176. Studies on Antifungal Agents

Part 25

1-[(3,5-Bisaryl-2-methylisoxazolidin-3-yl)methyl]-1*H*-1,2,4-triazoles

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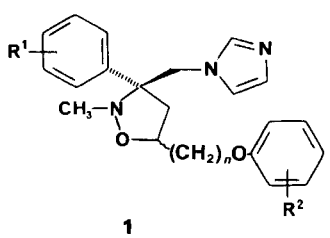
The synthesis and antifungal activity of a novel series of 1-[(3,5-bisaryl-2-methylisoxazolidin-3-yl)methyl]-1*H*-1,2,4-triazoles **6** and **7** (*i.e.* **8**–**19**) are discussed. The preparation of **8**–**19** was straightforward and highlighted by a regiospecific 1,3-dipolar cycloaddition of α-substituted (*E*)-ketonitrones **4** with appropriate styrene derivatives **5** that led to a *cis*/*trans*-diastereoisomeric mixture of the corresponding triazoles *(Scheme)*. The title compounds were evaluated for *in vitro* antifungal activity in solid agar cultures against a broad array of yeast and systemic mycoses and dermatophytes. The *in vivo* activity was determined in an immune-compromised mouse model of systemic candidiasis. While the *in vitro* activity was evident throughout the series, it was moderate in potency. However, some of the triazole derivatives demonstrated a potent *in vivo* activity comparable to that of the standard drug ketoconazole. Analogue **12** (PR 988-399) emerged as the best overall compound demonstrating potent antifungal activity in both *in vitro* and *in vivo* assays.

Recently [1], we have reported the synthesis and biological activity of a novel class of antifungal 3-aryl-5-[(aryloxy)alkyl]-3-[(1*H*-imidazol-1-yl)methyl]-2-methylisoxazolidines **1**. The preparation of compounds **1** involved a 1,3-dipolar cycloaddition of α-substituted (*E*)-ketonitrones with 1-alkenyl phenyl ethers. Previous reports [2–4] by a number of research laboratories indicated that substituting the 1*H*-imidazole ring with a 1*H*-1,2,4-triazole ring provided compounds having a more potent *in vivo* antifungal activity. This, coupled with a better pharmacokinetic profile made the 1*H*-1,2,4-triazole-containing azoles attractive and promising targets because of their lesser toxicity and better activity as systemic antifungal agents [5].



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The present study, which represents a further step in our search for more effective antifungal agents, describes the synthesis and both *in vitro* and *in vivo* antifungal activity of a novel series of *N*-methylisoxazolidines, the 1-[(3,5-bisaryl-2-methylisoxazolidin-3-yl)methyl]-1*H*-1,2,4-triazoles **6** and **7**.

Chemistry. – The synthesis of compounds **6** and **7** was accomplished in a straightforward manner as illustrated in the *Scheme*. Thus, condensation of 2-(1*H*-1,2,4-triazol-1-yl)acetophenones **2** with *N*-methylhydroxylamine hydrochloride (**3**) furnished the corresponding α-substituted (*E*)-ketonitrones **4**. The latter were subjected to a 1,3-dipolar cycloaddition with appropriate styrene derivatives **5**. The reaction which proceeded in a regiospecific manner produced a *cis*/*trans* diastereoisomeric mixture **6**/**7** in which the *cis*-isomer **6** was the major component. The two diastereoisomers were separated by flash chromatography on neutral silica gel yielding the *cis*-isomers **8**–**17** and *trans*-isomers **18** and **19** (see *Table 1*). The configuration of the two asymmetric centers in the isoxazolidine

Scheme

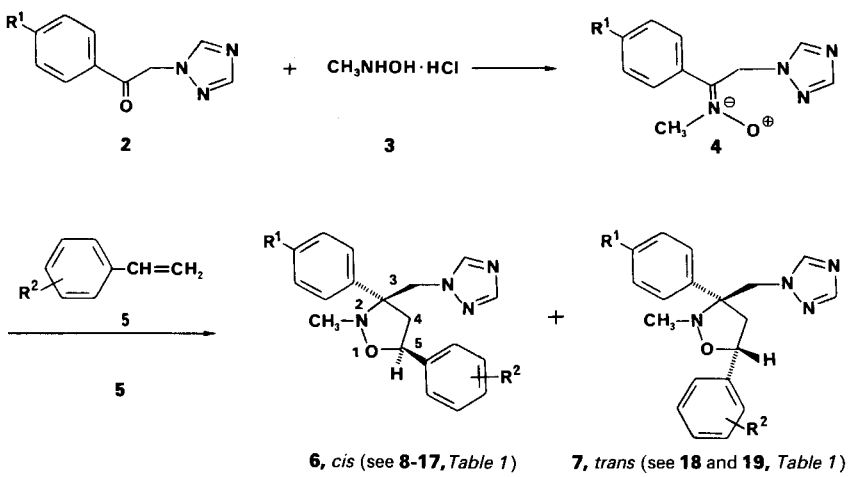

Table 1. *1-[(3,5-Bisaryl-2-methylisoxazolidin-3-yl)methyl]-1*H*-1,2,4-triazoles*

Compound	R^1	R^2	M.p. [°]	Recrystallization solvent	Yield [%]	Formula	Analyses
8	H	H	96–102	AcOEt	48.0	$C_{19}H_{20}N_4O$	C, H, N
9	H	4-F	105–109	AcOEt	37.0	$C_{19}H_{19}FN_4O$	C, H, F, N
10	H	4-CH_3	110–114	AcOEt	25.0	$C_{20}H_{22}N_4O$	C H, N
11	Cl	H	128–131	AcOEt/hexane 1:1	15.2	$C_{19}H_{19}ClN_4O$	C, H, Cl, N
12	Cl	4-Cl	117–120	AcOEt/hexane 1:1	34.0	$C_{19}H_{18}Cl_2N_4O$	C, H, Cl, N
13	Cl	4-F	138–140	AcOEt/hexane 1:1	16.2	$C_{19}H_{18}ClFN_4O$	C, H, Cl, F, N
14	F	H	122–125	AcOEt	8.2	$C_{19}H_{19}FN_4O$	C, H, F, N
15	F	4-F	98–103	AcOEt/hexane 1:1	14.8	$C_{19}H_{18}F_2N_4O$	C, H, F, N
16	CH_3O	3-NO_2	147–150	AcOEt	42.0	$C_{20}H_{21}N_5O_4$	C, H, N
17	CH_3O	3,4-$(CH_3O)_2$	177–179	AcOEt	25.6	$C_{22}H_{26}N_4O_4$	C, H, N
18	Cl	H	119–122	AcOEt/hexane 1:1	2.1	$C_{19}H_{19}ClN_4O$	C, H, Cl, N
19	Cl	4-Cl	144–147	AcOEt	9.6	$C_{19}H_{18}Cl_2N_4O$	C, H, Cl, N

rings of **6** and **7** was established by ^{1}H-NMR spectroscopy (the *J* values for each compound are listed in the *Exper. Part*). Previously, the configuration of the two asymmetric centers of a structurally similar *cis*-isoxazolidine derivative was determined by X-ray crystallography [1].

Results and Discussion. – The antifungal activity of the 1-[(3,5-bisaryl-2-methyl-isoxazolidin-3-yl)methyl]-1*H*-1,2,4-triazoles **8–19** was determined in both *in vitro* and *in vivo* assays. The *in vitro* testing was performed in solid agar 24-well tissue culture plates. The minimum inhibitory concentration (*MIC*) values (μg/ml) were interpreted as the lowest dilution at which no visible growth occurred. In all assays, ketoconazole was used as the positive standard drug. All results obtained during the current study are summarized in *Table 2*. In all tested compounds, the substituent at the 3′-aryl ring was kept at the 4-position, whereas the substitution at the 5′-aryl ring was varied. When compared to the *in vitro* antifungal activity of the corresponding 1*H*-imidazol analogues (reported earlier [6]), their 1*H*-1,2,4-triazole counterparts **8–19** showed less effective activity. This observation is not suprising and corroborates previous findings [2–4] concerning the *in vitro* potency of these two series of antifungal azoles. Lower *in vitro* activity was especially noticable among those of compounds **8–19** that had the 3′-aryl ring unsubstituted (**8–10**) or substituted with a 4-CH_3O group (**16**, **17**). In general, the *in vitro* potency of **8–19** was weak against *Aspergillus fumigatus* and *Microsporum* sp. and moderate to weak against *Candida* sp. By comparison, the activity of **8–19** against *Epidermophyton floccosum* and

Table 2. In vitro *Antifungal Activity of 1-[(3,5-Bisaryl-2-methylisoxazolidin-3-yl)methyl]-1*H*-1,2,4-triazoles*. Activity expressed as the minimum inhibitory concentration (*MIC*) in μg/ml.

Compd.	*T.m.* [a]	*T.r.* [b]	*T.t.* [c]	*T.s.* [d]	*E.f.* [e]	*M.a.* [f]	*M.c.* [g]	*A.f.* [h]	*C.a.* [i]	*C.s.* [j]
8	> 70.0	70.0	> 70.0	70.0	70.0	> 70.0	> 70.0	> 70.0	> 70.0	20.0
9	70.0	20.0	70.0	20.0	70.0	> 70.0	70.0	> 70.0	70.0	70.0
10	70.0	70.0	70.0	7.0	20.0	> 70.0	70.0	> 70.0	70.0	20.0
11	> 70.0	20.0	> 70.0	> 70.0	7.0	> 70.0	> 70.0	> 70.0	> 70.0	7.0
12	20.0	2.0	20.0	2.0	2.0	70.0	20.0	> 70.0	20.0	20.0
13	> 70.0	> 70.0	> 70.0	70.0	2.0	> 70.0	> 70.0	> 70.0	> 70.0	20.0
14	70.0	20.0	70.0	7.0	7.0	> 70.0	70.0	> 70.0	> 70.0	7.0
15	20.0	20.0	> 20.0	20.0	2.0	> 70.0	> 70.0	> 70.0	> 70.0	> 70.0
16	> 70.0	70.0	> 70.0	70.0	20.0	> 70.0	> 70.0	> 70.0	70.0	20.0
17	> 70.0	> 70.0	> 70.0	> 70.0	> 70.0	> 70.0	> 70.0	> 70.0	> 70.0	> 70.0
18	> 70.0	> 70.0	> 70.0	> 70.0	20.0	70.0	> 70.0	> 70.0	> 70.0	70.0
19	20.0	7.0	20.0	7.0	< 0.2	20.0	20.0	> 20.0	20.0	20.0
Ketoconazole	2.0	0.7	< 0.2	0.7	< 0.2	7.0	2.0	7.0	20.0	20.0

[a] *T.m.* = *Trichophyton mentagrophytes* ATCC 9533.
[b] *T.r.* = *Trichophyton rubrum* ATCC 18762.
[c] *T.t.* = *Trichophyton tonsurans* ATCC 9085.
[d] *T.s.* = *Trichophyton schoenleinii* ATCC 22775.
[e] *E.f.* = *Epidermophyton floccosum* ATCC E-18397.
[f] *M.a.* = *Microsporum audouinii* ATCC 9079.
[g] *M.c.* = *Microsporum canis* ATCC 44459.
[h] *A.f.* = *Aspergillus fumigatus* ATCC 28212.
[i] *C.a.* = *Candida albicans* ATCC 10259.
[j] *C.s.* = *Candida stellatoidea* ATCC 36232.

Trichophyton sp. was significantly higher (*Table 2*). The best *in vitro* activity was demonstrated with derivatives having a Cl substituent at the 3′-aryl ring (**11–13**). Overall, analogue **12** was found to be the most potent *in vitro* compound from this series. When compared to their *trans*-counterparts **18** and **19**, the *cis*-isomers **11** and **12** showed, in general, higher *in vitro* potency (especially evident with the activity against *Candida stellatoidea* of the **11**/**18** pair). The *in vitro* activity of ketoconazole was superior to that of all compounds tested. However, it is worth noting that both analogues **11** and **14** (having a halogen substituent at the 3′-aryl ring and an unsubstituted 5′-phenyl ring) compared favorably to ketoconazole against *C. stellatoidea;* the majority of the remaining compounds (**8**, **10**, **12**, **13**, **16**, **19**) showed equipotent activity against *C. stellatoidea.*

Table 3. In vivo *Antifungal Activity of 1-[(3,5-Bisaryl-2-methylisoxazolidin-3-yl)methyl]-1*H*-1,2,4-triazoles.* Activity expressed by the number of surviving animals (live-animal count) at day 5 following the challenge.

Compound	Oral dose [mg/kg][a])	Live-animal count					
		day 0	day 1	day 2	day 3	day 4	day 5
Controls[b])	–	8	6	0			
8	10	8	2	0			
	25	8	5	0			
	50	8	8	2	0		
10	10	8	6	2	0		
	25	8	6	0			
	50	8	8	0			
11	10	8	3	0			
	25	8	2	0			
	50	8	3	0			
12	5[c])	5	5	3	1	1	0
	10	8	8	8	4	4	2
	25	8	8	8	8	8	7
	50	8	8	7	7	7	7
13	10	8	8	7	1	1	0
	25	8	8	8	8	6	2
	50	8	8	8	6	6	5
15	10	8	8	8	2	1	1
	25	8	8	8	7	3	0
	50	8	8	8	8	8	8
16	10	8	6	0			
	25	8	8	2	0		
	50	8	6	0			
17	10	8	8	0			
	25	8	7	2	0		
	50	8	8	0			
19	10	8	2	0			
	25	8	4	0			
	50	8	3	0			
Ketoconazole	10	8	8	6	1	1	0
	25	8	8	8	7	7	6
	50	8	8	8	8	8	8

[a]) Groups of 8 mice were used in each assay.
[b]) Groups of 8 immune-normal control animals were challenged with $2.0 \cdot 10^7$ CFU's of *C. albicans* strain B-311.
[c]) Groups of 5 mice were tested at this dose.

The *in vivo* antifungal activity of the triazoles **8**–**19** was evaluated in an immune-compromised mouse model of systemic candidiasis [7–15], using orally dosed ketoconazole as the positive standard drug. Immune-normal mice were used as controls. The animals were dosed orally with the test compounds (at 10, 25, and 50 mg/kg) 1, 4, and 24 h after the challenge with *C. albicans* B-311. The activity is expressed by the number of surviving animals (live-animal count) at day 5 of the experiment. As seen from *Table 3*, the halogen-substituted aryl derivatives **12**, **13**, and **15** were the most potent *in vivo* analogues displaying activity equal to that of ketoconazole. Of the *cis*/*trans*-diastereoisomeric pair **12**/**19**, the *cis*-isomer **12** was superior, as with the case of the *in vitro* assay. Another interesting observation to note is that although, in general, *in vitro* activity of azole antifungals does not correlate well with their *in vivo* potency [2–4], the bis(4-chlorophenyl) compound **12** which was found to be the most potent *in vitro* agent from this series *(Table 2)* was also found to be adequately efficient in the *in vivo* screening. The two F-containing analogues **13** and **15** also demonstrated potent *in vivo* antifungal activity, especially the bis(4-fluorophenyl) compound **15**. The latter finding may indicate that the presence of a *para*-halogen substitution at both aryl rings in the *cis*-series **8**–**17** is a necessary feature for imparting of *in vivo* antifungal activity. Replacing the halogen atom on either or both aromatic rings with other substituents (**8**, **10**, **16**, **17**) led to compounds with a significantly diminished *in vivo* potency.

Thus, the 1-[(3,5-bisaryl-2-methylisoxazolidin-3-yl)methyl]-1*H*-1,2,4-triazoles **8**–**19** represent a novel class of potent antifungal agents. While their *in vitro* antifungal activity was less evident (as compared to that of the corresponding 1*H*-imidazole analogues), some 1*H*-triazoles **8**–**19** displayed a potent *in vivo* activity in an immune-compromised mouse model of systemic candidiasis. The bis(4-chlorophenyl) analogue **12** (PR 988-399) emerged as the best overall compound having demonstrated potent antifungal activity in both *in vitro* and *in vivo* screening.

Experimental Part

1. *General.* See [1]. FC = flash chromatography.

2. *1-Aryl-*N*-methyl-2-(1*H*-1,2,4-triazol-1-yl)ethanimine* N*-Oxides* **4** were prepared by previously known procedures [1] from the corresponding 1-aryl-2-(1*H*-1,2,4-triazol-1-yl)ethanones **2** and *N*-methylhydroxylamine hydrochloride (**3**).

3. *1-[(*cis*-2-Methyl-3,5-diphenylisoxazolidin-3-yl)methyl]-1*H*-1,2,4-triazole* (**8**). A soln. of 14.9 g (68.9 mmol) of *N*-methyl-1-phenyl-2-(1*H*-1,2,4-triazol-1-yl)ethanimine *N*-oxide (**4**; R^1 = H) and 11.8 ml (103 mmol) of styrene (**5**; R^2 = H) in 300 ml of toluene was heated to reflux under N_2 for 48 h. Upon cooling to r.t., the solvent was evaporated. Crystallization from AcOEt gave 10.6 g (48%) of **8** in a mixture with the corresponding *trans*-isomer. Recrystallization from AcOEt provided a pure sample of **8**. M.p. 96–102°. IR (KBr): 3120*m*, 3076*m*, 3038*m*, 3004*m*, 2972*m*, 2920*m*, 2904*m*, 1506*s*, 1450*s*, 1355*m*, 1279*s*, 1216*m*, 1137*s*, 1022*m*, 886*m*, 758*m*, 743*s*, 703*s*, 682*m*. ^{1}H-NMR (200 MHz, $CDCl_3$): 2.66 (*s*, CH_3N); 2.96 (*dd*, *J* = 5.5, 13.2, 1 H–C(4)); 3.16 (*dd*, *J* = 9.9, 13.2, 1 H–C(4)); 4.55 (*s*, CH_2N); 5.61 (*dd*, *J* = 5.5, 9.9, H–C(5)); 7.09–7.60 (*m*, 11 H); 7.71 (*s*, 1 H). Anal. calc. for $C_{19}H_{20}N_4O$: C 71.33, H 6.29, N 17.49; found: C 71.17, H 6.48, N 17.41.

The following 1-[(3,5-bisaryl-2-methylisoxazolidin-3-yl)methyl]-1*H*-1,2,4-triazoles were prepared by procedures similar to that described for **8** (yields and m.p.'s in *Table 1*).

*1-{[*cis*-5-(4-Fluorophenyl)-2-methyl-3-phenylisoxazolidin-3-yl]methyl}-1*H*-1,2,4-triazole* (**9**). From **4** (R^1 = H) and 4-fluorostyrene (**5**; R^2 = 4-F). Purification: FC on neutral silica gel ($CHCl_3$/MeOH 98:2). IR (KBr): 1607*w*, 1514*s*, 1507*s*, 1453*s*, 1275*m*, 1226*m*, 1207*m*, 1163*m*, 1140*m*, 1025*w*, 852*m*, 836*s*, 708*m*. ^{1}H-NMR (200 MHz, $CDCl_3$): 2.66 (*s*, CH_3N); 2.99 (*dd*, *J* = 5.5, 12.7, 1 H–C(4)); 3.13 (*dd*, *J* = 9.9, 12.7, 1 H–C(4)); 3.99 (*d*, *J* = 14.3,

1 H, CH_2N); 4.05 (*d*, *J* = 14.3, 1 H, CH_2N); 5.58 (*dd*, *J* = 5.5, 9.9, H–C(5)); 7.08–7.15 (*m*, 5 H); 7.26–7.36 (*m*, 3 H); 7.55–7.62 (*m*, 2 H). Anal. calc. for $C_{19}H_{19}FN_4O$: C 67.44, H 5.66, F 5.61, N 16.56; found: C 67.46, H 5.48, F 5.57, N 16.64.

*1-{[*cis*-2-Methyl-5-(4-methylphenyl)-3-phenylisoxazolidin-3-yl]methyl}-1*H*-1,2,4-triazole* (**10**). From **4** (R^1 = H) and 4-methylstyrene (**5**; R^2 = 4-CH_3). Purification: FC on neutral silica gel ($CHCl_3$/MeOH 98:2). IR (KBr): 1506*s*, 1481*m*, 1447*m*, 1273*s*, 1137*m*, 1014*m*, 919*m*, 809*m*, 761*m*, 705*s*. ^{1}H-NMR (200 MHz, $CDCl_3$): 2.37 (*s*, CH_3Ar); 2.66 (*s*, CH_3N); 2.96 (*dd*, *J* = 5.5, 13.2, 1 H–C(4)); 3.13 (*dd*, *J* = 9.9, 13.2, 1 H–C(4)); 4.56 (*s*, CH_2N); 5.59 (*dd*, *J* = 5.5, 9.9, H–C(5)); 7.05–7.48 (*m*, 10 H); 7.69 (*s*, 1 H). Anal. calc. for $C_{20}H_{22}N_4O$: C 71.83, H 6.63, N 16.75; found: C 71.64, H 6.66, N 16.67.

*1-{[*cis*-3-(4-Chlorophenyl)-2-methyl-5-phenylisoxazolidin-3-yl]methyl}-1*H*-1,2,4-triazole* (**11**). From 1-(4-chlorophenyl)-*N*-methyl-2-(1*H*-1,2,4-triazol-1-yl)ethanimine *N*-oxide (**4**; R^1 = Cl) and **5** (R^2 = H). Purification: FC on neutral silica gel ($CHCl_3$/MeOH 99:1). IR (KBr): 3121*m*, 1507*s*, 1492*s*, 1454*m*, 1275*m*, 1215*m*, 1140*s*, 1094*m*, 1015*s*, 859*m*, 763*m*, 746*m*, 706*m*, 679*m*. ^{1}H-NMR (200 MHz, $CDCl_3$): 2.65 (*s*, CH_3N); 2.90 (*dd*, *J* = 5.5, 13.2, 1 H–C(4)); 3.13 (*dd*, *J* = 9.9, 13.2, 1 H–C(4)); 4.49 (*d*, *J* = 14.3, 1 H, CH_2N); 4.52 (*d*, *J* = 14.3, 1 H, CH_2N); 5.57 (*dd*, *J* = 5.5, 9.9, H–C(5)); 7.03 (*d*, *J* = 8.3, 2 H); 7.25–7.56 (*m*, 8 H); 7.72 (*s*, 1 H). Anal. calc. for $C_{19}H_{19}ClN_4O$: C 64.31, H 5.40, Cl 9.99, N 15.79; found: C 64.28, H 5.42, Cl 10.11, N 15.78.

*1-{[*cis*-3,5-Bis(4-chlorophenyl)-2-methylisoxazolidin-3-yl]methyl}-1*H*-1,2,4-triazole* (**12**). From **4** (R^1 = Cl) and 4-chlorostyrene (**5**; R^2 = 4-Cl). Purification: FC on neutral silica gel ($CHCl_3$/MeOH 98:2). IR (KBr): 1510*m*, 1493*s*, 1466*m*, 1404*m*, 1274*s*, 1210*m*, 1148*m*, 1092*m*, 1013*s*, 918*m*, 836*s*, 820*m*, 739*m*, 680*m*. ^{1}H-NMR (200 MHz, $CDCl_3$): 2.64 (*s*, CH_3N); 2.95 (*dd*, *J* = 5.5, 13.8, 1 H–C(4)); 3.11 (*dd*, *J* = 9.9, 13.8, 1 H–C(4)); 4.49 (*s*, CH_2N); 5.54 (*dd*, *J* = 5.5, 9.9, H–C(5)); 7.03 (*d*, *J* = 8.3, 2 H); 7.26–7.52 (*m*, 7 H); 7.75 (*s*, 1 H). Anal. calc. for $C_{19}H_{18}Cl_2N_4O$: C 58.62, H 4.66, Cl 18.21, N 14.39; found: C 58.65, H 4.82, Cl 17.85, N 14.21.

*1-{[*cis*-3-(4-Chlorophenyl)-5-(4-fluorophenyl)-2-methylisoxazolidin-3-yl]methyl}-1*H*-1,2,4-triazole* (**13**). From **4** (R^1 = Cl) and **5** (R^2 = 4-F). Purification: FC on neutral silica gel ($CHCl_3$/MeOH 98:2). IR (KBr): 1602*m*, 1512*s*, 1507*s*, 1491*m*, 1264*m*, 1221*s*, 1157*m*, 1138*m*, 1096*m*, 1032*m*, 1014*m*, 860*m*, 832*s*. ^{1}H-NMR (200 MHz, $CDCl_3$): 2.65 (*s*, CH_3N); 2.96 (*dd*, *J* = 5.5, 13.2, 1 H–C(4)); 3.13 (*dd*, *J* = 9.9, 13.2, 1 H–C(4)); 4.51 (*d*, *J* = 14.3, 1 H, CH_2N); 4.53 (*d*, *J* = 14.3, 1 H, CH_2N); 5.54 (*dd*, *J* = 5.5, 9.9, H–C(5)); 7.02–7.16 (*m*, 4 H); 7.26–7.33 (*m*, 3 H); 7.51–7.58 (*m*, 2 H); 7.76 (*s*, 1 H). Anal. calc. for $C_{19}H_{18}ClFN_4O$: C 61.21, H 4.87, Cl 9.51, F 5.10, N 15.03; found: C 61.22, H 45.78, Cl 9.63, F 5.00, N 14.95.

*1-{[*cis*-3-(4-Fluorophenyl)-2-methyl-5-phenylisoxazolidin-3-yl]methyl}-1*H*-1,2,4-triazole* (**14**). From 1-(4-fluorophenyl)-*N*-methyl-2-(1*H*-1,2,4-triazol-1-yl)ethanimine *N*-oxide (**4**; R^1 = F) and **5** (R^2 = H). Purification: FC on neutral silica gel ($CHCl_3$/MeOH 98:2). IR (KBr): 1601*m*, 1509*s*, 1484*m*, 1450*m*, 1438*m*, 1275*s*, 1222*m*, 1142*m*, 1011*m*, 840*m*, 746*m*, 697*m*, 662*m*. ^{1}H-NMR (200 MHz, $CDCl_3$): 2.65 (*s*, CH_3N); 2.93 (*dd*, *J* = 5.5, 13.2, 1 H–C(4)); 3.15 (*dd*, *J* = 9.9, 13.2, 1 H–C(4)); 4.51 (*s*, CH_2N); 5.60 (*dd*, *J* = 5.5, 9.9, H–C(5)); 6.96–7.12 (*m*, 4 H); 7.34–7.57 (*m*, 6 H); 7.72 (*s*, 1 H). Anal. calc. for $C_{19}H_{19}FN_4O$: C 67.44, H 5.66, F 5.61, N 16.56; found: C 67.07, H 5.59, F 5.55, N 16.66.

*1-{[*cis*-3,5-Bis(4-fluorophenyl)-2-methylisoxazolidin-3-yl]methyl}-1*H*-1,2,4-triazole* (**15**). From **4** (R^1 = F) and **5** (R^2 = 4-F). Purification: FC on neutral silica gel ($CHCl_3$/MeOH 98:2). IR (KBr): 3105*w*, 2882*w*, 1604*m*, 1509*s*, 1437*m*, 1275*m*, 1269*m*, 1229*s*, 1219*m*, 1160*m*, 1142*m*, 1014*m*, 838*s*. ^{1}H-NMR (200 MHz, $CDCl_3$): 2.65 (*s*, CH_3N); 2.98 (*dd*, *J* = 5.5, 13.2, 1 H–C(4)); 3.11 (*dd*, *J* = 9.9, 13.2, 1 H–C(4)); 4.49 (*d*, *J* = 14.3, 1 H, CH_2N); 4.55 (*d*, *J* = 14.3, 1 H, CH_2N); 5.56 (*dd*, *J* = 5.5, 9.9, H–C(5)); 6.97–7.17 (*m*, 6 H); 7.29 (*s*, 1 H); 7.52–7.59 (*m*, 2 H); 7.75 (*s*, 1 H). Anal. calc. for $C_{19}H_{18}F_2N_4O$: C 64.04, H 5.09, F 10.66, N 15.72; found: C 63.93, H 5.05, F 10.59, N 15.68.

*1-{[*cis*-3-(4-Methoxyphenyl)-2-methyl-5-(3-nitrophenyl)isoxazolidin-3-yl]methyl}-1*H*-1,2,4-triazole* (**16**). From 1-(4-methoxyphenyl)-*N*-methyl-2-(1*H*-1,2,4-triazol-1-yl)ethanimine *N*-oxide (**4**; R^1 = CH_3O) and 3-nitrostyrene (**5**; R^2 = 3-NO_2). Purification: fractional crystallization from Et_2O. IR (KBr): 1617*m*, 1530*s*, 1514*s*, 1446*m*, 1350*s*, 1271*m*, 1265*m*, 1243*m*, 1182*m*, 1138*m*, 1039*m*, 998*w*, 858*m*, 807*m*, 739*m*, 680*m*. ^{1}H-NMR (200 MHz, $CDCl_3$): 2.65 (*s*, CH_3N); 3.04 (*dd*, *J* = 5.5, 12.7, 1 H–C(4)); 3.18 (*dd*, *J* = 9.9, 12.7, 1 H–C(4)); 3.80 (*s*, CH_3O); 4.48 (*d*, *J* = 13.8, 1 H, CH_2N); 4.52 (*d*, *J* = 13.8, 1 H, CH_2N); 5.65 (*dd*, *J* = 5.5, 9.9, H–C(5)); 6.96 (*d*, *J* = 8.3, 2 H); 7.07 (*d*, *J* = 8.3, 2 H); 7.17 (*s*, 1 H); 7.57–7.69 (*m*, 1 H); 7.71 (*s*, 1 H); 7.94 (*d*, *J* = 8.3, 1 H); 8.21 (*d*, *J* = 8.3, 1 H); 8.43 (*s*, 1 H). Anal. calc. for $C_{20}H_{21}N_5O_4$: C 60.75, H 5.35, N 17.71; found: C 60.61, H 5.35, N 17.52.

*1-{[*cis*-5-(3,4-Dimethoxyphenyl)-3-(4-methoxyphenyl)-2-methylisoxazolidin-3-yl]methyl}-1*H*-1,2,4-triazole* (**17**). From **4** (R^1 = CH_3O) and 3,4-dimethoxystyrene (**5**; R^2 = 3,4-$(CH_3O)_2$). Purification: fractional crystallization from AcOEt. IR (KBr): 1609*m*, 1522*s*, 1512*s*, 1464*m*, 1450*m*, 1273*s*, 1234*s*, 1182*m*, 1166*m*, 1138*m*, 1025*s*, 850*m*, 823*m*. ^{1}H-NMR (200 MHz, $CDCl_3$): 2.65 (*s*, CH_3N); 3.03 (*d*, *J* = 7.7, 2 H–C(4)); 3.81 (*s*, CH_3O); 3.92 (*s*, CH_3O); 3.98 (*s*, CH_3O); 4.53 (*d*, *J* = 13.8, 1 H, CH_2N); 4.74 (*d*, *J* = 13.8, 1 H, CH_2N); 5.57 (*t*, *J* = 7.7, H–C(5));

6.84–6.95 (*m*, 3 H); 7.05–7.18 (*m*, 4 H); 7.33 (*s*, 1 H); 7.77 (*s*, 1 H). Anal. calc. for $C_{22}H_{26}N_4O_4$: C 64.38, H 6.38, N 13.65; found: C 64.01, H 6.51, N 13.60.

1-{[trans-3-(4-Chlorophenyl)-2-methyl-5-phenylisoxazolidin-3-yl]methyl}-1H-1,2,4-triazole (**18**). From **4** (R^1 = Cl) and **5** (R^2 = H). Purification: FC on neutral silica gel ($CHCl_3$/MeOH 99:1). IR (KBr): 1516*m*, 1492*s*, 1447*m*, 1272*s*, 1219*m*, 1156*m*, 1096*m*, 1017*m*, 917*m*, 848*m*, 758*s*, 705*m*, 678*m*. ^{1}H-NMR (200 MHz, $CDCl_3$): 2.53 (*s*, CH_3N); 2.67 (*dd*, *J* = 8.8, 12.7, 1 H–C(4)); 3.26 (*dd*, *J* = 7.7, 12.7, 1 H–C(4)); 4.54 (*d*, *J* = 14.3, 1 H, CH_2N); 4.76 (*d*, *J* = 14.3, 1 H, CH_2N); 5.52 (*dd*, *J* = 7.7, 8.8, H–C(5)); 7.09 (*d*, *J* = 8.8, 2 H); 7.25–7.37 (*m*, 8 H); 7.86 (*s*, 1 H). Anal. calc. for $C_{19}H_{19}ClN_4O$: C 64.31, H 5.40, Cl 9.99, N 15.79; found: C 64.24, H 5.49, Cl 10.08, N 15.78.

1-{[trans-3,5-Bis(4-chlorophenyl)-2-methylisoxazolidin-3-yl]methyl}-1H-1,2,4-triazole (**19**). From **4** (R^1 = Cl) and **5** (R^2 = 4-Cl). Purification: FC on neutral silica gel ($CHCl_3$/MeOH 98:2). IR (KBr): 1506*m*, 1485*s*, 1454*m*, 1439*m*, 1276*s*, 1136*m*, 1090*s*, 1013*m*, 841*s*, 830*s*, 745*m*, 730*m*, 679*m*, 656*m*. ^{1}H-NMR (200 MHz, $CDCl_3$): 2.52 (*s*, CH_3N); 2.63 (*dd*, *J* = 8.8, 12.7, 1 H–C(4)); 3.14 (*dd*, *J* = 7.7, 12.7, 1 H–C(4)); 4.54 (*d*, *J* = 14.3, 1 H, CH_2N); 4.75 (*d*, *J* = 14.3, 1 H, CH_2N); 5.48 (*dd*, *J* = 7.7, 8.8, H–C(5)); 7.09 (*d*, *J* = 8.8, 2 H); 7.25–7.35 (*m*, 7 H); 7.86 (*s*, 1 H). Anal. calc. for $C_{19}H_{18}Cl_2N_4O$: C 58.62, H 4.66, Cl 18.21, N 14.39; found: C 58.54, H 4.73, Cl 18.10, N 14.38.

4. In vitro *Assay for Antifungal Activity*. The antifungal activity was assayed *in vitro* in solid agar tests performed in 24-well tissue culture plates. The test medium was prepared by diluting the test compound 10-fold into 'antibiotic medium 3' +2% agar. The testing was accomplished by either using a 4-point (70, 20, 2, and 0.2 μg/ml) or a 6-point (70, 20, 7, 2, 0.7, and 0.2 μg/ml) dilution scheme, with ketoconazole as a control drug in all assays. All test organisms were grown on potato-flake agar at 26°. *Candida albicans* was grown overnight, *Aspergillus fumigatus* was grown for *ca.* 1 week and *Trichophyton rubrum* for *ca.* 2 weeks. The cells were either removed from the plates with a sterile cotton swab and suspended in sterile H_2O *(C. albicans, A. fumigatus)* or washed from the surface of the plate with sterile H_2O and diluted in sterile H_2O *(T. rubrum)*. The actual counts were performed using a hemacytometer, and the suspensions were diluted to 1×10^4 cells/ml. The test and control plates were inoculated with 0.05 ml of the fungal suspension and were incubated at 26° until visible growth in the compound-free control plates was evident. The minimum inhibitory concentration *(MIC)* values were interpreted as the lowest dilution at which no visible growth occurred.

5. *Immune-Compromised Mouse Model of Systemic Candidiasis*. Groups of 8 male ICR mice weighing between 20 and 25 g were immune-compromised by dosing 3 days prior to challenge with 200 mg/kg cyclophosphamide (intraperitoneal administration). Immune-normal mice were used as controls. *Candida albicans* strain B-311, at a dose of $2.0 \cdot 10^7$ or $2.0 \cdot 10^5$ CFU's in 0.5 ml of saline, was used to challenge the immune-normal or immune-compromised animals, respectively. The mice were dosed orally at 1, 4, and 24 h after the challenge with the test compounds and ketoconazole which was used as the positive standard drug, at doses of 10, 25, and 50 mg/kg. All tested compounds were suspended in 1% carboxymethylcellulose no more than 3 days prior to the first dosing and were refrigerated prior to and between the dosing. In the cyclophosphamide-suppressed animals, the WBC counts remained low for the duration of the experiment. The activity was expressed by the number of surviving animals (live-animal count) at day 5 following the challenge with *C. albicans*.

REFERENCES

[1] G. B. Mullen, P. A. Swift, D. M. Maryniak, S. D. Allen, J. T. Mitchell, C. R. Kinsolving, V. St. Georgiev, *Helv. Chim. Acta* **1988**, *71*, 718.

[2] G. B. Mullen, P. A. Swift, G. A. Bennett, T. R. DeCory, D. M. Maryniak, P. G. Dormer, V. St. Georgiev, in 'Antifungal Drugs', Ed. V. St. Georgiev, Annals of the New York Academy of Sciences, New York, 1988, in press.

[3] F. T. Boyle, J. F. Riley, R. G. Wilson, in 'Recent Trends in the Discovery, Development and Evaluation of Antifungal Agents', Ed. R. A. Fromtling, J. R. Prous Publishers, Barcelona, 1987, p. S1:31.

[4] D. Berg, M. Plempel, K.-H. Büchel, G. Holmwood, K. Stroech, in [2].

[5] K. Richardson, M. S. Marriott, *Annu. Rep. Med. Chem.* **1987**, *22*, 159.

[6] T. R. DeCory, G. B. Mullen, J. T. Mitchell, S. D. Allen, C. R. Kinsolving, V. St. Georgiev, in [2].

[7] S. D. Allen, W. E. Jones, J. T. Mitchell, IVth Annual Conference of the Inter-American Society for Chemotherapy, Clearwater Beach, Florida, Jan. 10–13, 1988.

[8] J. E. Cutler, *J. Reticuloend. Soc.* **1976**, *19*, 121.

[9] M.N. Guentzel, C. Herrera, *Infect. Immunity* **1982,** *35,* 222.
[10] A. Polak, F.D. Odds, E. Lüdin, H.J. Scholer, *Chemotherapy* **1985,** *31,* 395.
[11] S. Rabinovich, B.D. Shaw, T. Bryant, S.T. Donta, *J. Infect. Dis.* **1974,** *130,* 28.
[12] K. Richardson, K.W. Brammer, M.S. Marriott, P.F. Troke, *Antimicrob. Agents Chemother.* **1985,** *27,* 832.
[13] R.L. Stiller, J.E. Bennett, H.J. Scholer, M. Wall, A. Polak, D.A. Stevens, *J. Infect. Dis.* **1983,** *147,* 1070.
[14] P.F. Troke, ASM Conference on the Biology and Pathogenicity of *Candida albicans,* Palm Springs, Cal., May 14–16, 1987.
[15] M.N. Guentzel, ASM Conference on the Biology and Pathogenicity of *Candida albicans,* Palm Springs, Cal., May 14–16, 1987.