The Synthesis of Aminimide-Containing Azole Antifungals

Mark D. Abel, Randy T. Hewgill, Katherine J. Malczyk, Ronald G. Micetich*, and Mohsen Daneshtalab* [1]

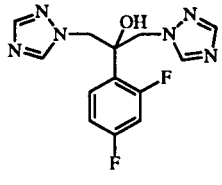
SynPhar Laboratories Inc. #2, 4290-91A Street, Edmonton, Alberta, Canada T6E 5V2

Received July 22, 1997

The synthesis of a series of azole antifungal compounds which incorporate the aminimide functional moiety is described. The procedure involves the reaction of an epoxide intermediate with 1,1-disubstituted hydrazines to form aminimines which are subsequently treated with acyl chlorides resulting in the desired zwitterionic aminimides. The aminimides were tested for *in vitro* antifungal activity and found to be moderately active against *Candida* and *Cryptococcus* species, but inactive versus *Aspergillus*.

J. Heterocyclic Chem., **35**, 193 (1998).

The recent increase in systemic fungal infections, particularly among AIDS and other immunocompromised patients, has led to considerable interest in the discovery of safer, more effective antifungal agents. While members of the azole class of antifungals, including Fluconazole **1**, have



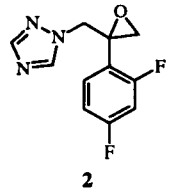
1: Fluconazole

proven to be highly effective against yeasts such as *Candida albicans*, they often exhibit little or no efficacy against filamentous fungi such as *Aspergillus fumigatus* or *Aspergillus niger*. During the course of our work involving azole compounds, it was observed that typically, as the lipophilicity of our molecules was increased, improved *in vitro* activity against filamentous fungi and retained or improved activity against yeasts was noted [2]. On the other hand, the increased lipophilicity often resulted in diminished water solubility and/or poor *in vivo* bioavailability characteristics. Thus, there was a need to correctly balance the lipophilic and hydrophilic character of the azole antifungals in order to obtain potent, broad spectrum compounds.

Recently, there have been reports describing the use of the aminimide functional moiety in peptidomimetic inhibitors of elastase [3] and HIV-1 protease [4]. The zwitterionic character of aminimides was reported to result in enhanced solubility in protic, aprotic and non-polar media [3]. This interesting property led us to consider the use of the aminimide moiety in Fluconazole-like molecules with the hope of obtaining potent, broad spectrum antifungal compounds with a suitable lipophilic/hydrophilic balance. We report herein the synthesis and antifungal activity of a series of aminimide-containing azole antimycotics.

Chemistry.

The chemistry of aminimides has been the subject of various review articles [5,6] and these compounds can be synthesized through a number of methods. The method that was of particular interest to us produces aminimides from the reaction of carboxylic esters, unsymmetrically disubstituted hydrazines and epoxides in a one-pot procedure [7]. The mechanism for this reaction was reported to involve the initial attack of a hydrazine on the epoxide to form an intermediate aminimine which subsequently reacts with an ester to form the aminimide. This particular reaction was appealing due to the common usage of epoxide intermediates, such as **2**, in the synthesis of azole antifungals.



2

Epoxide **2** was prepared according to a published method [8]. We initially attempted the reaction of **2**, 1,1-dimethylhydrazine **3a** and ethyl acetate in methanol at room temperature as described in the literature [7]. Rather than obtaining the desired aminimide, we isolated a very hygroscopic compound that nmr and mass spectral data revealed to be aminimine **4a**. Thus, it seems clear that in our particular case the initial aminimine formation proceeded, but no subsequent acylation took place. Indeed, when **2** and **3a** were stirred in methanol with no ethyl acetate present, the product was the same aminimine **4a** as previously obtained. Similar results were obtained regardless of which disubstituted hydrazine was employed, however longer reaction times and lower yields were observed for 1-aminopiperidine **3b** and 1-ethyl-1-methylhydrazine **3c**, perhaps owing to greater steric hindrance when compared to 1,1-dimethylhydrazine **3a**.

Aminimine **4a** was then treated with acetyl chloride **5a** to give aminimide **6** as supported by nmr and mass sprectral data. In addition, the X-ray crystal structure of **6** was determined (Figure 1). The crystal structure unequivocably verified it as the desired aminimide with water coordinated at two-thirds water per aminimide molecule.

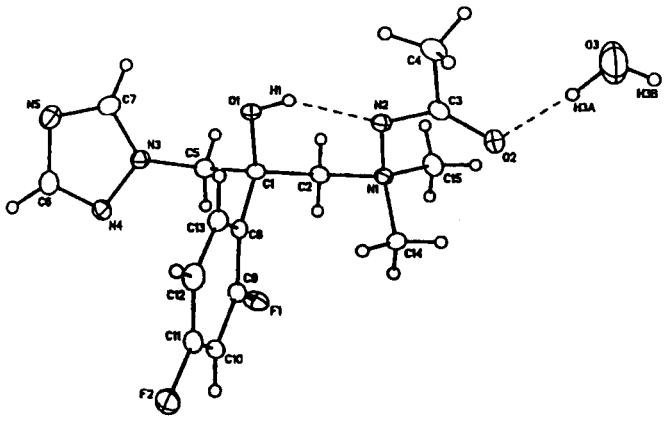


Figure 1. Perspective view of **6** and the associated cocrystallized water molecule. Non-hydrogen atoms are represented by Gaussian ellipsoids at the 20% probability level. Hydrogen atoms are shown with arbitrarily small thermal parameters. Hydrogen-bonded interactions between H1 and N2 and between H3A and O2 are indicated wtih dashed lines.

Based on these initial studies, a general method for the preparation of aminimide-containing azole antifungal compounds was established as depicted in Scheme 1. Thus, epoxide **2** was treated with various 1,1-disubstituted hydrazines **3a-c** in methanol at room temperature to give aminimines **4a-c** in 23% to 78% yields. The aminimines **4a-c** were then acylated with acyl chlorides **5a-l** in the presence of triethylamine and catalytic 4-dimethylaminopyridine resulting in aminimides **6-22** (Table 1) in yields ranging between 5% and 56%. In the case of less reactive acyl chlorides, the use of 4-dimethylaminopyridine was particularly crucial. For instance, aminimine **4a** was stirred with cyclohexanecarbonyl chloride **5e** for 3 days with no product being formed. However, when the same reaction was performed in the presence of 0.05 equivalents 4-dimethylaminopyridine, aminimide **10** was formed in 50% yield.

Catalytic hydrogenation of the 4-nitrobenzoyl derivative **12** resulted in the isolation of the 4-aminobenzoyl analog **13** in 45% yield.

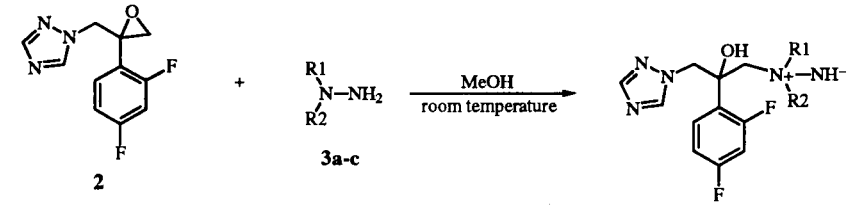
Compounds **6-20** were isolated as racemates while silica gel chromatography of **21** and **22** resulted in the isolation of two pairs of diastereomers, respectively, which were arbitrarily designated **21a,b** and **22a,b** to differentiate between pairs of isomers. In this initial study, no further effort was made to isolate pure enantiomers or diastereomers and the compounds were tested for antifungal activity as mixtures.

Results and Discussion.

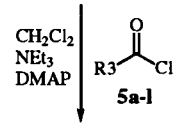
The results of *in vitro* screening of aminimides **6-22** against various *Candida* species are summarized in Table 2. It should be noted that *Candida glabrata* is typically less susceptible to azole antifungals than the other species included in the panel. In this study, we investigated the effect on *in vitro* activity of varying the nature of the acyl moiety or, alternately, the substituents on the quaternary

Scheme 1

Synthesis of Aminimide Antifungal Compounds



No.	R1	R2
3a, 4a	CH_3	CH_3
3b, 4b	$-(CH_2)_5-$	
3c, 4c	CH_3	CH_2CH_3



No.	R3	No.	R3
5a	CH_3	5g	$-NO_2$
5b	$(CH_2)_3H_3$	5h	$-F$
5c	$(CH_2)_8CH_3$	5i	$-CF_3$
5d		5j	$-CF_3$
5e		5k	$-OCF_3$
5f		5l	$-OCH_2CF_2CHF_2$

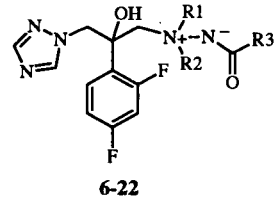
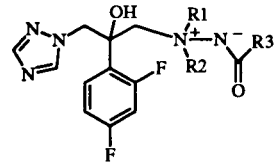
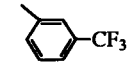
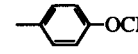
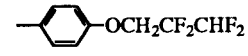
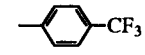
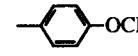
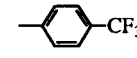
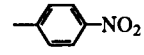
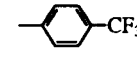
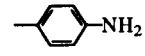
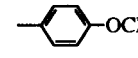
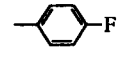
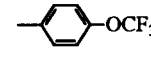
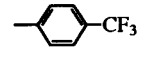


Table 1
Aminimide Antifungal Compounds **6-22**



No.	R1	R2	R3	No.	R1	R2	R3
6	CH_3	CH_3	CH_3	**16**	CH_3	CH_3	
7	CH_3	CH_3	$-(CH_2)_3CH_3$	**17**	CH_3	CH_3	
8	CH_3	CH_3	$-(CH_2)_8CH_3$	**18**	CH_3	CH_3	
9	CH_3	CH_3		**19**	$-(CH_2)_3-$		
10	CH_3	CH_3		**20**	$-(CH_2)_3-$		
11	CH_3	CH_3		**21a** [a]	CH_3	CH_2CH_3	
12	CH_3	CH_3		**21b** [a]	CH_3	CH_2CH_3	
13	CH_3	CH_3		**22a** [a]	CH_3	CH_2CH_3	
14	CH_3	CH_3		**22b** [a]	CH_3	CH_2CH_3	
15	CH_3	CH_3					

[a] Compounds **21** and **22** were each isolated as two pairs of diastereomers.

nitrogen. The tertiary alcohol, triazole and difluorophenyl moieties, which are well known to be important for antifungal activity, were kept constant for all compounds studied.

In compounds **6-18**, various acyl groups were incorporated into the molecule while, in all cases, there was dimethyl substitution on the quaternary nitrogen. While all the compounds exhibited moderate activity against most *Candida* species, it was possible to identify certain qualitative trends in structure-activity relationships.

When comparing the acetyl **6**, pentanoyl **7** and decanoyl **8** derivatives it is clear that while similar activity was observed for **6** and **7**, as the length of the alkyl chain increased to ten carbon atoms a significant increase in antifungal potency was observed. Indeed, compound **8** turned out to be the most potent analog synthesized. It is possible that this may be at least partially due to the greater lipophilic character imparted to the molecule by the longer alkyl chain. In addition, the cylcloalkane carbonyl derivatives **9** and **10** were noticeably less active than **8**.

In general, moderate potency was exhibited by the benzoyl and substituted benzoyl derivatives **11-18**. The weakest activity was associated with the 4-aminobenzoyl compound **13**. The hydrophilic nature of the amino group may at least partially account for this observation. The most active benzoyl derivatives were those containing the 3-trifluoromethyl **16** and 4-trifluoromethoxy **17** substituents. Overall, **16** was the most potent benzoyl derivative, but was slightly less effective than the decanoyl compound **8**.

We then turned our attention to the effect on antifungal activity of varying the substituents on the quaternary nitrogen atom. When comparing the piperidinyl compounds **19** and **20** with their *N,N*-dimethyl analogs **15** and **17**, it was readily apparent that reduced potency was associated with the piperidinyl moiety.

A comparison of **15** and **17** with their *N*-ethyl-*N*-methyl counterparts **21a,b** and **22a,b** was considerably more complicated due to the diastereomeric nature of the unsymmetrically substituted derivatives. Silica gel chromatography of **21** and **22** allowed for the separation of two pairs of diastereomers for each of the derivatives which were then tested for antifungal activity. In the case of the 4-(trifluoromethoxy)benzoyl pairs **22a** and **22b**, no noticeable difference in MIC values was observed. However, in the case of the 4-(trifluoromethyl)benzoyl pairs **21a** and **21b**, a dramatic difference in activity was apparent. This was a clear indication that the absolute configuration of the chiral cen-

Table 2

In vitro Antifungal Activity Against *Candida* Species

	MIC$_{50}$ (μg/ml) [a] [b]					
No.	*C. albicans* ATCC 14053	*C. albicans* 200/175	*C. albicans* Y01-09	*C. tropicalis* ATCC 13803	*C. kefyr* ATCC 38296	*C. glabrata* ATCC 90030
6	3.12	3.12	1.56	6.25	1.56	>100
7	6.25	6.25	6.25	12.5	3.12	>100
8	0.78	0.78	1.56	3.12	<0.19	>100
9	6.25	6.25	12.5	12.5	12.5	>100
10	6.25	6.25	3.12	12.5	3.12	>100
11	6.25	6.25	12.5	12.5	25	>100
12	12.5	12.5	12.5	25	25	>100
13	12.5	12.5	12.5	>100	12.5	>100
14	6.25	1.56	3.12	6.25	3.12	>100
15	12.5	6.25	3.12	3.12	3.12	>100
16	1.56	1.56	0.78	3.12	1.56	>100
17	1.56	1.56	1.56	6.25	3.12	>100
18	6.25	1.56	1.56	6.25	3.12	>100
19	12.5	12.5	25	25	6.25	>100
20	6.25	6.25	12.5	>100	0.78	>100
21a	3.12	3.12	1.56	1.56	1.56	>100
21b	>100	>100	>100	>100	12.5	>100
22a	12.5	12.5	25	>100	12.5	>100
22b	12.5	12.5	25	>100	25	>100
FLU [c]	0.78	0.39	0.39	0.39	0.39	12.5

[a] Minimum inhibitory concentration. [b] Confidence limit equals ± 1 dilution. [c] Fluconazole.

ters can be crucial in terms of antifungal activity. Thus, at the present time a direct comparison of the *N,N*-dimethyl and *N*-ethyl-*N*-methyl compounds would be highly speculative. Future preparation of chirally pure derivatives would be very useful in the investigation of this aspect of structure-activity relationships.

The discussion so far has dealt only with the activity of the aminimide derivatives against *Candida* species. As mentioned previously, our goal was the discovery of compounds with broad spectrum activity that included *Aspergillus* species. In this regard, **6-18** were also tested against a panel of various *Cryptococcus* and *Aspergillus* organisms. In general, no activity against these fungi was observed. The only exception was the decanoyl derivative **8** which exhibited reasonable activity against the two *Cryptococcus neoformans* strains, but none versus *Aspergillus* (Table 3).

Thus, we have demonstrated that the aminimide functional moiety can be successfully incorporated into the structure of azole antifungals. This incorporation can lead to compounds effective against yeasts such as *Candida albicans* or *Candida kefyr*. However, the compounds synthesized to date have exhibited a limited spectrum of activity and therefore considerable chemical modification is necessary to expand their range of activity to include filamentous fungi such as *Aspergillus* species.

Table 3

In vitro Activity of **8** Against *Cryptococcus* and *Aspergillus* Species

	MIC$_{50}$ (μg/ml) [a] [b]				
No.	*Cryptococcus neoformans* KF-33	*Cryptococcus neoformans* PLM 589	*Aspergillus niger* PLM 1140	*Aspergillus fumigatus* PLM 712	*Aspergillus fumigatus* ATCC 13073
8	1.56	6.25	>100	>100	>100
FLU [c]	1.56	3.12	>100	>100	>100

[a] Minimum inhibitory concentration. [b] Confidence limit equals ± 1 dilution. [c] Fluconazole.

EXPERIMENTAL

The compounds were assessed for *in vitro* antifungal activity using a protocol outlined by the National Committee for Clinical Laboratory Standards [9].

Melting points were determined on an Electrothermal digital melting point apparatus and are uncorrected. The ^{1}H nmr spectra were acquired on a Bruker AC-E 200 FT nmr spectrometer. Mass spectra and X-ray crystallography were performed by the Chemistry Department of The University of Alberta, Edmonton, Alberta, Canada. Dichloromethane was distilled over phosphorous pentoxide and stored over molecular sieves. Methanol was distilled over sodium. Silica gel used was Kieselgel 60 from Merck, 15-40 mesh for flash and 40-60 mesh for gravity chromatography.

1-[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-1,1-dimethylhydrazinium Inner Salt (**4a**).

Epoxide **2** (5.00 g, 21.08 mmoles) and 1,1-dimethylhydrazine **3a** (1.27 g, 21.08 mmoles) were dissolved in 20 ml of methanol and stirred at room temperature for 8 days. The methanol was evaporated and chloroform (100 ml) added to the residue. The chloroform solution was then extracted three times with 50 ml of water. The combined aqueous extracts were then washed with 75 ml of chloroform and the water removed on a freeze dryer to give 5.21 g (78%) of the product as a pale yellow solid, mp 60-65°; ^{1}H nmr (deuteriochloroform): δ 2.95 (s, 3H), 3.16 (s, 3H), 4.02 (m, 2H), 4.24 (d, J = 13.7 Hz, 1H), 4.68 (d, J = 13.7 Hz, 1H), 5.4-6.6 (br, 2H), 6.7-7.0 (m, 2H), 7.77 (s, 1H), 7.6-8.0 (m, 1H), 8.47 (s, 1H); ms: (FAB) M^+ = 297.8.

Anal. Calcd. for $C_{13}H_{17}F_2N_5O$: C, 52.51; H, 5.77; N, 23.56. Found: C, 52.18; H, 5.98; N, 23.21.

1-Amino-1-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]hexahydropyridinium Inner Salt (**4b**).

In a similar manner, **4b** was obtained from **2** and 1-aminopiperidine **3b** in 28% yield as a pale, yellow solid, mp 56-59°; ^{1}H nmr (deuteriochloroform): δ 1.3-1.5 (m, 2H), 1.6-1.8 (m, 2H), 1.9-2.1 (m, 1H), 2.1-2.4, (m, 1H), 2.7-2.9 (m, 1H), 3.1-3.4 (m, 2H), 3.7-3.9 (m, 1H), 4.0-4.9 (m, 4H), 6.8-6.9 (m, 2H), 7.5-7.7 (m, 1H), 7.63 (s, 1H), 8.51 (s, 1H); ms: (FAB) M^+ = 337.9.

Anal. Calcd. for $C_{16}H_{21}F_2N_5O$: C, 56.96; H, 6.27; N, 20.76. Found: C, 56.61; H, 6.08; N, 20.54.

1-[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-1-ethyl-1-methylhydrazinium Inner Salt (**4c**).

In a similar manner, **4c** was obtained from **2** and 1-methyl-1-ethylhydrazine **3c** as a mixture of diastereomers in 23% yield as a pale, yellow solid, mp 62-70°; ^{1}H nmr (deuteriochloroform): δ 1.03 (t, J = 7.2 Hz, 1.5H), 1.36 (t, J = 7.2 Hz, 1.5H), 2.85 (s, 1.5H), 2.97 (s, 1.5H), 3.2-3.4 (m, 2H), 3.7-4.0 (m, 2H), 4.1-4.2 (m, 1H), 4.6-4.9 (m, 1H), 5.4-6.3 (br, 2H), 6.7-7.0 (m, 2H), 7.79 (s, 0.5H), 7.81 (s, 0.5H), 7.9-8.1 (m, 1H), 8.54 (s, 1H); ms: (FAB) M^+ = 312.0.

Anal. Calcd. for $C_{14}H_{19}F_2N_5O$: C, 54.01; H, 6.15; N, 22.50. Found: C, 53.68; H, 6.31; N, 22.14.

1-[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-1,1-dimethyl-2-[4-(trifluoromethyl)benzoyl]-hydrazinium Inner Salt (**15**).

To a solution of **4a** (0.300 g, 0.911 mmole), triethylamine (0.165 ml, 1.184 mmoles) and 4-dimethylaminopyridine (0.006 g, 0.046 mmole) in 10 ml dry dichloromethane at 0°, 4-(trifluoromethyl)benzoyl chloride **5i** (0.149 ml, 1.002 mmoles) was added under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 36 hours and then 20 ml of water added. The organic phase was separated and the aqueous portion washed with an additional two 20 ml portions of dichloromethane. The combined organic extracts were then washed with brine, dried over sodium sulphate and evaporated to yield crude product. Flash chromatography through a silica gel column using 1% methanol-99% chloroform gave 0.239 g (56%) of **15** as a colourless solid, mp 188-189°; ^{1}H nmr (deuteriochloroform): δ 3.25 (s, 3H), 3.6-3.7 (m, 4H), 4.04 (d, J = 13.4 Hz, 1H), 4.25 (d, J = 14.3 Hz, 1H), 4.71 (d, J = 14.3 Hz, 1H), 6.8-7.0 (m, 2H), 7.4-8.1 (m, 5H), 7.94 (s, 1H), 8.34 (s, 1H), 9.65 (s, 1H); ms: (FAB) M^+ = 469.7.

Anal. Calcd. for $C_{21}H_{20}F_5N_5O_2$: C, 53.73; H, 4.29; N, 14.92. Found: C, 53.62; H, 4.04; N, 14.57.

2-Acetyl-1-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-1,1-dimethylhydrazinium Inner Salt (**6**).

In a similar manner, the reaction of **4a** and acetyl chloride **5a** resulted in **6** in 39% yield as a colourless solid, mp 111-114°; ^{1}H nmr (deuteriochloroform): δ 1.68 (s, 3H), 3.00 (s, 3H), 3.37 (d, J = 13.8 Hz, 1H), 3.41 (s, 3H), 3.78 (d, J = 13.4 Hz, 1H), 4.13 (d, J = 14.2 Hz, 1H), 4.58 (d, J = 14.2 Hz, 1H), 6.8-7.0 (m, 2H), 7.7-7.8 (m, 1H), 7.83 (s, 1H), 8.21 (s, 1H), 10.15 (br, 1H); ms: (FAB) M^+ = 340.0.

Anal. Calcd. for $C_{15}H_{19}F_2N_5O_2$: C, 53.09; H, 5.64; N, 20.64. Found: C, 52.86; H, 5.71; N, 20.33.

1-[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-1,1-dimethyl-2-pentanoylhydrazinium Inner Salt (**7**).

In a similar manner, the reaction of **4a** and pentanoyl chloride **5b** resulted in **7** in 54% yield as a colourless solid, mp 162-163°; ^{1}H nmr (deuteriochloroform): δ 0.91 (t, J = 7.1 Hz, 3H), 1.2-1.4 (m, 2H), 1.4-1.6 (m, 2H), 1.98 (t, J = 7.4 Hz, 2H), 3.06 (s, 3H), 3.41 (d, J = 13.4 Hz, 1H), 3.48 (s, 3H), 3.85 (d, J = 13.3 Hz, 1H), 4.20 (d, J = 14.2 Hz, 1H), 4.65 (d, J = 14.2 Hz, 1H), 6.8-7.1 (m, 2H), 7.7-7.9 (m, 1H), 7.91 (s, 1H), 8.30 (s, 1H), 10.10 (br, 1H); ms: (FAB) M^+ = 382.2.

Anal. Calcd. for $C_{18}H_{25}F_2N_5O_2$: C, 56.68; H, 6.61; N, 18.36. Found: C, 56.57; H, 6.65; N, 17.98.

1-[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-1,1-dimethyl-2-decanoylhydrazinium Inner Salt (**8**).

In a similar manner, the reaction of **4a** and decanoyl chloride **5c** resulted in **8** in 5% yield as a yellowish solid, mp 110-111°; ^{1}H nmr (deuteriochloroform): δ 0.89 (t, J = 6.3 Hz, 3H), 1.2-1.3 (m, 12H), 1.4-1.6 (m, 2H), 1.9 (t, J = 7.4 Hz, 2H), 3.07 (s, 3H), 3.41 (d, J = 13.3 Hz, 1H), 3.48 (s, 3H), 3.85 (d, J = 13.3 Hz, 1H), 4.20 (d, J = 14.2 Hz, 1H), 4.65 (d, J = 14.2 Hz, 1H), 6.8-7.1 (m, 2H), 7.7-7.9 (m, 1H), 7.91 (s, 1H), 8.30 (s, 1H), 10.30 (br, 1H); ms: (FAB) M^+ = 451.8.

Anal. Calcd. for $C_{23}H_{35}F_2N_5O_2$: C, 61.17; H, 7.81; N, 15.51. Found: C, 60.92; H, 8.00; N, 15.18.

2-Cyclopropanecarbonyl-1-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-1,1-dimethylhydrazinium Inner Salt (**9**).

In a similar manner, the reaction of **4a** and cyclopropanecarbonyl chloride **5d** resulted in **9** in 47% yield as a pale, yellow solid, mp 155-159°; ^{1}H nmr (deuteriochloroform): δ 0.4-0.6 (m, 4H), 1.2-1.3 (m, 1H), 3.00 (s, 3H), 3.40 (s, 3H), 3.46 (d, J = 13.7 Hz, 1H), 3.81 (d, J = 13.3 Hz, 1H), 4.15 (d, J = 14.2 Hz, 1H), 4.58 (d, J = 14.2 Hz, 1H), 6.7-7.0 (m, 2H), 7.7-7.9 (m, 1H), 7.82 (s, 1H), 8.23 (s, 1H), 9.50 (br, 1H); ms: (FAB) M^+ = 366.1.

Anal. Calcd. for $C_{17}H_{21}F_2N_5O_2$: C, 55.88; H, 5.79; N, 19.17. Found: C, 55.59; H, 6.02; N, 18.96.

2-Cyclohexanecarbonyl-1-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-1,1-dimethylhydrazinium Inner Salt (**10**).

In a similar manner, the reaction of **4a** and cyclohexanecarbonyl chloride **5e** resulted in **10** in 50% yield as a colourless solid, mp 160-161°; ^{1}H nmr (deuteriochloroform): δ 1.1-1.4 (m, 4H), 1.6-1.8 (m, 4H), 1.9-2.0 (m, 1H), 3.05 (s, 3H), 3.40 (d, J = 13.4 Hz, 1H), 3.46 (s, 3H), 3.85 (d, J = 13.3 Hz, 1H), 4.20 (d, J = 14.2 Hz, 1H), 4.66 (d, J = 14.2 Hz, 1H), 6.8-7.1 (m, 2H), 7.7-7.9 (m, 1H), 7.91 (s, 1H), 8.31 (s, 1H), 10.50 (br, 1H); ms: (FAB) M^+ = 408.2.

Anal. Calcd. for $C_{20}H_{27}F_2N_5O_2$: C, 58.95; H, 6.68; N, 17.19. Found: C, 58.62; H, 6.88; N, 17.53.

1-[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-1,1-dimethyl-2-benzoylhydrazinium Inner Salt (**11**).

In a similar manner, the reaction of **4a** and benzoyl chloride **5f** resulted in **11** in 20% yield as a colourless solid, mp 179-182°; ^{1}H nmr (deuteriochloroform): δ 3.16 (s, 3H), 3.56 (s, 3H), 3.57 (d, J = 13.2 Hz, 1H), 3.93 (d, J = 13.3 Hz, 1H), 4.17 (d, J = 14.2 Hz, 1H), 4.64 (d, J = 14.2 Hz, 1H), 6.7-6.9 (m, 2H), 7.3-7.4 (m, 3H), 7.7-7.8 (m, 3H), 7.86 (s, 1H), 8.28 (s, 1H), 10.15 (br, 1H); ms: (FAB) M^+ = 402.2.

Anal. Calcd. for $C_{20}H_{21}F_2N_5O_2$: C, 59.84; H, 5.27; N, 17.45. Found: C, 59.73; H, 5.61; N, 17.56.

1-[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-1,1-dimethyl-2-(4-nitrobenzoyl)hydrazinium Inner Salt (**12**).

In a similar manner, the reaction of **4a** and 4-nitrobenzoyl chloride **5g** resulted in **12** in 45% yield as a colourless solid, mp 190-191°; ^{1}H nmr (deuteriochloroform): δ 3.25 (s, 3H), 3.63 (s, 3H), 3.70 (d, J = 13.5 Hz, 1H), 4.06 (d, J = 13.4 Hz, 1H), 4.25 (d, J = 14.3 Hz, 1H), 4.72 (d, J = 14.3 Hz, 1H), 6.8-7.1 (m, 2H), 7.7-7.9 (m, 3H), 7.95 (s, 1H), 8.2-8.3 (m, 2H), 8.32 (s, 1H), 9.45 (br, 1H); ms: (FAB) M^+ = 447.3.

Anal. Calcd. for $C_{20}H_{20}F_2N_6O_4$: C, 53.81; H, 4.52; N, 18.83. Found: C, 53.63; H, 4.42; N, 18.94.

1-[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-1,1-dimethyl-2-(4-fluorobenzoyl)hydrazinium Inner Salt (**14**).

In a similar manner, the reaction of **4a** and 4-fluorobenzoyl chloride **5h** resulted in **14** in 23% yield as a colourless solid, mp 172-179°; ^{1}H nmr (deuteriochloroform): δ 3.22 (s, 3H), 3.6-3.7 (m, 4H), 4.00 (d, J = 13.3 Hz, 1H), 4.23 (d, J = 14.3 Hz, 1H), 4.71 (d, J = 14.3 Hz, 1H), 6.8-7.1 (m, 4H), 7.7-7.9 (m, 3H), 7.94 (s, 1H), 8.34 (s, 1H), 9.93 (br, 1H); ms: (FAB) M^+ = 420.0.

Anal. Calcd. for $C_{20}H_{20}F_3N_5O_2$: C, 57.27; H, 4.81; N, 16.70. Found: C, 57.45; H, 4.50; N, 16.38.

1-[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-1,1-dimethyl-2-[3-(trifluoromethyl)benzoyl]-hydrazinium Inner Salt (**16**).

In a similar manner, the reaction of **4a** and 3-(trifluoromethyl)benzoyl chloride **5j** resulted in **16** in 17% yield as a colourless solid, mp 193-195°; ^{1}H nmr (deuteriochloroform): δ 3.25 (s, 3H), 3.6-3.7 (m, 4H), 4.04 (d, J = 13.4 Hz, 1H), 4.25 (d, J = 14.3 Hz, 1H), 4.72 (d, J = 14.3 Hz, 1H), 6.8-7.0 (m, 2H), 7.4-7.6 (m, 1H), 7.6-7.9 (m, 3H), 7.94 (s, 1H), 8.07 (s, 1H), 8.34 (s, 1H), 9.65 (s, 1H); ms: (FAB) M^+ = 469.9.

Anal. Calcd. for $C_{21}H_{20}F_5N_5O_2$: C, 53.73; H, 4.29; N, 14.92. Found: C, 53.37; H, 4.09; N, 14.66.

1-[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-1,1-dimethyl-2-[4-(trifluoromethoxy)benzoyl]-hydrazinium Inner Salt (**17**).

In a similar manner, the reaction of **4a** and 4-(trifluoromethoxy)benzoyl chloride **5k** resulted in **17** in 42% yield as a colourless solid, mp 153-155°; ^{1}H nmr (deuteriochloroform): δ 3.22 (s, 3H), 3.6-3.7 (m, 4H), 4.20 (d, J = 13.6 Hz, 1H), 4.23 (d, J = 14.3 Hz, 1H), 4.71 (d, J = 14.1 Hz, 1H), 6.8-7.0 (m, 2H), 7.1-7.3 (m, 2H), 7.7-7.9 (m, 3H), 7.94 (s, 1H), 8.34 (s, 1H), 9.95 (br, 1H); ms: (FAB) M^+ = 485.9.

Anal. Calcd. for $C_{21}H_{20}F_5N_5O_3$: C, 51.96; H, 4.15; N, 14.43. Found: C, 52.06; H, 4.13; N, 14.46.

1-[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-1,1-dimethyl-2-[4-(2,2,3,3-tetrafluoropropyloxy)benzoyl]hydrazinium Inner Salt (**18**).

In a similar manner, the reaction of **4a** and 4-(2,2,3,3-tetrafluoropropyloxy)benzoyl chloride **5l** resulted in **18** in 36% yield as a colourless solid, mp 71-75°; ^{1}H nmr (deuteriochloroform): δ 3.22 (s, 3H), 3.5-3.8 (m, 4H), 3.99 (d, J = 13.3 Hz, 1H), 4.23 (d, J = 14.3 Hz, 1H), 4.37 (t, J = 11.8 Hz, 2H), 4.71 (d, J = 14.1 Hz, 1H), 6.08 (tt, J = 53.0 and 5.0 Hz, 1H), 6.8-7.0 (m, 2H), 7.7-7.9 (m, 5H), 7.94 (s, 1H), 8.34 (s, 1H), 10.15 (br, 1H); ms: (FAB) M^+ = 531.9.

Anal. Calcd. for $C_{23}H_{23}F_6N_5O_3$: C, 51.98; H, 4.36; N, 13.18. Found: C, 52.10; H, 4.10; N, 13.42.

1-[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-1-[[4-(trifluoromethyl)benzoyl]amino]hexahydropyridinium Inner Salt (**19**).

In a similar manner, the reaction of **4b** and 4-(trifluoromethyl)benzoyl chloride **5i** resulted in **19** in 13% yield as a colourless solid, mp 78-82°; ^{1}H nmr (deuteriochloroform): δ 1.5-2.0 (m, 6H), 2.7-2.9 (m, 1H), 2.9-3.1 (m, 1H), 4.04 (d, J = 13.7 Hz, 1H), 4.31 (d, J = 14.2 Hz, 1H), 4.35 (d, J = 13.7 Hz, 1H), 4.5-4.7 (m, 1H), 4.71 (d, J = 14.2 Hz, 1H), 5.0-5.2 (m, 1H), 6.8-7.0 (m, 2H), 7.5-7.7 (m, 3H), 7.8-7.9 (m, 3H), 8.18 (s, 1H), 8.80 (br, 1H); ms: (FAB) M^+ = 510.3.

Anal. Calcd. for $C_{24}H_{24}F_5N_5O_2$: C, 56.58; H, 4.75; N, 13.75. Found: C, 56.86; H, 5.09; N, 13.83.

1-[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-1-[[4-(trifluoromethoxy)benzoyl]amino]hexahydropyridinium Inner Salt (**20**).

In a similar manner, the reaction of **4b** and 4-(trifluoromethoxy)benzoyl chloride **5k** resulted in **20** in 29% yield as a colourless solid, mp 95-97°; ^{1}H nmr (deuteriochloroform): δ 1.5-2.1 (m, 6H), 2.7-2.9 (m, 1H), 2.9-3.1 (m, 1H), 4.01 (d, J = 13.6 Hz, 1H), 4.30 (d, J = 14.2 Hz, 1H), 4.35 (d, J = 13.6 Hz, 1H), 4.4-4.7 (m, 1H), 4.70 (d, J = 14.2 Hz, 1H), 5.0-5.2 (m, 1H), 6.7-7.0 (m, 2H), 7.1-7.2 (m, 2H), 7.5-7.7 (m, 1H), 7.7-7.9 (m, 3H), 8.20 (s, 1H), 8.94 (br, 1H); ms: (FAB) M^+ = 525.9.

Anal. Calcd. for $C_{24}H_{24}F_5N_5O_3$: C, 54.85; H, 4.60; N, 13.33. Found: C, 54.51; H, 4.49; N, 12.99.

1-[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-1-ethyl-1-methyl-2-[4-(trifluoromethyl)benzoyl]-hydrazinium Inner Salt (**21a,b**).

In a similar manner, the reaction of **4c** with 4-(trifluoromethyl)benzoyl chloride **5i** resulted in two pairs of diastereomers designated **21a** and **21b**. No attempt was made to further resolve these mixtures and they were tested as such. Mixture

21a was obtained in 21% yield as a colourless solid, mp 166-167°; ^{1}H nmr (deuteriochloroform): δ 1.20 (t, J = 7.3 Hz, 3H), 3.20 (s, 3H), 3.4-3.6 (m, 1H), 3.6-3.9 (m, 2H), 4.22 (d, J = 14.3 Hz, 1H), 4.4-4.6 (m, 1H), 4.70 (d, J = 14.3 Hz, 1H), 6.8-7.0 (m, 2H), 7.4-7.6 (m, 1H), 7.6-7.7 (m, 1H), 7.7-7.9 (m, 2H), 7.95 (s, 1H), 8.06 (s, 1H), 8.34 (s, 1H), 9.85 (br, 1H); ms: (FAB) M^+ = 484.1.

Anal. Calcd. for $C_{22}H_{22}F_5N_5O_2$: C, 54.65; H, 4.59; N, 14.49. Found: C, 54.43; H, 4.42; N, 14.21.

Mixture **21b** was obtained in 20% yield as a colourless solid, mp 63-65°; ^{1}H nmr (deuteriochloroform): δ 1.10 (t, J = 7.3 Hz, 3H), 3.0-3.3 (m, 1H), 3.50 (s, 3H), 3.65 (d, J = 13.6 Hz, 1H), 4.19 (d, J = 13.7 Hz, 1H), 4.34 (d, J = 14.2 Hz, 1H), 4.4-4.6 (m, 1H), 4.78 (d, J = 14.2 Hz, 1H), 6.8-7.0 (m, 2H), 7.4-7.7 (m, 2H), 7.7-7.9 (m, 2H), 7.93 (s, 1H), 8.05 (s, 1H), 8.33 (s, 1H), 9.55 (br, 1H); ms: (FAB) M^+ = 484.2.

Anal. Calcd. for $C_{22}H_{22}F_5N_5O_2$: C, 54.65; H, 4.59; N, 14.49. Found: C, 54.30; H, 4.64; N, 14.36.

1-[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-1-ethyl-1-methyl-2-[4-(trifluoromethoxy)benzoyl]-hydrazinium Inner Salt (**22a,b**).

In a similar manner, the reaction of **4c** with 4-(trifluoromethoxy)benzoyl chloride **5k** resulted in two pairs of diastereomers designated **22a** and **22b**. No attempt was made to further resolve these mixtures and they were tested as such. Mixture **22a** was obtained in 12% yield as a colourless solid, mp 163-164°; ^{1}H nmr (deuteriochloroform): δ 1.19 (t, J = 7.2 Hz, 3H), 3.18 (s, 3H), 3.4-3.6 (m, 1H), 3.6-3.8 (m, 2H), 4.21 (d, J = 14.3 Hz, 1H), 4.4-4.6 (m, 1H), 4.70 (d, J = 14.2 Hz, 1H), 6.8-7.0 (m, 2H), 7.1-7.3 (m, 2H), 7.7-7.9 (m, 3H), 7.95 (s, 1H), 8.34 (s, 1H), 9.90 (br, 1H); ms: (FAB) M^+ = 500.1.

Anal. Calcd. for $C_{22}H_{22}F_5N_5O_3$: C, 52.90; H, 4.44; N, 14.02. Found: C, 52.58; H, 4.29; N, 13.92.

Mixture **22b** was obtained in 11% yield as a colourless solid, mp 75-77°; ^{1}H nmr (deuteriochloroform): δ 1.05 (t, J = 7.3 Hz, 3H), 2.9-3.1 (m, 1H), 3.51 (s, 3H), 3.60 (d, J = 13.7 Hz, 1H), 4.13 (d, J = 13.7 Hz, 1H), 4.30 (d, J = 14.2 Hz, 1H), 4.4-4.6 (m, 1H), 4.78 (d, J = 14.2 Hz, 1H), 6.8-7.0 (m, 2H), 7.1-7.3 (m, 2H), 7.7-7.9 (m, 3H), 7.92 (s, 1H), 8.32 (s, 1H), 10.15 (br, 1H); ms: (FAB) M^+ = 500.1.

Anal. Calcd. for $C_{22}H_{22}F_5N_5O_3$: C, 52.90; H, 4.44; N, 14.02. Found: C, 52.71; H, 4.21; N, 14.20.

2-(4-Aminobenzoyl)-1-[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-1,1-dimethylhydrazinium Inner Salt (**13**).

Compound **12** (0.090 g, 0.20 mmole) and 0.015 g of 10% palladium on activated carbon were added to 15 ml of ethanol and hydrogenated in a Parr apparatus at 45 psi for 3 hours. The mixture was filtered and the filtrate evaporated to yield a crude product. Elution through a silica gel column using methanol/chloroform (1:10) as eluent gave 0.037 g (45%) of **13** as a colourless solid, mp 93-98°; ^{1}H nmr (deuteriochloroform): δ 3.21 (s, 3H), 3.5-3.7 (m, 4H), 3.85 (br, 2H), 3.97 (d, J = 13.3 Hz, 1H), 4.24 (d, J = 14.2 Hz, 1H), 4.70 (d, J = 14.2 Hz, 1H), 6.6-6.7 (m, 2H), 6.8-7.0 (m, 2H), 7.5-7.7 (m, 2H), 7.7-7.9 (m, 1H), 7.93 (s, 1H), 8.36 (s, 1H), 9.80 (br, 1H); ms: (FAB) M^+ = 417.1.

Anal. Calcd. for $C_{20}H_{22}F_2N_6O_2$: C, 57.68; H, 5.33; N, 20.18. Found: C, 57.41; H, 5.65; N, 20.04.

Acknowledgements.

The authors are grateful to Mrs. Anita Gandhi and Dr. Sameeh Salama for performing *in vitro* antifungal assessments, Mrs. Joyce Ng for conducting hplc and elemental analyses, Ms. Gerdy Aarts for performing nmr experiments, and Dr. Bob McDonald of the University of Alberta for conducting X-ray crystallographic studies.

REFERENCES AND NOTES

[1] Author to whom correspondence should be addressed.

[2] M. Daneshtalab, unpublished results.

[3] E. Peisach, D. Casebier, S. L. Gallion, P. Furth, G. A. Petsko, J. C. Hogan Jr. and D. Ringe, *Science*, **269**, 66 (1995).

[4] E. E. Rutenber, F. McPhee, A. P. Kaplan, S. L. Gallion, J. C. Hogan Jr., C. S. Craik and R. M. Stroud, *Bioorg. Med. Chem.*, **4**, 1545 (1996).

[5] W. J. McKillip, E. A. Sedor, B. M. Culbertson and S. Wawzonek, *Chem. Rev.*, **73**, 255 (1973).

[6] S. Wawzonek, *Ind. Eng. Chem. Prod. Res. Dev.*, **19**, 338 (1980).

[7] R. C. Slagel, *J. Org. Chem.*, **33**, 1374 (1968).

[8] K. Richardson, U.K. Patent 2 099 818 A (1982); *Chem. Abstr.*, **99**, 38467q (1983).

[9] Reference method for broth dilution antifungal susceptibility testing for yeasts. Proposed standard M27-P. National Committee for Clinical Laboratory Standards, 1992.