3-Aryl-5-[(aryloxy)methyl]-3-[(1*H*-1,2,4-triazol-1-yl)methyl]-2-methylisoxazolidine Derivatives. Synthesis and Antifungal Activity

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The synthesis and antifungal activity of a novel series of 3-aryl-5-[(aryloxy)methyl]-3-[(1*H*-1,2,4-triazol-1-yl)-methyl]-2-methylisoxazolidines are described. The *in vitro* activity was evaluated in solid agar cultures against a variety of dermatophytes and yeast fungi, while *in vivo* activity was measured in an immune-compromised mouse model of systemic candidiasis. The activity of the title series was compared to that of ketoconazole and one derivative, the *cis*-3-(4-chlorophenyl)-5-(4-chlorophenyloxy)methyl analogue **5f** was found to possess a similar potency in the *in vivo* assay. Structure-activity relationship correlations are also discussed.

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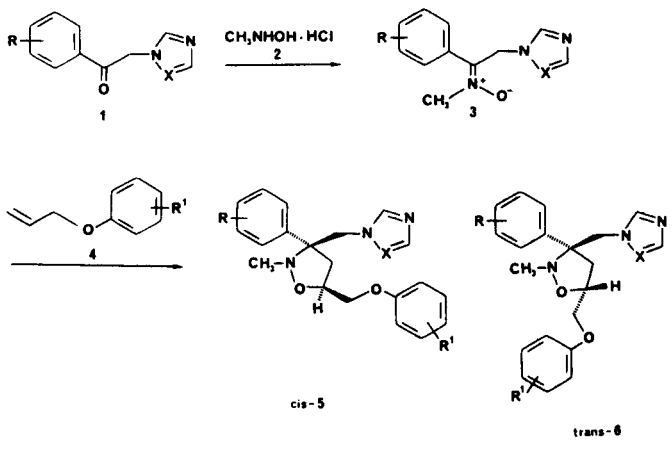
Recently [1], we reported the synthesis and *in vitro* antifungal activity of a novel class of antimycotic agents, the 3-aryl-5-[(aryloxy)methyl]-3-[(1*H*-imidazol-1-yl)methyl]-2-methylisoxazolidines **5** and **6** (X = CH). When tested in solid agar cultures, the latter exerted a potent activity against a number of dermatophytes and yeast fungi. The most active derivative of this series was found to be the *cis*-3-(4-chlorophenyl)-5-[(4-chlorophenyloxy)methyl] analogue PR 967-248 (**5**, R = R¹ = 4-Cl, X = CH) which, when compared ot ketoconazole, elicited a superior activity against *Candida* and *Microsporum* sp. coupled with a very potent activity against *Epidermophyton floccosum* and *Trychophyton* sp. and a moderate activity against *Aspergillus fumigatus*. Its minimum inhibitory concentration (MIC) values ranged between 0.2 and 2.0 μg/ml, as compared to 0.2-20.0 μg/ml for ketoconazole [1]. However, when tested *in vivo* in an immune-compromised mouse model of systemic candidiasis, these 3-[(1*H*-imidazol-1-yl)-methyl] derivatives did not show any meaningful activity.

Previously [2], it has been reported on several occasions that 3-[(1*H*-imidazol-1-yl)methyl]-containing antimycotics may undergo extensive protein binding and a facile first-pass metabolism in the liver thus resulting in a low drug concentration in the systemic circulation. In turn, that may translate into lower *in vivo* activity. However, substituting the 1*H*-imidazole with 1*H*-1,2,4-triazole ring leads, in general, to a much improved pharmacokinetics profile and *in vivo* potency, as amply demonstrated by data from clinical trials of fluconazole and itraconazole, two novel antimycotics containing 3-[(1*H*-1,2,4-triazol-1-yl)methyl] moiety [3].

Therefore, it was of interest to investigate the antifungal activity of the 1*H*-1,2,4-triazole analogues of **5** and **6** (X = CH), namely the 3-aryl-5-[(aryloxy)methyl]-3-[(1*H*-1,2,4-triazol-1-yl)methyl]-2-methylisoxazolidines **5** and **6** (X = N). In the present communication we discuss our results in this direction. The antifungal activity of derivatives **5** and **6** (X = N) was determined *in vitro* in solid agar cultures, while the *in vivo* activity was evaluated in an immune-compromised mouse model of systemic candidiasis. Some structure-activity relationship correlations were also determined.

The synthesis of the title 3-aryl-5-[(aryloxy)methyl]-3-[(1*H*-1,2,4-triazol-1-yl)methyl]-2-methylisoxazolidines is presented in Scheme I. Condensation of 2-(1*H*-1,2,4-triazol-1-yl)acetophenone (**1**, X = N) with *N*-methylhydroxylamine hydrochloride (**2**) to afford the corresponding α-substituted ketonitrones **3** (X = N), was carried out as described earlier [4]. Next, the 1,3-dipolar cycloaddition reaction of compounds **3** with an appropriate olefin dipolarophile **4** proceeded regioselectively to furnish the desired 3,5-substituted isoxazolidine derivatives as mixtures of the corresponding *cis* and *trans* diastereomers **5** and **6**, respectively. The *cis* isomers **5**, which were the major product of cycloaddition, were separated from their *trans*-counterparts by flash chromatography on neutral silica gel. All isoxazolidine compounds synthesized during

Scheme I



the present study are listed in Table I. The configuration of the two asymmetric centers of **5** and **6** (X = N) was established as previously described [1,5] by using 200-MHz nmr spectroscopy.

Table I

3-Aryl-5-[(aryloxy)methyl]-3-[(1*H*-azol-1-yl)methyl]-2-methylisoxazolidines **5** and **6**

Compound	R	R^1	X
cis-**5a**	H	H	N
cis-**5b**	H	4-Cl	N
cis-**5c**	H	4-F	N
cis-**5d**	H	4-$NHCOCH_3$	N
cis-**5e**	4-Cl	H	N
cis-**5f**	4-Cl	4-Cl	N
cis-**5g**	4-Cl	4-OCH_3	N
cis-**5h**	4-Cl	3,4-CH_3(Cl)	N
cis-**5i**	4-Cl	4-$NHCOCH_3$	N
cis-**5j**	4-Cl	4-CO_2CH_3	N
cis-**5k**	4-OCH_3	4-Cl	N
cis-**5l**	3-CH_3	2-NO_2	N
cis-**5m**	4-Cl	4-COOH	N
trans-**6a**	4-Cl	H	N
trans-**6b**	4-Cl	4-Cl	N
trans-**6c**	4-Cl	4-CO_2CH_3	N

The antifungal activity of compounds **5** and **6** (X = N) was assayed in both *in vitro* and *in vivo* experiments. The *in vitro* potency was evaluated in solid agar tests using 24-well tissue culture plates. The observed activity was expressed as the minimum inhibitory concentration (MIC) in μg/ml (Table II). When compared to the earlier reported [1] 3-(1*H*-imidazol-1-ylmethyl) analogues **5/6** (X = CH), the antifungal potency *in vitro* of 3-(1*H*-1,2,4-triazol-1-ylmethyl) derivatives **5/6** (X = N) was noticeably lower. Of all tested compounds from the present series, only the *cis*-3-(4-chlorophenyloxy)methyl analogue **5f** was consistently active against most of the fungi, especially *Trichophyton* sp. and *Epidermophyton floccosum*. This is in agreement with a similar observation made previously [1] for its 3-(1*H*-imidazol-1-ylmethyl) analogue **5** (R = R^1 = 4-Cl, X = CH). Potent activity against *Candida stellatoidea* was found for compounds **5f-5h**; all three contain a 3-(4-chlorophenyl) group. By comparison, the effectiveness of **5f-5h** against *C. albicans* was considerably lower. The lack of substituent(s) on the 3-phenyl ring, of compounds **5a-5d**, also resulted in a decrease in potency. The introduction of 4-acetamido **5i**, 5-methoxycarbonyl **5j**, or 4-carboxy **5m**, groups in place of the 4-chloro substituent (R^1 in **5/6**) provided derivatives with weak *in vitro* activity. Similarly, the replacement of 4-Cl with 4-OCH_3 group on the 3-phenyl ring had the same negative effect, **5f** *versus* **5k**. As expected [1], the *trans*-diastereomers **6b** and **6c** were less potent *in vitro* than the *cis* counterparts **5f** and **5j**.

The *in vivo* antifungal activity of triazoles **5** and **6** was evaluated in an immune-compromised mouse model of systemic candidiasis [4] using orally dosed ketoconazole as the positive standard drug. Immune-normal mice were used as controls. The animals were given oral doses (10, 25 and 50 mg/kg) of test compounds at 1, 4 and 24 hours after the challenge with *C. albicans* strain B-311, and the observed activity was expressed as the number of surviving animals (live-animal count) at day 4 of the experiment (Table III). Of the two tested compounds, **5f** was found to exert *in vivo* activity as potent as ketoconazole, while **5g** was inactive at doses of 10 and 25 mg/kg, and was only marginally active at the 50-mg/kg dose. As observed previously [4] with other 3,5-substituted isoxazolidines, the *in vitro* activity of **5f** correlated with its *in vivo* potency.

The experimental results from the present study indicate that the title 3-aryl-5-[(aryloxy)methyl]-3-[(1*H*-1,2,4-triazol-1-yl)methyl]-2-methylisoxazolidines represent a novel series of antifungal agents having both *in vitro* and *in vivo* efficacy. The *cis*-3-(4-chlorophenyl)-5-[(4-chlorophenyloxy)methyl analogue **5f** emerged as the most potent overall compound with *in vivo* activity equal in potency to that of the standard drug ketoconazole [6].

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The infrared (ir) spectra were obtained on a Nicolet MX-1 FT spectrometer as potassium bromide discs. The proton nuclear magnetic resonance (^{1}H nmr) spectra were taken on a Varian EM-360A (60 MHz) spectrometer using tetramethylsilane (TMS) as an internal standard; the 200-MHz nmr spectra were recorded on a Bruker-IBM-200-SY Fourier-transform spectrometer with TMS as the internal standard. All spectra were consistent with the assigned structures.

cis-N-[[2-Methyl-5-(phenoxy)methyl-3-phenylisoxazolidin-3-yl]-methyl]-1*H*-1,2,4-triazole (**5a**).

General Procedure.

Under a nitrogen atmosphere, a solution of 5.34 g (25 mmoles)

Table II

In Vitro Antifungal Activity of 3-Aryl-5-[(aryloxy)methyl]-3-[(1*H*-1,2,4-triazol-1-yl)methyl]-2-methylisoxazolidines [expressed as the minimum inhibitory concentration (MIC), in μg/ml]

Compound	T.a. [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]
cis-**5a**	>70.0	20.0	>70.0	20.0	70.0	70.0	>70.0	>70.0	>70.0	20.0
cis-**5b**	>70.0	7.0	>70.0	7.0	7.0	>70.0	>70.0	>70.0	>70.0	>70.0
cis-**5c**	70.0	20.0	70.0	20.0	20.0	70.0	>70.0	>70.0	70.0	70.0
cis-**5d**		>500.0						>500.0	>500.0	
cis-**5e**	20.0	20.0	>70.0	>70.0	7.0	>70.0	20.0	>70.0	>70.0	20.0
cis-**5f**	7.0	2.0	0.7	>70.0	2.0	70.0	7.0	>70.0	7.0	0.7
cis-**5g**	2.0	7.0	2.0	70.0	7.0	70.0	70.0	>70.0	20.0	0.7
cis-**5h**	20.0	2.0	20.0	>70.0	2.0	>70.0	7.0	>70.0	20.0	2.0
cis-**5i**	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0	>70.0	>20.0	>20.0
cis-**5j**	7.0	>70.0	70.0	20.0	7.0	>70.0	>70.0	>70.0	>70.0	>70.0
cis-**5k**	>70.0	>70.0	>70.0	>70.0	>70.0	>70.0	>70.0	>70.0	>70.0	>70.0
cis-**5l**		<2.0						>500.0	>500.0	
cis-**5m**	>70.0	>70.0	>70.0	>70.0	>70.0	>70.0	>70.0	>70.0	>70.0	>70.0
trans-**6b**		<2.0						>70.0	20.0	
trans-**6c**	70.0	>70.0	>70.0	>70.0	20.0	>70.0	70.0	>70.0	>70.0	>70.0
Ketoconazole	2.0	0.7	<0.2	0.7	<0.2	7.0	2.0	7.0	20.0	20.0

[a] T.a. = *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] *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.

of *N*-methyl-1-phenyl-2-(1*H*-1,2,4-triazol-1-yl)ethanimine *N*-oxide (**3**, R = H) and 6.2 ml (45 mmoles) of allyl phenyl ether (**4**, R^1 = H) in 250 ml of toluene was heated to reflux and stirred for 48 hours. Upon cooling to ambient temperature, the solvent was removed under reduced pressure to give a dark-colored viscous oil which was flash-chromatographed on neutral silica gel using a 98:2 mixture of chloroform and methanol as the eluent. Crystallization from ethyl acetate-hexane (1:1) furnished 1.94 (22%) of *cis*-**5a**, mp 68-75°; ir (potassium bromide): 1599 (m), 1505 (s), 1452 (m), 1275 (m), 1249 (s), 1141 (m), 1028 (w), 1010 (w), 753 (m), 709 (m), 691 (m) and 680 (m) cm^{-1}; ^{1}H nmr (deuteriochloroform): 200 MHz, ppm 2.59 (s, 3H, NCH_3), 2.81 (d, 2H, J = 7.7 Hz, OCH-CH_2), 4.35 (d, 2H, J = 4.4 Hz, OCH_2), 4.62 (d, 1H, J = 13.8 Hz, NHCH), 4.75 (d, 1H, J = 13.8 Hz, NHC*H*), 4.86-4.99 (m, 1H, OCH), 6.96-7.14 (m, 5H), 7.27-7.36 (m, 6H), 7.77 (s, 1H).

Anal. Calcd. for $C_{20}H_{22}N_4O_2$: C, 68.55; H, 6.33; N, 15.99. Found: C, 68.70; H, 6.44; N, 15.95.

Derivatives **5b-5l** and **6a-6c** were synthesized by procedures similar to that described for **5a**.

cis-*N*-[[5-(4-Chlorophenyloxy)methyl-2-methyl-3-phenylisoxazolidin-3-yl]methyl]-1*H*-1,2,4-triazole (**5b**).

Compound **5b** was obtained by reacting *N*-methyl-1-phenyl-2-(1*H*-1,2,4-triazol-1-yl)ethanimine *N*-oxide (**3**, R = H) and allyl 4-chlorophenyl ether (**4**, R^1 = 4-Cl), and was purified by flash chromatography on neutral silica gel using chloroform-methanol (98:2) as eluent, yield 20%, mp 144-147° (ethyl acetate); ir (potassium bromide): 1597 (m), 1578 (w), 1505 (m), 1492 (s), 1451 (m), 1440 (m), 1283 (m), 1249 (s), 1140 (m), 1003 (m), 856 (m), 826 (s) and 705 (s) cm^{-1}; ^{1}H nmr (deuteriochloroform): 200 MHz, ppm 2.56 (s, 3H, NCH_3), 2.84 (d, 2H, J = 7.7 Hz, OCH-CH_2), 4.28 (d, 2H, J = 4.4 Hz, OCH_2), 4.57 (d, 1H, J = 13.8 Hz, NHCH), 4.73 (d, 1H, J = 13.8 Hz, NHC*H*), 4.83-4.96 (m, 1H, OCH), 6.94 (d, 2H, J = 8.8 Hz), 7.06-7.12 (m, 2H), 7.23-7.33 (m, 6H), 7.77 (s, 1H).

Anal. Calcd. for $C_{20}H_{21}ClN_4O_2$: C, 62.42; H, 5.50; Cl, 9.21; N, 14.56. Found: C, 62.40; H, 5.54; Cl, 9.14; N, 14.54.

cis-*N*-[[5-(4-Fluorophenyloxy)methyl-2-methyl-3-phenylisoxazolidin-3-yl]methyl]-1*H*-1,2,4-triazole (**5c**).

Compound **5c** was obtained from *N*-methyl-1-phenyl-2-(1*H*-1,2,4-triazol-1-yl)ethanimine *N*-oxide (**3**, R = H) and allyl 4-fluorophenyl ether (**4**, R^1 = 4-F) and was purified by flash chromatography on neutral silica gel using chloroform-methanol (98:2), yield 29%, mp 115-120° (ethyl acetate); ir (potassium bromide):

Table III

In vivo Antifungal Activity of 3-Aryl-5-[(aryloxy)methyl]-3-[(1*H*-1,2,4-triazol-1-yl)methyl]-2-methylisoxazolidines [expressed by number of surviving animals (live-animal count) at day 4 after the challenge]

Compounds	Oral dose (mg/kg) [a]	Live-animal count day 0	day 1	day 2	day 3	day 4
controls [b]	—	8	6	0		
cis-**5f**	10	8	7	4	0	
	25	8	8	8	8	7
	50	8	8	8	8	8
cis-**5g**	10	8	5	0		
	25	8	4	0		
	50	8	7	2	0	
Ketoconazole	10	8	7	5	1	0
	25	8	8	8	8	8
	50	8	8	8	7	7

[a] Groups of 8 mice were used in each experiment. [b] Groups of 8 immune-normal control mice were challenged with 2.0 x 10^7 CFU's of *C. albicans* strain B-311.

3126 (w), 3100 (w), 3067 (w), 3000 (w), 2968 (m), 2925 (m), 2907 (m), 2859 (w), 1598 (w), 1508 (s), 1474 (m), 1450 (m), 1303 (m), 1278 (m), 1252 (s), 1245 (s), 1211 (s), 1142 (m), 1048 (m), 1025 (m), 1008 (m), 896 (m), 828 (s), 761 (m), 748 (m), 704 (s), 685 (m) cm^{-1}; ^1H nmr (deuteriochloroform): 200 MHz, ppm 2.56 (s, 3H, NCH_3), 2.84 (d, 2H, J = 7.2 Hz, OCH-C*H*$_2$), 4.28 (d, 2H, J = 4.4 Hz, OCH_2), 4.58 (d, 1H, J = 14.3 Hz, N*H*CH), 4.74 (d, 1H, J = 14.3 Hz, NHC*H*), 4.84-4.96 (m, 1H, OCH), 6.91-7.12 (m, 6H), 7.26-7.35 (m, 4H), 7.77 (s, 1H).

Anal. Calcd. for $C_{20}H_{21}FN_4O_2$: C, 65.20; H, 5.75; F, 5.16; N, 15.21. Found: C, 65.13; H, 5.79; F, 5.26; N, 15.13.

cis-*N*-[[5-(4-Acetamidophenyloxy)methyl-2-methyl-3-phenylisoxazolidin-3-yl]methyl]-1*H*-1,2,4-triazole (**5d**).

Analogue **5d** was prepared by reacting *N*-methyl-1-phenyl-2-(1*H*-1,2,4-triazol-1-yl)ethanimine *N*-oxide (**3**, R = H) and 4-acetamidophenyl allyl ether (**4**, R^1 = 4-$NHCOCH_3$), and was purified by flash chromatography on neutral silica gel using a 97:3 mixture of chloroform and methanol as eluent, yield 34%, mp 186-189° (benzene-methanol, 1:1); ir (potassium bromide): 3252 (w), 3056 (m), 1678 (s), 1554 (m), 1510 (s), 1267 (m), 1243 (s), 1135 (m), 1037 (m), 1014 (w), 834 (m), 745 (w), 708 (m) cm^{-1}; ^1H nmr (deuteriochloroform): 200 MHz, ppm 2.15 (s, 3H, $COCH_3$), 2.56 (s, 3H, NCH_3), 2.84 (d, 2H, J = 7.7 Hz, OCH-C*H*$_2$), 4.30 (d, 2H, J = 5.0 Hz, OCH_2), 4.60 (d, 1H, J = 13.8 Hz, N*H*CH), 4.72 (d, 1H, J = 13.8 Hz, NHC*H*), 4.84-4.96 (m, 1H, OCH), 6.96 (d, 2H, J = 9.9 Hz), 7.06-7.10 (m, 2H), 7.27-7.36 (m, 5H, 4 x aromatic H, NH), 7.42 (d, 2H, J = 9.9 Hz), 7.77 (s, 1H).

Anal. Calcd. for $C_{22}H_{25}N_5O_3$: C, 64.85; H, 6.18; N, 17.19. Found: C, 64.80; H, 6.26; N, 17.35.

N-[[3-(4-Chlorophenyl)-2-methyl-5-(phenyloxy)methylisoxazolidin-3-yl]methyl]-1*H*-1,2,4-triazole (*cis*-**5e** and *trans*-**6a**).

Compounds *cis*-**5e** and *trans*-**6a** were synthesized from 1-(4-chlorophenyl)-*N*-methyl-2-(1*H*-1,2,4-triazol-1-yl)ethanimine *N*-oxide (**3**, R = 4-Cl) and allyl phenyl ether (**4**, R^1 = H), and the resulting diastereomeric mixture was separated by flash chromatography on neutral silica gel using a 98:2 mixture of chloroform and methanol as the eluent, *cis*-**5e**, yield 17%, mp 135-139° (ethyl acetate-hexane, 1:1); ir (potassium bromide): 1602 (m), 1507 (m), 1497 (s), 1458 (m), 1273 (m), 1244 (s), 1205 (m), 1135 (m), 1092 (m), 1011 (m), 910 (m), 828 (m), 753 (m), 677 (m) cm^{-1}; ^1H nmr (deuteriochloroform): 200 MHz, ppm 2.56 (s, 3H, NCH_3), 2.82 (dd, 1H, J = 9.4, 13.2 Hz, OCH-*H*CH), 2.87 (dd, 1H, J = 6.1, 13.2 Hz, OCH-HC*H*), 4.30 (d, 2H, J = 5.0 Hz, OCH_2), 4.63 (d, 1H, J = 14.3 Hz, N*H*CH), 4.68 (d, 1H, J = 14.3 Hz, NHC*H*), 4.82-4.94 (m, 1H, OCH), 6.97-7.02 (m, 5H), 7.26-7.35 (m, 4H), 7.54 (s, 1H), 7.77 (s, 1H).

Anal. Calcd. for $C_{20}H_{21}ClN_4O_2$: C, 62.42; H, 5.50; Cl, 9.21; N, 14.56. Found: C, 62.37; H, 5.64; Cl, 9.54; N, 14.47.

trans-**6a**.

This compound was obtained in 3.4% yield, mp 97-100° (ethyl acetate-hexane, 1:1); ^1H nmr (deuteriochloroform): 200 MHz, ppm 2.57 (s, 3H, NCH_3), 2.72 (dd, 1H, J = 8.8, 12.7 Hz, OCH-*H*CH), 2.89 (dd, 1H, J = 7.7, 12.7 Hz, OCH-HC*H*), 4.09-4.17 (m, 2H, OCH_2), 4.48 (d, 1H, J = 14.3 Hz, N*H*CH), 4.65 (d, 1H, J = 14.3 Hz, NHC*H*), 4.71-4.84 (m, 1H, OCH), 6.78-7.00 (m, 3H), 7.11 (d, 2H, J = 8.8 Hz), 7.24-7.35 (m, 5H), 7.84 (s, 1H).

N-[[5-(4-Chlorophenyloxy)methyl-3-(4-chlorophenyl)-2-methylisoxazolidin-3-yl]methyl]-1*H*-1,2,4-triazole (*cis*-**5f** and *trans*-**6b**).

Compounds *cis*-**5f** and *trans*-**6b** were prepared from 1-(4-chlorophenyl)-*N*-methyl-2-(1*H*-1,2,4-triazol-1-yl)ethanimine *N*-oxide (**3**, R = 4-Cl) and allyl 4-chlorophenyl ether (**4**, R^1 = 4-Cl), and were separated by flash chromatography on neutral silica gel using chloroform-methanol (98:2) as the eluent, *cis*-**5f**, yield 49%, mp 125-129° (ethyl acetate); ir (potassium bromide): 1595 (m), 1505 (m), 1492 (s), 1447 (m), 1270 (m), 1246 (s), 1141 (m), 1097 (m), 1014 (m), 826 (m) cm^{-1}; ^1H nmr (deuteriochloroform): 200 MHz, ppm 2.55 (s, 3H, NCH_3), 2.80 (dd, 1H, J = 9.4, 13.2 Hz, OCH-*H*CH), 2.89 (dd, 1H, J = 5.5, 13.2 Hz, OCH-HC*H*), 4.27 (d, 2H, J = 4.4 Hz, OCH_2), 4.58 (d, 1H, J = 14.3 Hz, N*H*CH), 4.69 (d, 1H, J = 14.3 Hz, NHC*H*), 4.81-4.93 (m, 1H, OCH), 6.93 (d, 2H, J = 8.8 Hz), 7.02 (d, 2H, J = 8.8 Hz), 7.25 (d, 2H, J = 8.8 Hz), 7.29 (d, 2H, J = 8.8 Hz), 7.45 (s, 1H), 7.78 (s, 1H).

Anal. Calcd. for $C_{20}H_{20}Cl_2N_4O_2$: C, 57.29; H, 4.81; Cl, 16.91; N, 13.36. Found: C, 57.30; H, 4.89; Cl, 16.74; N, 13.37.

trans-**6b**.

This compound was obtained in 4.6% yield, mp 105-109° (ethyl acetate-hexane, 1:1); ir (potassium bromide): 1592 (w), 1507 (m), 1493 (s), 1458 (m), 1275 (m), 1237 (s), 1138 (m), 1097 (m), 1013 (m), 820 (m) cm^{-1}; ^1H nmr (deuteriochloroform): 200 MHz, ppm 2.56 (s, 3H, NCH_3), 2.69 (dd, 1H, J = 8.8, 12.7 Hz, OCH-*H*CH), 2.89 (dd, 1H, J = 7.7, 12.7 Hz, OCH-HC*H*), 4.11 (d, 2H, J = 5.0 Hz, OCH_2), 4.48 (d, 1H, J = 14.3 Hz, N*H*CH), 4.65 (d, 1H, J = 14.3 Hz, NHC*H*), 4.69-4.83 (m, 1H, OCH), 6.81 (d, 2H, J = 8.8

Hz), 7.11 (d, 2H, J = 8.3 Hz), 7.21-7.34 (m, 5H), 7.84 (s, 1H).

Anal. Calcd. for $C_{20}H_{20}Cl_2N_4O_2$: C, 57.29; H, 4.81; Cl, 16.91; N, 13.36. Found: C, 57.22; H, 4.90; Cl, 16.88; N, 13.34.

cis-N-[[3-(4-Chlorophenyl)-5-(4-methoxyphenyloxy)methyl-2-methylisoxazolidin-3-yl]methyl]-1*H*-1,2,4-triazole (**5g**).

Analogue **5g** was made by reacting 1-(4-chlorophenyl)-*N*-methyl-2-(1*H*-1,2,4-triazol-1-yl)ethanimine *N*-oxide (**3**, R = 4-Cl) and allyl 4-methoxyphenyl ether (**4**, R^1 = 4-OCH_3), and was purified by flash chromatography on neutral silica gel using chloroform-methanol (98:2) as eluent, yield 13%, mp 109-111° (ethyl acetate-hexane, 1:1); ir (potassium bromide): 1507 (s), 1493 (m), 1229 (s), 1207 (m), 1131 (m), 1093 (m), 1048 (m), 1042 (m), 1014 (m), 826 (m), 746 (m) cm^{-1}; ^1H nmr (deuteriochloroform): 200 MHz, ppm 2.55 (s, 3H, NCH_3), 2.79 (dd, 1H, J = 9.4, 12.7 Hz, OCH-*H*CH), 2.84 (dd, 1H, J = 6.6, 12.7 Hz, OCH-HC*H*), 3.77 (s, 3H, OCH_3), 4.24 (d, 2H, J = 4.4 Hz, OCH_2), 4.61 (d, 1H, J = 14.3 Hz, N*H*CH), 4.66 (d, 1H, J = 14.3 Hz, NHC*H*), 4.78-4.91 (m, 1H, OCH), 6.84 (d, 2H, J = 9.4 Hz), 6.93 (d, 2H, J = 9.4 Hz), 6.99 (d, 2H, J = 8.3 Hz), 7.27 (d, 2H, J = 8.3 Hz), 7.55 (s, 1H), 7.76 (s, 1H).

Anal. Calcd. for $C_{21}H_{23}ClN_4O_3$: C, 60.79; H, 5.59; Cl, 8.55. Found: C, 60.75; H, 5.69; Cl, 8.59; N, 13.48.

cis-N-[[5-(4-Chloro-3-methylphenyloxy)methyl-3-(4-chlorophenyl)-2-methylisoxazolidin-3-yl]methyl]-1*H*-1,2,4-triazole (**5h**).

Derivative **5h** was obtained from 1-(4-chlorophenyl)-*N*-methyl-2-(1*H*-1,2,4-triazol-1-yl)ethanimine *N*-oxide (**3**, R = 4-Cl) and allyl 4-chloro-3-methylphenyl ether [**4**, R^1 = 3,4-CH_3(Cl)], and was purified by flash chromatography on neutral silica gel using chloroform-methanol (98:2) as eluent, yield 12%, mp 135-139° (ethyl acetate-hexane, 1:1); ir (potassium bromide): 3102 (m), 2960 (m), 2887 (m), 1576 (m), 1514 (m), 1494 (m), 1482 (s), 1451 (m), 1399 (m), 1291 (s), 1282 (s), 1274 (s), 1243 (m), 1172 (s), 1141 (m), 1092 (m), 1013 (m), 858 (m), 817 (w), 798 (m), 685 (m) cm^{-1}; ^1H nmr (deuteriochloroform): 200 MHz, ppm 2.34 (s, 3H, $ArCH_3$), 2.55 (s, 3H, NCH_3), 2.79 (dd, 1H, J = 9.9, 13.2 Hz, OCH-*H*CH), 2.85 (dd, 1H, J = 6.1, 13.2 Hz, OC*H*-HC*H*), 4.25 (d, 2H, J = 4.4 Hz, OCH_2), 4.59 (d, 1H, J = 13.8 Hz, N*H*CH), 4.66 (d, 1H, J = 13.8 Hz, NHC*H*), 4.79-4.91 (m, 1H, OCH), 6.75 (dd, 1H, J = 3.3, 8.8 Hz), 6.86 (d, 1H, J = 3.3 Hz), 6.99 (d, 2H, J = 8.8 Hz), 7.20 (d, 1H, J = 8.8 Hz), 7.28 (d, 2H, J = 8.8 Hz), 7.49 (s, 1H), 7.77 (s, 1H).

Anal. Calcd. for $C_{21}H_{22}Cl_2N_4O_2$: C, 58.21; H, 5.12; N, 12.93. Found: C, 58.41; H, 5.29; N, 12.89.

cis-N-[[5-(4-Acetamidophenyloxy)methyl-3-(4-chlorophenyl)-2-methylisoxazolidin-3-yl]methyl]-1*H*-1,2,4-triazole (**5i**).

Compound **5i** was synthesized starting from 1-(4-chlorophenyl)-*N*-methyl-2-(1*H*-1,2,4-triazol-1-yl)ethanimine *N*-oxide (**3**, R = 4-Cl) and 4-acetamidophenyl allyl ether (**4**, R^1 = 4-$NHCOCH_3$), and was purified by flash chromatography on neutral silica gel using a 99:1 mixture of chloroform and methanol as the eluent, yield 24%, mp 150-152° (benzene); ir (potassium bromide): 1682 (s), 1608 (m), 1552 (s), 1508 (s), 1493 (m), 1454 (m), 1409 (m), 1266 (m), 1246 (s), 1134 (m), 1095 (m), 1014 (m), 835 (m) cm^{-1}; ^1H nmr (deuteriochloroform): 200 MHz, ppm 2.15 (s, 3H, $COCH_3$), 2.55 (s, 3H, NCH_3), 2.74-2.93 (m, 2H, OCH-CH_2), 4.27 (d, 2H, OCH_2), 4.61 (d, 1H, J = 14.3 Hz, N*H*CH), 4.66 (d, 1H, J = 14.3 Hz, NHC*H*), 4.81-4.93 (m, 1H, OCH), 6.95 (d, 2H, J = 8.8 Hz), 7.00 (d, 2H, J = 8.8 Hz), 7.02-7.13 (m, 1H, NH), 7.28 (d, 2H, J = 8.8 Hz), 7.41 (d, 2H, J = 8.8 Hz), 7.53 (s, 1H), 7.78 (s, 1H).

Anal. Calcd. for $C_{22}H_{24}ClN_5O_3$: C, 59.79; H, 5.47; Cl, 8.02; N, 15.85. Found: C, 59.68; H, 5.72; Cl, 7.73; N, 15.44.

Methyl 4-[[3-(4-Chlorophenyl)-2-methyl-3-[(1*H*-1,2,4-triazol-1-yl)methyl]isoxazolidin-5-yl]methoxy]benzoate (*cis*-**5j** and *trans*-**6c**).

Analogues *cis*-**5j** and *trans*-**6c** were obtained by reaction of 1-(4-chlorophenyl)-*N*-methyl-2-(1*H*-1,2,4-triazol-1-yl)ethanimine *N*-oxide (**3**, R = 4-Cl) and methyl 4-(2-propenyloxy)benzoate (**4**, R^1 = 4-CO_2CH_3), and were separated by flash chromatography on neutral silica gel using chloroform-methanol (98:2) as the eluent, *cis*-**5j**, yield 29%, mp 153-156° (ethyl acetate); ir (potassium bromide): 1712 (s), 1606 (s), 1511 (s), 1452 (m), 1437 (m), 1286 (s), 1271 (s), 1259 (s), 1175 (m), 1098 (m), 1013 (m), 854 (m), 770 (m) cm^{-1}; ^1H nmr (deuteriochloroform): 200 MHz, ppm 2.55 (s, 3H, NCH_3), 2.81 (dd, 1H, J = 9.4, 13.2 Hz, OCH-*H*CH), 2.89 (dd, 1H, J = 6.1, 13.2 Hz, OCH-HC*H*), 3.89 (s, 3H, OCH_3), 4.35 (d, 2H, J = 4.4 Hz, OCH_2), 4.57 (d, 1H, J = 13.8 Hz, N*H*CH), 4.69 (d, 1H, J = 13.8 Hz, NHC*H*), 4.82-4.95 (m, 1H, OCH), 7.00 (d, 4H, J = 8.8 Hz), 7.28 (d, 2H, J = 8.8 Hz), 7.43 (s, 1H), 7.78 (s, 1H), 8.00 (d, 2H, J = 8.8 Hz).

Anal. Calcd. for $C_{22}H_{23}ClN_4O_4$: C, 59.66; H, 5.23; Cl, 8.00; N, 12.65. Found: C, 59.64; H, 5.32; Cl, 8.09; N, 12.58.

trans-**6c**.

This compound was obtained in 5.8% yield, mp 145-148° (ethyl acetate-hexane, 1:1); ir (potassium bromide): 1707 (s), 1606 (s), 1512 (m), 1506 (m), 1437 (m), 1282 (s), 1250 (s), 1180 (m), 1118 (m), 1109 (m), 1028 (m), 1010 (m), 771 (m) cm^{-1}; ^1H nmr (deuteriochloroform): 200 MHz, ppm 2.58 (s, 3H, NCH_3), 2.71 (dd, 1H, J = 9.4, 13.2 Hz, OCH-*H*CH), 2.92 (dd, 1H, J = 7.2, 13.2 Hz, OCH-HC*H*), 3.89 (s, 3H, OCH_3), 4.19 (d, 2H, J = 4.4 Hz, OCH_2), 4.48 (d, 1H, J = 14.3 Hz, N*H*CH), 4.66 (d, 1H, J = 14.3 Hz, NHC*H*), 4.72-4.86 (m, 1H, OCH), 6.89 (d, 2H, J = 8.8 Hz), 7.11 (d, 2H, J = 8.3 Hz), 7.32 (d, 2H, J = 8.3 Hz), 7.33 (s, 1H), 7.84 (s, 1H), 7.97 (d, 2H, J = 8.8 Hz).

Anal. Calcd. for $C_{22}H_{23}ClN_4O_4$: C, 59.66; H, 5.23; Cl, 8.00; N, 12.65. Found: C, 59.59; H, 5.40; Cl, 7.93; N, 12.53.

cis-N-[[5-[(4-Chlorophenyloxy)methyl]-3-(4-methoxyphenyl)-2-methylisoxazolidin-3-yl]methyl]-1*H*-1,2,4-triazole (**5k**).

Compound **5k** was made from 1-(4-methoxyphenyl)-*N*-methyl-2-(1*H*-1,2,4-triazol-1-yl)ethanimine *N*-oxide (**3**, R = 4-OCH_3) and allyl 4-chlorophenyl ether (**4**, R^1 = 4-Cl), and was purified by flash chromatography on neutral silica gel using a 98:1 mixture of chloroform and methanol as the eluent, yield 6%, mp 143-146° (ethyl acetate); ir (potassium bromide): 2959 (m), 1616 (w), 1596 (m), 1510 (m), 1505 (m), 1493 (s), 1454 (m), 1271 (s), 1184 (m), 1140 (m), 1027 (m), 1004 (m), 833 (m) cm^{-1}; ^1H nmr (deuteriochloroform): 200 MHz, ppm 2.54 (s, 3H, NCH_3), 2.79 (d, 2H, J = 7.2 Hz, OCH-CH_2), 3.80 (s, 3H, OCH_3), 4.28 (d, 2H, J = 4.4 Hz, OCH_2), 4.52 (d, 1H, J = 14.3 Hz, N*H*CH), 4.71 (d, 1H, J = 14.3 Hz, NHC*H*), 4.82-4.94 (m, 1H, OCH), 6.83 (d, 2H, J = 8.3 Hz), 6.94 (d, 2H, J = 8.8 Hz), 7.00 (d, 2H, J = 8.3 Hz), 7.25 (d, 2H, J = 8.8 Hz), 7.26 (s, 1H), 7.78 (s, 1H).

Anal. Calcd. for $C_{21}H_{23}ClN_4O_3$: C, 60.79; H, 5.59; Cl, 8.55; N, 13.50. Found: C, 60.92; H, 5.67; Cl, 8.64; N, 13.56.

cis-N-[[2-Methyl-3-(3-methylphenyl)-5-[(2-nitrophenyloxy)methyl]-methylisoxazolidin-3-yl]methyl]-1*H*-1,2,4-triazole (**5l**).

Derivative **5l** was made from *N*-methyl-1-(3-methylphenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanimine *N*-oxide (**3**, R = 3-CH_3) and allyl

2-nitrophenyl ether (**4**, R^1 = 2-NO_2), yield 62%, mp 149-152° (ethyl acetate); ir (potassium bromide): 2947 (m), 1604 (m), 1586 (m), 1533 (s), 1505 (s), 1491 (s), 1454 (s), 1378 (m), 1287 (s), 1275 (s), 1253 (s), 1208 (m), 1134 (m), 1011 (m), 868 (m), 858 (m), 769 (m), 746 (m), 678 (m), 668 (m) cm^{-1}; ^1H nmr (deuteriochloroform): 200 MHz, ppm 2.32 (s, 3H, $ArCH_3$), 2.43 (s, 3H, NCH_3), 2.76 (dd, 1H, J = 5.5, 13.2 Hz, OCH-*H*CH), 2.87 (dd, 1H, J = 9.9, 13.2 Hz, OCH-HC*H*), 4.44 (d, 2H, J = 4.4 Hz, OCH_2), 4.58 (s, 2H, NCH_2), 4.83-4.97 (m, 1H, OCH), 6.89 (s, 2H), 7.05-7.25 (m, 5H), 7.57 (t, 1H, J = 7.7 Hz), 7.75 (s, 1H), 7.85 (d, 1H, J = 7.7 Hz).

Anal. Calcd. for $C_{21}H_{23}N_5O_4$: C, 61.60; H, 5.66; N, 17.10. Found: C, 61.27; H, 5.93; N, 16.94.

cis-4-[[3-(4-Chlorophenyl)-2-methyl-3-[(1*H*-1,2,4-triazol-1-yl)methyl]isoxazolidin-5-yl]methoxy]benzoic Acid (**5m**).

A solution of 5.2 g (12 mmoles) of *cis*-**5j** and 1.0 g (17 mmoles) of potassium hydroxide in 50 ml of 95% ethanol was refluxed for 90 minutes under a nitrogen atmosphere. Upon cooling to ambient temperature, the solvent was evaporated under reduced pressure leaving a solid residue which was dissolved in water and then acidified with 5% sulfuric acid (*p*H 5). The white precipitate was collected and recrystallized from aqueous ethanol to give 3.48 (68%) of *cis*-**5m**, mp 240-242°; ir (potassium bromide): 3020-2740 (br w, OH), 1700 (m), 1685 (m), 1605 (s), 1514 (m), 1289 (m), 1256 (s), 1170 (m), 1132 (m), 1104 (m), 1012 (w), 853 (w), 774 (w); ^1H nmr (dimethyl sulfoxide-d_6): 200 MHz, ppm 2.54 (s, 3H, NCH_3), 2.88 (d, 2H, J = 7.7 Hz, OCH-C*H*$_2$), 4.31 (d, 2H, J = 4.4 Hz, OCH_2), 4.69 (s, 2H, NCH_2), 4.72-4.86 (m, 1H, OCH), 7.02 (d, 2H, J = 8.8 Hz), 7.13 (d, 2H, J = 8.8 Hz), 7.29 (d, 2H, J = 8.8 Hz), 7.65 (s, 1H), 7.75 (s, 1H), 7.94 (d, 2H, J = 8.8 Hz).

Anal. Calcd. for $C_{21}H_{21}ClN_4O_4$: C, 58.81; H, 4.94; Cl, 8.27; N, 13.06. Found: C, 58.96; H, 5.05; Cl, 8.39; N, 13.08.

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 being used as the control drug in all assays. All test compounds were grown on potato flake agar at 26°. *Candida albicans* was grown overnight, *Aspergillus fumigatus* was grown for approximately one week, and *Trichophyton rubrum* for about two weeks. The cells were either removed from the plates with a sterile cotton swab and suspended in sterile water (*C. albicans*, *A. fumigatus*) or washed from the surface of the plate with sterile water and diluted in sterile water (*T. rubrum*). The actual counts were performed using hemacytometer, and the suspensions were diluted to 1 x 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 occured.

Immune-Compromised Mouse Model of Systemic Candidiasis.

Groups of 8 male ICR mice weighing between 20 and 25 g were immune-compromised by dosing them 3 days prior to challenge with 200 mg/kg cyclophosphamide (intraperitoneal administration). Immune-normal mice were used as controls. *C. albicans* strain B-311, at a concentration of 2.0 x 10^7 or 2.0 x 10^5 colony-forming units in 0.5 ml of saline, was used to challenge the immune-normal and immune-compromised animals, respectively. The mice were dosed orally at 1, 4, and 24 hours 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 test compounds were suspended in 1% carboxymethylcellulose no more than 3 days prior to the first dosing, and were refrigerated prior and between the dosing. In the cyclophosphamide-suppressed animals, the WBC counts remained low for the duration of the experiments. The activity was expressed by the number of surviving animals (live-animal count) at day 4 after the challenge with *C. albicans*.

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