Figure 1 and represented by **13a**. Furthermore, during the course of this work computer-assisted molecular design (CAMD) techniques became available to us, and determination of the energetic requirement for 1 to attain a conformation that mimics the isobenzofuran structure, as well as a comparison between these two structures, was now possible. In addition, the results of these studies could be compared to the structural data determined for 1[21] and ketoconazole[22] by X-ray crystallography and computer modeling studies reported by others.[23-26]

Implicit in the design of conformationally constrained analogues of flexible molecules is the hypothesis that it is the minimum-energy conformation of the analogue, or more likely, a conformation accessible from the minimum, that determines the pharmacological activity of the analogue. This hypothesis has been used previously with success in the design and synthesis of conformationally constrained analogues exhibiting enhanced gastric antisecretory potency relative to nonrigid analogues.[27]

If this hypothesis was applicable to the present study, identification of the global minimum energy conformation of selected compounds might reveal some correlation between conformation and antifungal potency. It is assumed for the sake of this study that, for most azoles of a similar structural class, differences in minimal inhibitory concentration (MIC) and topical activity reflect primarily differences in intrinsic activity of the various compounds rather than differences in dermal penetration or cellular absorption. In this study, the activity of interest was the inhibition of the vaginal *Candida* infection in hamsters. The compounds compared were miconazole (1), **13a**, **13b**, and **13c**.

The results in Table IV show that the flexible tetrachloro-substituted compound, 1, and the conformationally constrained monochloro-substituted analogue, **13b** exhibit moderate antifungal activity, while the constrained tetrachloro-substituted analogue **13a** is inactive and the dichloro-substituted analogue **13c** is very potent. Comparison of the global minimum energy conformation of **13c** with that of 1 shows that there is only a partial similarity between these two structures (Figure 2). However, a conformational search of 1 using the distance defined by the position between the centroids of the imidazole and the two different substituted-phenyl rings, as well as the distance defined by the position between the centroids of the two different substituted-phenyl rings in **13c**, as distance constraints in the search identified a subset of conformations of 1, which when minimized identified a local minimum energy conformation residing only 0.58 kcal/mol above the global minimum. This energetically accessible local minimum energy conformation of 1 (1-LMEC) more closely mimics the global minimum energy conformation of **13c** (Figure 3). This rationalization is consistent with the two compounds being

(20) Note that the stereochemical designator for **13b** created from the ***R*-13c** template is ***S*-13b**, a consequence of nomenclature rules though three-dimensional geometrical relationships of atoms is the same.

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(26) Lewis, D. F. V.; Rodrigues, A. D.; Ioannides, C.; Parke, D. V. Adverse Reactions of Imidazole Antifungal Agents: Computer Graphic Studies of Cytochrome P-450 Interactions. *J. Biochem. Toxicol.* **1989**, *4*, 231–234.

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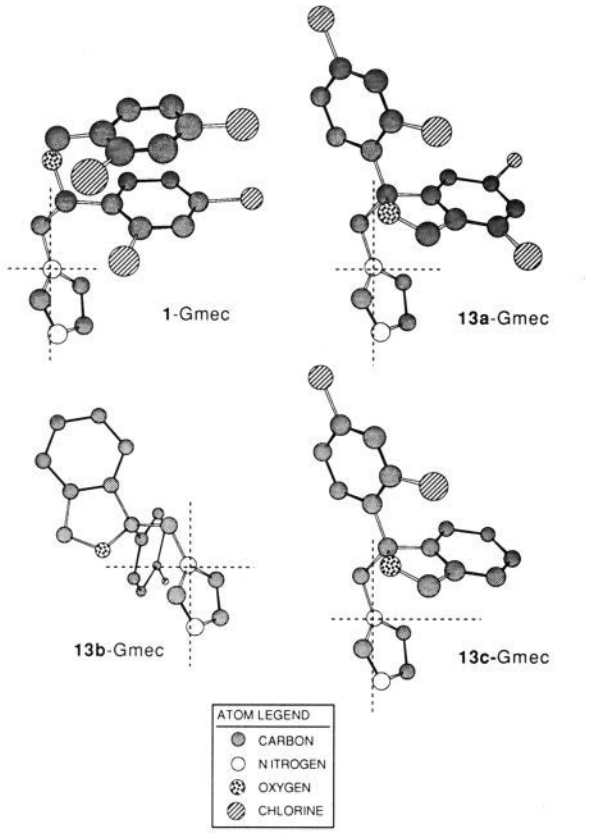

Figure 2. Global minimum energy conformations for 1 and **13a–c**, fit-aligned on the azole ring.

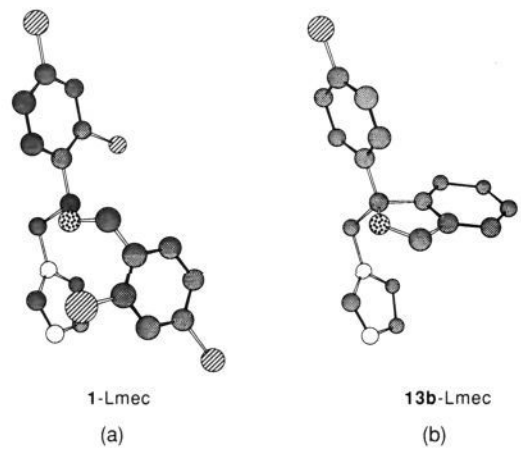

Figure 3. Local minimum energy conformations of (a) 1 at 0.58 kcal/mol above the global minimum and (b) **13b** at 0.72 kcal/mol above the global minimums.

capable of adopting a common binding conformation at the cytochrome P-450 receptor and the marginal differences in antifungal potency may be reflecting other factors.

Compound **13b** (*S* isomer)[20] also exhibits a global minimum energy conformation that is distinctly different from that of 1 or **13c** (Figure 2). However, a slightly higher energy conformation of **13b** (**13b**-LMEC) does exist, residing only 0.72 kcal/mol above the global minimum, that mimics the global minimum energy conformation of **13c** (Figure 3). Once again, this energetically accessible conformation could rationalize the slight differences observed in antifungal potency between analogues if it is postulated that the global minimum energy conformation

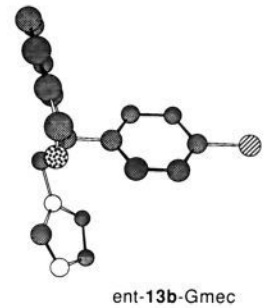

Figure 4. Global minimum energy conformation of (*R*)-**13b** derived from the "incorrect" enantiomer template.

of **13c** is representative of the optimum bioactive conformation for this limited series of azole antifungals.

Therefore, the lower antifungal potency of 1 and **13b** relative to **13c** may be a result of altered kinetic transport properties and/or altered entropic effects incurred by 1 and **13b** for adopting slightly higher energy binding conformations. Alternatively, it may reflect less efficient binding of their respective global minimum energy conformations.

Interestingly, the global minimum energy conformation of the other enantiomer of **13b**, the R isomer (*ent*-**13b**), does mimic that of **13c** (Figure 4). Since only racemic mixtures, and not individual optical antipodes, were independently prepared and tested, it is not apparent whether this result is relevant or simply fortuitous.

The global minimum energy conformation of inactive analogue **13a** was practically identical to the global minimum energy conformation of the most potent isobenzofuran analogue **13c**. This observation was unexpected and is inconsistent with the hypothesis that antifungal activity in this series is solely a result of adopting an energeticaly accessible bioactive conformation which is available to active analogues 1, **13b**, and **13c**, but is not available to inactive analogue **13a**. However, the lack of antifungal activity of **13a** might be a result of rigid steric and/or electronic requirements operative at the receptor, rather than broad structural differences between **13a** and **13c**. *This latter proposal is consistent with, and supported* by, the similar antifungal potency observed and the similar global and local conformations available to 1, **13b**, and **13c**.

Since **13c** has been proposed to mimic the bioactive conformation for this limited series of azole antifungals, the active analogue approach[28–30] was applied to 1, **13a**, **13b**, and **13c**. The conformation of 1, **13a**, and **13b** that each mimics the global minimum energy conformation of **13c** was compared to **13c** by fitting the atoms of the imidazole ring using the FIT command within SYBYL. In all cases, the root mean square (RMS) deviation of fit was <0.005 Å. By using the compared structures described above, the total molecular volume of the active analogues 1, **13b**, and **13c**, and the total molecular volume of the inactive analogue **13a** was generated using the MVOLUME command within SYBYL and visualized.

Graphical representation of excluded and included molecular volume maps can be interpreted as a hypo-

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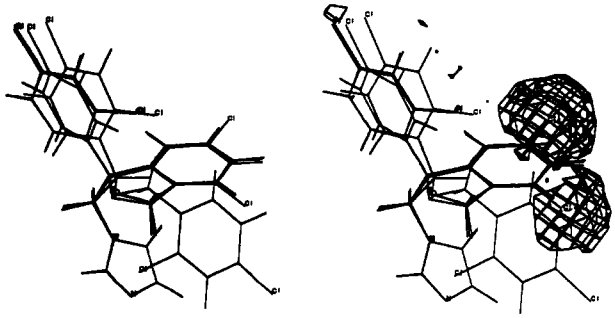
(30) Marshall, G. R. *Ann. N.Y. Acad. Sci.* **1985**, 439 (Macromol. Struct. Specif.), 162–169.



(a) (b)

Figure 5. (a) Molecular skeletons of 1-Lmec, **13a**-Lmec, **13b**-Gmec, and **13c**-Gmec, fit-aligned on the imidazole ring; (b) excluded molecular volume of **13a**-Lmec by difference from the common included molecular volumes of 1-Lmec, **13b**-Gmec, and **13c**-Gmec, superimposed on (a).

thetical description of the pharmacophore at the active site, in this case whereby the azole antifungals interact with cytochrome P-450 enzyme at the P-450$_{14\alpha DM}$ binding site. Comparison by difference of the molecular volume of **13a** relative to the included volume of **1**, **13b**, and **13c** generated an excluded volume map which revealed an excluded volume in **13a** (Figure 5). The two chlorine atoms in the isobenzofuran ring occupy a unique volume within this set of compounds. The additional molecular volume alone, the electrostatic nature of the chlorine atoms, or some combination of both could possibly account for the inactivity of **13a**.

The global minimum energy conformation of **13c** more reasonably agrees with the "footlike" model for cytochrome P-450 inhibitors proposed by Nakayayma than 1 does.[25] The "footlike" model, incorporating both molecular modeling and extensive QSAR analysis, suggests spatial attributes beyond the previous schematic representations. It describes a binding pocket shaped like a foot over the heme iron of the P-450 enzyme with an aryl ring in the "ankle", an iron-binding azole at the "heel", and a lipophilic component along the "arch" to the "toe". Moreover, this model appears to be quite consistent with X-ray data[22,23] and conclusions of other azole antifungal modeling studies[24,25,27] as they attempt to correlate relative antifungal activity to structure.

Since the binding site–substrate complex of P-450$_{14\alpha DM}$ remains undefined, the conformation–activity relationships discussed and the data previously published and cited may contribute further toward deduction of additional characteristics and requirements of the binding site and/or pharmacophore. Knowledge of this information may assist in the discovery of more specific and efficacious antifungal agents. The limited scope of the present study does not permit broader conclusions about either the binding site characteristics or the pharmacophore requirements of P-450$_{14\alpha DM}$.

Experimental Section

Biological Methods. In vitro activity was assayed against isolates of *Candida albicans* (strains C40, C41, C42, C43), *Candida tropicalis*, *Candida stellatoidea*, *Candida parapsilosis*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum gypseum*, *Microsporum canis*, and *Epidermophyton floccosum*. Minimum inhibitory concentrations (MIC) were determined in Sabouraud dextrose broth (SDB), pH 5.7, for *Candida* species and dermatophytes and in Eagle's minimum essential medium (EMEM), pH 7.0, for *Candida* species. The MIC was defined as the lowest concentration of test compound at which fungal growth was completely inhibited when cultures were examined visually in a 96-well microtiter plate. Test compounds were dissolved in DMSO or ethanol, serially diluted in growth medium, inoculated, and incubated at 37 °C in a CO_2 environment for EMEM and at 27 °C for SDB. Growth MIC was determined at 48 h for *Candida* species and at 72 h for dermatophytes.

Topical in vivo activity was determined using a hamster vaginal infection model employing *Candida albicans* strains C43 and C60 (10 animals per test compound). The test compound was dissolved in EtOH–PEG 400–glycerol (10:45:45 v/v/v) and applied intravaginally by inserting a cotton pellet containing 0.15 mL of sample at a concentration of 0.05% or 0.01% (w/w) once daily for 4 or 8 days, beginning on the fifth day postinfection. On days 4 and 8 postinfection, cultures were taken of the vaginal cavity by inserting a cotton swab, agitating the swab in saline, and filtering through a 22-μm membrane filter. The filter was placed onto a Sabouraud dextrose agar (SDA) plate and incubated at 37 °C for 48 h at which time colonies were counted. The percent cultures negative at each time was calculated, and the area under the percent culture–time curve (AUCs) was determined.

Topical in vivo activity was determined using a *Trichophyton mentagrophytes* strain D24 infection in guinea pigs (five animals per test compound). Infection was established by swabbing a homogenate of the microorganism onto the shaven and abraded skin of the animals. The test compound was dissolved or suspended in ethanol–PEG–glycerol (10:45:45 v/v/v) at a concentration of 0.25% (w/v), and 0.3 mL of the solution was applied to the infected area and rubbed in using a glass rod. Treatment was administered twice daily for 10 days, starting on the fourth day postinfection. Multiple hair samples were taken from the infected site of each animal periodically for up to 16 days, placed onto SDA plates, and incubated at 27 °C. Plates were examined visually on days 4 and day 8 and scored as negative if there was no visible growth. The number of negative cultures per group of animals as a percent of total cultures per group was then determined. The day that all cultures for an individual animal were negative was also recorded.

Oral activity was determined by infecting mice with a measured inoculum of *C. albicans*. Two hours later, compound was administered by gavage at 50 mg/kg. At selected time intervals, mice were sacrificed, their kidneys homogenized, and the homogenate was serially diluted. The homogenate was applied to an agar plate and incubated at 37 °C for 48 h at which time colonies were counted. The number of viable colonies per plate was extrapolated to the number of colonies per animal at the time of sacrifice.

All microorganisms used were from an in-house collection derived from clinical isolates.

Computer Modeling Methods. Entry of structural information was done using the features of the program SYBYL 5.32 (Tripos Associates, St. Louis, MO) as described in the text. Energy minimization of SYBYL-generated structures was done using the program MacroModel v3.1 (Still, W. C.; et al., Columbia University, New York, NY) as described in the text. Energy-minimized conformations were aligned in SYBYL using its FIT command to produce fit-aligned models for file storage in MOL file format. Graphical screen representations of fit-aligned stored models were manipulated using the rotational and sizing features of the program Chem3D Plus (Cambridge Scientific Computing Inc., Cambridge, MA), all models being manipulated concurrently to maintain fit alignment. Printed representations were produced with Chem3D Plus.

Chemistry. Reagents, reactants, and solvents obtained from commercial sources were the best available grades and were used without further purification. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Proton NMR spectra were obtained on either a Varian CFT-20 or a Varian EM 390 spectrometer, using tetramethylsilane as an internal reference. Mass spectra were obtained on a Varian MAT CH5 spectrometer.

2-Bromo-4,6-dichlorobenzyl Alcohol (6). Bromine (10 g, 63 mmol) was added in 1-g portions to a mixture of 4,6-dichlorotoluene (32 g, 190 mmol) and anhydrous $FeCl_3$ (0.3 g) while a temperature of 20–25 °C was maintained. Additional bromine (22 g, 138 mmol) in CCl_4 (100 mL) was added slowly at

20–25 °C, and the mixture was stirred for 15 min and then diluted with CH_2Cl_2 (200 mL). The organic layer was washed with 5% aqueous sodium thiosulfate, dried over anhydrous K_2CO_3, filtered, and evaporated to dryness. The residue was crystallized from CH_3CN to give 2-bromo-4,6-dichlorotoluene (**22**) (32 g, 67%), mp 84–5°C. Anal. Calcd ($C_7H_5BrCl_2$): C, 35.94; H, 2.10. Found: C, 35.56; H, 2.54.

An intimate mixture of **22** (220 g, 920 mmol), *N*-bromosuccinimide (167 g, 940 mmol), and benzoyl peroxide (1 g) in a 5-L flask (equipped with a condenser and stirrer) was immersed in a 145 °C oil bath and slowly stirred. After 10–20 min a vigorous eruptive reaction occurred and the mixture liquified. After removal of the oil bath and stirring for an additional 5 min, CCl_4 (1.5 L) was added slowly through the condenser. The cooled mixture was suction filtered, the filtrate was washed with 5% aqueous sodium thiosulfate, and the organic layer was dried over anhydrous K_2CO_3. The mixture was filtered, the solvent was evaporated, and the residue was fractionally distilled to give 2-bromo-4,6-dichlorobenzyl bromide (**23**) (134 g, 46%), bp 94–7 °C (0.2 Torr), which contained ca. 20 mol % of the α,α-dibromo compound by ^{1}H NMR analysis, but which was suitable for solvolysis in the next step.

A mixture of crude **23** as obtained from the previous step (22 g, 69 mmol), anhydrous sodium acetate (30 g, 365 mmol), and glacial acetic acid (80 mL) was heated for 18 h at 100 °C and then evaporated to leave a dry residue. The residue was treated with ethanol (200 mL) and 2 N NaOH (200 mL) and stirred at reflux for 3 h. After cooling, the mixture was concentrated and extracted with Et_2O. The ethereal layer was dried over anhydrous K_2CO_3 and filtered, and the filtrate was evaporated to dryness. The residue was crystallized from toluene–hexane to give **6** (10 g, 57%): mp 111–2 °C; ^{1}H NMR ($CDCl_3$) δ 7.69 (s, 1 H), 7.45 (s, 1 H), 4.72 (d, 2 H), 2.00 (t, 1 H). Anal. ($C_7H_5BrCl_2O$): C, H.

Method A: General Procedure for the Preparation of Isobenzofurans. 2-Bromobenzyl 2-Tetrahydropyranyl Ether (7). To a solution of **2** (25 g, 134 mmol) in CH_2Cl_2 (400 mL) at 10 °C were added *p*-toluenesulfonic acid (0.25 g) and then dihydropyran (17 g, 202 mmol) in one portion. The solution was stirred for 30 min at ambient temperature, washed with 5% aqueous K_2CO_3 and then saturated brine, and dried over anhydrous K_2CO_3. The mixture was filtered, and the filtrate was evaporated and fractionally distilled to give **7** (33 g, 91%), bp 119–22 °C (0.8 Torr), which was suitable for the subsequent reaction: ^{1}H NMR ($CDCl_3$) δ 7.6–7.0 (m, 4 H), 4.6 (m, 3 H), 3.6 (m, 2 H), 1.9–1.2 (m, 9 H).

Compounds **8–10** were prepared similarly.

2-[[2-(1-Phenyloxiranyl)phenyl]methoxy]tetrahydropyran (11h). Magnesium shavings (0.54 g, 22 mmol) were added to a solution of **7** (6.0 g, 22 mmol) in THF (10 mL). The mixture was gently warmed to 45–50 °C at which the reaction commenced. The temperature was moderated below reflux to maintain the reaction until the magnesium was nearly consumed. The Grignard reagent so formed was added dropwise over a period of 15 min at 0–5 °C to a solution of phenacyl chloride (3.25 g, 21 mmol) in THF (100 mL), and the mixture was allowed to warm slowly to room temperature and stirred for 18 h. The mixture was cooled to 0 °C and vigorously stirred and saturated aqueous NH_4Cl (50 mL) added. The aqueous layer was separated and extracted with EtOAc, and the combined organic layer and extracts were dried over anhydrous Na_2SO_4. The mixture was filtered and the filtrate evaporated to leave crude **11h** (6.8 g, >100%), which was used immediately in the next step: ^{1}H NMR ($CDCl_3$) δ 7.1–7.8 (m, 9 H), 4.5–4.8 (m, 3 H), 3.1–3.5 (m) and 3.3 (dd) [4 H total], 1.3–2.0 (m, 6 H).

Compounds **11b–g** were prepared similarly. Preparation of **11a** is described below.

[2-[1-(2,4-Dichlorophenyl)oxiranyl]-4,6-dichlorophenyl]methanol (11a). A solution of 1.6 M *n*-butyllithium in hexane (132 mL) was added to a solution of **6** (25 g, 98 mmol) in THF (250 mL) over 2 h at −65 to −70 °C. The cold bath was removed and a solution of 2,4-dichlorophenacyl chloride (22 g, 98 mmol) in THF (75 mL) was added rapidly. The mixture was stirred an additional 20 min without further cooling and cooled again to 5 °C, and 5% aqueous K_2CO_3 (350 mL) was added with vigorous stirring. The organic layer was separated and the aqueous layer extracted with CH_2Cl_2. The organic layer and extract were combined and dried over anhydrous K_2CO_3. The mixture was filtered and the filtrate evaporated in vacuo. The residue was chromatographed on silica gel and eluted with EtOAc–CH_2Cl_2 to afford crude **11a** (9.1 g, 26%): ^{1}H NMR ($CDCl_3$ + D_2O) δ 3.3 (m, 2 H), 4.7 (s, 2 H), 7.3 (m, 4 H), 7.7 (d, 1 H).

2-(1*H*-Imidazol-1-yl)-1-phenyl-1-[2-[(2-tetrahydropyranyloxy)methyl]phenyl]ethanol (12h). Crude **11h** (7.3 g, ca. 24 mmol) in THF (10 mL) was added at 20 °C to a stirred suspension of sodium imidazole (4.4 g, 48 mmol) in DMF (prepared by mixing 2.0 g of 60% oil dispersion of NaH and 3.4 g of imidazole at 5–20 °C in 50 mL of DMF for 1 h). The mixture was then heated for 2 h at 85 °C, poured into ice water, and suction filtered to leave a precipitate. The filtrate was extracted with EtOAc, and the extract washed with brine and dried over anhydrous Na_2SO_4. The mixture was filtered and the filtrate evaporated to leave a residue. The residue and the original precipitate were combined and dried in vacuo, thoroughly triturated under EtOAc, and filtered to leave crude **12h** (4.7 g, 53%), which was suitable for the subsequent reaction: ^{1}H NMR ($CDCl_3$) δ 7.0–7.8 (m, 9 H), 4.7 (AB q), and 4.4 (m) [4 H total], 3.77 (d) and 3.4–3.8 (m) [3 H total], 2.0–1.3 (m, 6 H).

Compounds **12a–g** were prepared similarly.

1-Phenyl-1-[(1*H*-imidazol-1-yl)methyl]-1,3-dihydroisobenzofuran (13h). A mixture of **12h** (4.6 g, 12 mmol) in 6 N hydrochloric acid (50 mL) was heated at reflux for 1.5 h. The hot solution was poured into a mixture of 3 N NaOH (90 mL), 10% K_2CO_3 (100 mL), and ice (100 g) and extracted with $CHCl_3$. The extract was dried over anhydrous Na_2SO_4, filtered, and evaporated to a residue. The residue was chromatographed on silica gel eluting with CH_2Cl_2–EtOAc to afford **13h** (3.3 g, 100%). For characterization the product was dissolved in Et_2O and treated with anhydrous HCl in dioxane solution and evaporated to dryness, and the residue was recrystallized from CH_3CN to give **13h**·HCl (2.2 g, 59%): ^{1}H NMR (DMSO-d_6) δ 9.09 (s, 1 H), 7.6 (m, 3 H), 7.3 (m, 8 H), 5.14 (AB q, 2 H), 5.08 (s, 2 H).

Compounds **13a–g** were prepared similarly.

1-(4-Chlorophenylmethyl)-1-[(1*H*-imidazol-1-yl)methyl]1,3-dihydroisobenzofuran (19). Compound **7** was reacted with 3-chloro-1-(4-chlorophenyl)propanone[14] as described in method A to afford 2-[[2-[1-[(4-chlorophenyl)methyl]-oxiranyl]phenyl]methoxy]tetrahydrofuran as a minor component, by NMR, in a complex mixture. This mixture and the subsequent crude product mixtures were treated sequentially as described above in method A to afford, following chromatography and treatment with HCl–dioxane, **19**·HCl (0.09 g, 0.5% overall): ^{1}H NMR ($CDCl_3$) δ 7.4–6.8 (m, 10 H), 6.66 (s, 1 H), 4.58 (AB q, 2 H), 4.29 (m, 2 H), 3.10 (s, 2 H).

1-(4-Chlorophenyl)-1-[(1*H*-imidazol-1-yl)methyl]-3,4-dihydro-1*H*-2-benzopyran (20). Compound **9** (24.5 g, 105 mmol) was reacted with 4-chlorophenacyl chloride as described in method A to afford 2-[2-[2-[1-(4-chlorophenyl)oxiranyl]-phenyl]ethoxy]tetrahydropyran as a component in a mixture. This mixture and the subsequent crude product mixtures were treated sequentially as described above in method A to afford, following chromatography and treatment with HCl–dioxane, **20**·HCl·$^1/_2H_2O$: ^{1}H NMR (DMSO-d_6) δ 9.02 (s, 1 H), 7.8–7.1 (m, 10 H), 5.08 (s, 2 H), 3.90 (m, 1 H), 3.55 (m, 1 H), 2.62 (m, 2 H).

1-Phenyl-1-[(1*H*-imidazol-1-yl)methyl]-1,3-dihydroisobenzothiophene (21). Compound **10** was reacted with phenacyl chloride as described in method A to afford 2-[[2-[1-(phenylmethyl)oxiranyl]phenyl]methoxy]-1,3-dihydroisobenzothiophene as a minor component, by TLC, in a complex mixture. This mixture and the subsequent crude product mixtures were treated sequentially as described above in method A to afford, following chromatography and treatment with HCl–dioxane, **21**·HCl: ^{1}H NMR (DMSO-d_6) δ 7.9 (s, 1 H), 7.38 (m, 10 H), 7.01 (t, 1 H), 5.35 (s, 2 H), 4.22 (d, 1 H), 3.74 (d, 1 H).

Method B. Alternate Synthesis of Isobenzofurans. 1-[2-[1-(4-Chlorophenyl)oxiranyl]phenyl]-1,3-dioxolane (16). Using the procedure described above for **11h**, the Grignard reagent of 2-(2-bromophenyl)-1,3-dioxolane[31] (16 g, 70 mmol) was pre-

(31) Watanabe, T.; Soma, N. Studies on Seven-membered Ring Compounds. XXXVI. Synthesis of Azulene Derivatives by Ring Closure of β-Cycloheptatrienyl-substituted Carbonyl Compounds. *Chem. Pharm. Bull. Jpn.* **1971**, *19*, 2215–2221.

pared and reacted with 4-chlorophenacyl bromide (14.9 g, 64 mmol) to afford crude **16**. This was chromatographed on silica gel eluting with EtOAc/hexane–1% Et_3N (v/v) to afford **16** (13 g, 66%), homogeneous by TLC: ^1H NMR ($CDCl_3$) δ 7.0–7.7 (m, 8 H), 6.0 (s, 1 H), 4.0 (m, 4 H), 3.3 (q, 2 H). Anal. ($C_{17}H_{15}ClO_3$): C, H.

1-(4-Chlorophenyl)-1-[2-(1,3-dioxolan-2-yl)phenyl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (17). Using the procedure described above for **12h**, **16** (13.3 g, 44 mmol) was treated with sodium 1*H*-1,2,4-triazole (4.6 g, 50 mmol). The residue of the extract was chromatographed on silica gel eluting with acetone–CH_2Cl_2, and the product so obtained was crystallized from CH_3CN to give **17** (10.8 g, 58%), mp 161–163 °C. Anal. ($C_{19}H_{18}ClN_3O_3 \cdot H_2O$): C, H, N.

1-(4-Chlorophenyl)-3-hydroxy-1-[(1*H*-1,2,4-triazol-1-yl)methyl]-1,3-dihydroisobenzofuran (18). A mixture of **17** (6.0 g, 16 mmol), dioxane (60 mL), and 10% aqueous oxalic acid (60 mL) was heated at reflux for 8 h. The mixture was cooled and poured into a solution of brine–H_2O–10% aqueous K_2CO_3 (10:10:1 v/v/v) (300 mL), which was then extracted with EtOAc. The combined extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was chromatographed on silica gel eluting with EtOAc–CH_2Cl_2 to obtain a solid. The solid was triturated with diisopropyl ether, filtered, and dried to leave **18** (3.8 g, 72%).

1-(4-Chlorophenyl)-1-[(1*H*-1,2,4-triazol-1-yl)-methyl]-1,3-dihydroisobenzofuran (13f). A stirred solution of **18** (1.4 g, 4.3 mmol) in 10% aqueous EtOH (200 mL) was treated with 0.2-g portions of $NaBH_4$ at 1-h intervals for 4 h (total 0.8 g, 2.2 mmol). Solvent was removed on a rotary evaporator, and the remains were partitioned between EtOAc-saturated brine. The EtOAc over dried over anhydrous Na_2SO_4, filtered, and evaporated to give dryness. The residue was chromatographed on silica gel eluting with EtOAc–CH_2Cl_2 to afford 1-(4-chlorophenyl)-1-[2-(hydroxymethyl)phenyl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (0.21 g, 15%), mp 130–193 °C. Anal. ($C_{17}H_{16}ClN_3O_2$): C, H, N. The residue (0.85 g) of the remainder of the chromatography isolates was dissolved in Et_2O and treated with anhydrous HCl in dioxane solution and evaporated to dryness, and the residue was recrystallized from CH_3CN to give **13f**·HCl (0.43 g, 34%): ^1H NMR (DMSO-d_6) δ 7.98 (m, 3 H), 7.64 (m, 3 H), 7.23 (m, 4 H), 5.00 (m, 4 H).

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