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Synthesis, Specificity, and Antifungal Activity of Inhibitors of the *Candida albicans* Δ^{24}-Sterol Methyltransferase

Mark A. Ator,*[†,‡] Stanley J. Schmidt,[†,‡] Jerry L. Adams,[†] Roland E. Dolle,[†,‡] Lawrence I. Kruse,[†,‡] Carrie L. Frey,[§] and Janice M. Barone[§]

Departments of Medicinal Chemistry and Anti-Infectives, Research and Development Division, Smith Kline Beecham Pharmaceuticals, P.O. Box 1539, King of Prussia, Pennsylvania 19406-0939. *Received March 21, 1991*

A series of side chain modified analogues of cholesterol and lanosterol (1–10) have been synthesized and evaluated as inhibitors of the *Candida albicans* Δ^{24}-sterol methyltransferase. Two sterol substrate analogues 1 and 2 which contained a 24-thia substituent were relatively modest inhibitors of the enzyme (K_i = 1.5–72 μM). Compounds which mimic the carbocation intermediates proposed for the methyltransferase reaction, including sulfonium salts 4–6, amidines 7 and 8, and imidazoles 9 and 10 were substantially more potent inhibitors (K_i = 5–500 nM). All of the sterol analogues examined displayed less than 10-fold selectivity for inhibition of the methyltransferase versus the rat liver Δ^{24}-sterol reductase. The sterol analogues were tested for in vitro antifungal activity against *C. albicans*, *Candida tropicalis*, and *Torulopsis glabrata*. The minimum inhibitory concentrations versus *C. albicans* correlated well with the K_i values for methyltransferase inhibition, and the potency of several compounds approached that of amphotericin B, although only modest fungicidal activity was observed.

Current therapy for the treatment of systemic fungal infections is largely limited to two classes of compounds, the polyene antibiotics and the azoles.[1,2] Amphotericin B, a polyene antibiotic, is believed to exert its powerful fungicidal effect by complexing with ergosterol in the cytoplasmic membrane, resulting in the formation of channels which disrupt normal membrane proton gradients.[1] The utility of amphotericin B is limited by the necessity for parenteral administration and by its substantial toxicity, which may derive from its modest selectivity for combination with ergosterol relative to cholesterol, a component of mammalian cell membranes.[3] The second major class of antifungal drugs is the azoles, currently represented clinically by ketoconazole. The azoles interrupt the synthesis of ergosterol by inhibition of lanosterol 14α-demethylase, a cytochrome P-450 enzyme, by coordination of an imidazole or triazole moiety with the iron of the enzyme's heme prosthetic group.[1] While the azoles have the advantage of oral administration, they are not fungicidal and possess some side effects related to their limited selectivity for the fungal demethylase versus mammalian cytochrome P-450s, although the selectivity of fluconazole, a second-generation azole, is markedly improved.[4]

The mechanisms of action of the polyene antibiotics and the azoles clearly demonstrate that disruption of the production and function of ergosterol provides a valid target for antifungal therapy. In our efforts to identify novel, selective antifungal agents,[5] we have pursued the inhibition of *S*-adenosyl-L-methionine:Δ^{24}-sterol methyltransferase (EC 2.1.1.41), which catalyzes the addition of a C_1 group to the sterol side chain as an early step in ergosterol biosynthesis.[6] Culture of *Saccharomyces cerevisiae* in the presence of Δ^{24}-sterol methyltransferase inhibitors results in the decreased production of ergosterol, the accumulation of ergosterol precursors, and the inhibition of growth,[6] supporting its identification as a critical metabolic step. In addition, there is no direct counterpart for Δ^{24}-sterol methyltransferase in mammalian biochemistry, apparently providing the promise of specificity, although the Δ^{24}-sterol reductase of the mammalian cholesterol biosynthetic pathway is probably mechanistically related.[7]

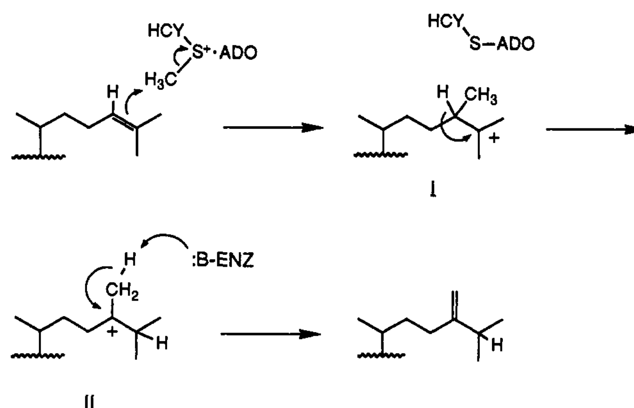
* Address correspondence to this author at Sterling Drug, Inc., 25 Great Valley Parkway, Malvern, PA 19355.

† Department of Medicinal Chemistry.

‡ Present address: Sterling Drug, Inc., 25 Great Valley Parkway, Malvern, PA 19355.

§ Department of Anti-Infectives.

Scheme I



The unusual reaction catalyzed by the methyltransferase is believed to proceed via nucleophilic attack of the π electrons of the sterol's Δ^{24} double bond on the methyl group of *S*-adenosyl-L-methionine (SAM) to yield a C-25 carbocation intermediate (i) (Scheme I).[8,9] A 24,25-hydride shift produces a C-24 carbocation (ii) (Scheme I) which is subsequently converted to the $\Delta^{24(28)}$ product by

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(2) Walsh, T. J.; Pizzo, A. Treatment of Systemic Fungal Infections: Recent Progress and Current Problems. *Eur. J. Clin. Microbiol. Infect. Dis.* 1988, *7*, 460–475.
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(6) Oehlschlager, A. C.; Angus, R. H.; Pierce, A. M.; Pierce, H. D., Jr.; Srinivasan, R. Azasterol Inhibition of Δ^{24}-Sterol Methyltransferase in *Saccharomyces cerevisiae*. *Biochemistry* 1984, *23*, 3582–3589.
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(8) Lederer, E. Biological C-Alkylation Reactions. In *The Biochemistry of Adenosylmethionine*; Salvatore, F., Borek, E., Zappia, V., Williams-Ashman, H. G., Schlenk, F., Eds.; Columbia University Press: New York, 1977; pp 89–126.
(9) Arigoni, D. Stereochemical Studies of Enzymic C-methylations. *Ciba Found. Symp.* 1977, *60*, 243–261.

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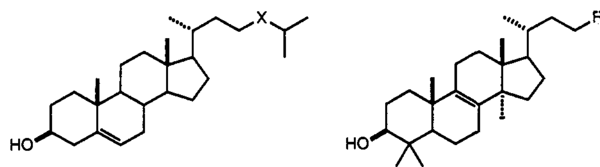
abstraction of a proton from the methyl group. An attractive strategy for inhibition of the methyltransferase involves the design of stable analogues of these highly reactive carbocation intermediates. Since enzymes bind intermediates much more tightly than substrates, analogues which can participate in the specific interactions employed to stabilize intermediates should be potent inhibitors of the enzyme.[10] This hypothesis is supported by the observation that a number of sterol analogues which contain positively charged functional groups in place of C-24 and C-25 are effective inhibitors of Δ^{24}-sterol methyltransferases.[6,11]

Herein we report the synthesis of a series of novel 24- and 25-substituted analogues of lanosterol and cholesterol **1–10**, evaluation of their inhibitory activity toward the *Candida albicans* Δ^{24}-sterol methyltransferase and the rat liver Δ^{24}-sterol reductase, and assessment of their in vitro antifungal activity against *C. albicans*, *Candida tropicalis*, and *Torulopsis glabrata*.



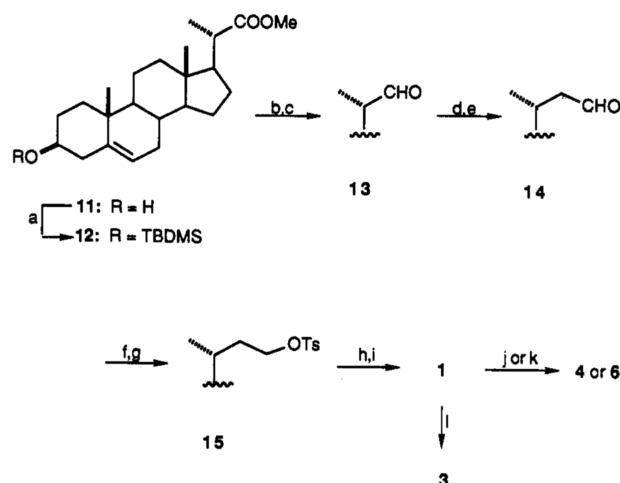
1: X = S
3: X = S(O)
4: X = $\overset{+}{S}CH_3I^-$
6: X = $\overset{+}{S}CH_2CH_3I^-$

2: R = $SCH(CH_3)_2$
5: R = $CH_3\overset{+}{S}CH(CH_3)_2I^-$
7: R = $C(NH_2){=}\overset{+}{N}H_2$ OTf^-
8: R = $C(NH_2){=}\overset{+}{N}(CH_3)_2$ OTf^-
9: R = 2-imidazolyl (NH)
10: R = 1-methyl-2-imidazolyl (N-CH_3)

Chemistry

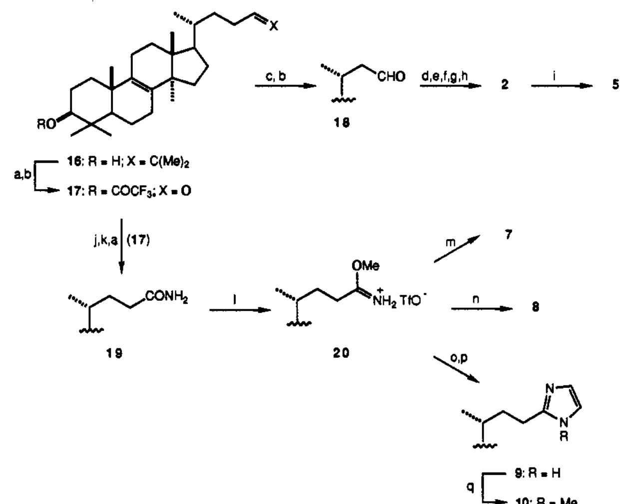
The synthesis of a series of 24-thia cholesterol analogues **1**, **3**, **4**, and **6** was performed as illustrated in Scheme II. The bisnorcholenic acid methyl ester **11**[12] was converted to **12** following etherification of the 3-hydroxyl group with *tert*-butyldimethylsilyl trifluoromethanesulfonate in the presence of 2,6-lutidine. Methyl ester **12** was subjected to lithium aluminum hydride reduction and Swern oxidation yielding aldehyde **13** (82% from **11**). One-carbon homologation of **13** to aldehyde **14** was accomplished via Wittig condensation with 4.0 equiv of $(Ph)_3P{=}CHOMe$ in toluene and hydrolysis of the intermediate enol ether ($Hg(OAc)_2$ in $THF-H_2O$, 9:1; 66% yield). The homologated aldehyde **14** was converted to tosylate **15** in two steps using standard methodology. Displacement of the nucleofuge with the potassium salt of $(CH_3)_2CHSH$ in DMF followed by acid hydrolysis of the *tert*-butyldimethylsilyl protecting group furnished **1**. Oxidation of **1** in CH_2Cl_2 with MCPBA yielded sulfoxide **3**, while sulfonium salts **4** and **6** were prepared by reaction of the sulfide with an

Scheme II



^{a} Reagents and conditions: (a) 2,6-lutidine, TBDMSiOTf; (b) LAH, THF; (c) DMSO, $(COCl)_2$, Et_3N; (d) $Ph_3P^+CH_2OCH_3Br^-$, potassium amylate, $PhCH_3$; (e) $Hg(OAc)_2$; (f) t-$BuNH_2{\cdot}BH_3$, CH_2Cl_2; (g) TsCl, pyr; (h) $KSCHMe_2$; (i) $THF-H_2O-HOAc$ 1:1:1.5; (j) MeI, CH_2Cl_2; (k) EtI, CH_2Cl_2; (l) MCPBA, CH_2Cl_2.

Scheme III



^{a} Reagents and conditions: (a) $(CF_3CO)_2O$, pyr; (b) O_3, CH_2Cl_2, then Me_2S; (c) TBDMSiOTf, Et_3N, CH_2Cl_2; (d) t-$BuNH_2{\cdot}BH_3$, CH_2Cl_2; (e) 2,6-lutidine, CH_2Cl_2, Tf_2O; (f) NaI, CH_3COCH_3; (g) $NaSCHMe_2$, DMF; (h) K_2CO_3, MeOH, CH_2Cl_2; (i) MeI, CH_2Cl_2; (j) CrO_3; (k) $(COCl)_2$, CH_2Cl_2, then NH_3; (l) MeOTf, CH_2Cl_2; (m) NH_3, EtOH; (n) Me_2NH, EtOH; (o) $NH_2CH_2CH(OMe)_2$, EtOH; (p) HCl, THF; (q) NaH, DMF, MeI.

excess of the appropriate alkyl iodide in methylene chloride.[13] A similar method has been reported recently for the preparation of 24-thia-5α-cholestan-3β-ol.[14]

The 24-thia lanosterol analogues **2** and **5** were prepared (Scheme III), using commercially available lanosterol **16** (Sigma Chemical Co.) as the starting material. The 3β-hydroxyl group present in **16** was protected as the tri-

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(13) Sulfoxide **3** and sulfonium salts **4–6** represent diastereomeric pairs due to the presence of the optically active sulfur atom. No attempt was made to separate **3–6** into their respective diastereomers. Enzyme kinetic and MIC/MLC data were obtained using the diastereomeric mixtures. Since no information exists regarding the relative potency of the individual isomers, the data are not corrected for the presence of the mixture.

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Table I. Inhibition of Δ^{24}-Sterol Methyltransferase and Δ^{24}-Sterol Reductase by Sterol Side-Chain Analogues

	Δ^{24}-sterol methyltransferase				Δ^{24}-sterol reductase:
compd[a]	pattern[b]	K_{is}	K_{ii}	α[c]	apparent K_i
1	C	1.5 μM			2 μM
2	C	72 μM			ND[d]
3	C	20 μM			ND
4	PC	16 nM		0.05	4 nM
5	PC	21 nM		0.02	ND
6	PC	0.5 μM			ND
7	PC	11 nM		0.06	7 nM
8	PC	6 nM			12 nM
9	NC	11 nM	25 nM		33 nM
10	PC	5 nM			48 nM

[a] See ref 13. [b] C, competitive; PC, parabolic competitive; NC, noncompetitive. [c] α is a factor which describes the cooperativity of binding of two identical inhibitor molecules, as described in the text. [d] Not determined.

fluoroacetate and the Δ^{24} double bond was cleaved by ozonolysis in CH_2Cl_2 to yield aldehyde **17**. Further degradation of the side chain (**17** → **18**) was accomplished by conversion of **17** to its corresponding *tert*-butyldimethylsilyl enol ether and subsequent ozonolysis. Gas chromatography indicated that the resulting aldehyde **18** contained only 2% of the starting aldehyde. Aldehyde **18** was ultimately converted to sulfide **19** by a procedure similarly used for **14** → **1**. Reaction of **19** with excess potassium carbonate in $MeOH-CH_2Cl_2$ afforded the target sulfide **2**. The methylsulfonium iodide **5** was prepared from **2** as described for the cholesterol analogue **4**.[13]

A series of nitrogen-containing analogues **7–10** were also derived from aldehyde **17** (Scheme III). Jones oxidation of **17** produced an intermediate carboxylic acid, which upon conversion to its acid chloride and treatment with an ice-cold saturated solution of ammonia in THF gave amide **19**. The trifluoroacetyl group was partially removed during amide formation, but it was restored by treatment of the crude amide mixture with excess trifluoroacetic anhydride and a few drops of triflic acid. Methyl imidate **20** was derived from **19** by the addition of excess methyl triflate to a solution of the amide in CH_2Cl_2, and it was used directly without purification. Amidines **7** and **8** were generated from imidate **20** by reaction with excess ammonia or dimethylamine in ethanol. In each case, the addition of **20** to the amine also resulted in the loss of the trifluoroacetyl group. All amidines were obtained after silica gel chromatography as their rather insoluble triflate salts. Imidazole **9** was prepared upon reaction of imidate **20** with aminoacetaldehyde dimethyl acetal and treatment with 4 N aqueous HCl at 0 °C for 2 h. Methylation of the imidazole moiety in the presence of the unprotected 3-hydroxyl was achieved using 2 equiv of NaH and excess MeI in DMF, yielding **10**.

Biological Results and Discussion

Compounds **1–10** were examined as reversible inhibitors of the microsomal *C. albicans* Δ^{24}-sterol methyltransferase using desmosterol as substrate.[15] The inhibition patterns and constants obtained are summarized in Table I. The 24-thia-substituted cholesterol analogue **1** was a modest competitive inhibitor of the enzyme, as expected for a molecule isoelectronic with the substrate. A 50-fold loss of potency was observed with lanosterol analogue **2**, consistent with the enzyme's preference for 4,4,14-desmethyl sterols as substrates.[15] Compounds **1** and **2** are also time-dependent inactivators of the methyltransferase, as reported in detail elsewhere.[15] The potent inhibition of the Δ^{24}-cycloartenol methyltransferase by a dipolar 25-*N*-oxide analogue of cycloartenol[11] suggested that oxidation of sulfide **1** to sulfoxide **3** might produce a more effective inhibitor. Instead, a significant decrease in affinity was observed.

Methylation of the sulfide **1** yields methylsulfonium derivative **4**,[13] which would be expected to bind avidly to the methyltransferase by virtue of its resemblance to intermediate (ii) of Scheme I. The observed 100-fold decrease in K_i is in accord with this prediction (Table I). Surprisingly, no difference in affinity was apparent between the cholesterol and lanosterol methylsulfonium analogues **4** and **5**, in contrast with the corresponding 24-thia derivatives, suggesting that the binding affinity of the sulfonium analogues is derived primarily from the side chain. Extension of the methylsulfonium analogue **4** to its ethylsulfonium congener **6** yields a 30-fold decrease in affinity, demonstrating that the portion of the active site which binds the sterol side chain is sterically constrained. This limitation might decrease the usefulness of a bisubstrate inhibitor,[16] in which elements of both the sterol substrate and SAM would be incorporated.

A series of nitrogen-substituted analogues of the C-25 carbocation (i) (Scheme I) have also been prepared and evaluated. These compounds require protonation in order to bear the positive charge required of an intermediate analogue, unlike the charged sulfonium species **4–6**. Amidine analogues **7** and **8** as well as the imidazole analogues **9** and **10** were extremely effective inhibitors of the enzyme (Table I). Assuming that the imidazolium ions of **9** and **10** are the true inhibitors and that their pK_a values are comparable to the value of 6.0 reported for histidine,[17] it might be expected that a decrease in the assay pH from its standard value of 7.5 would increase the concentration of the active protonated species and enhance the apparent potency of the compounds. The activity of the methyltransferase decreases dramatically with decreasing pH, however, so the experiment could not be performed.[18,19]

While simple competitive inhibition kinetics were observed with the ground-state analogues **1–3**, all of the carbocation analogues except for **9** were parabolic competitive inhibitors of the methyltransferase. Parabolic inhibition kinetics are indicative of binding of a second molecule of inhibitor to the E·I complex.[20] The data for compounds **4**, **5**, and **7** were not satisfactorily fit to an equation which describes parabolic competitive inhibition,

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(17) Dawson, R. M. C.; Elliott, D. C.; Elliott, W. H.; Jones, K. M. *Data for Biochemical Research*; Clarendon Press: Oxford, 1986; pp 16–17.
(18) Moore, J. T., Jr.; Gaylor, J. L. Isolation and Purification of an S-Adenosylmethionine:Δ^{24}-Sterol Methyltransferase from Yeast. *J. Biol. Chem.* **1969**, *244*, 6334–6340.
(19) We found that 25-azacholesterol (Counsell, R. E.; Klimstra, P. D.; Nysted, L. N.; Ranney, R. E. Hypocholesterolemic Agents. V. Isomeric Azacholesterols. *J. Med. Chem.* **1965**, *8*, 45–48.) was also a potent inhibitor of the methyltransferase: K_{is} = 11 nM (parabolic competitive inhibition pattern).[15] This finding is in agreement with literature reports for inhibition of the *S. cerevisiae*[6] and maize[11] enzymes by other 25-azasterols. The MICs of iii against *C. albicans*, *C. tropicalis*, and *T. glabrata* were 0.2, 50, and 3.1 μg/mL, respectively, while the MLCs against each of the organisms were >100 μg/mL.
(20) Cleland, W. W. Steady-State Kinetics. In *The Enzymes*, 3rd ed.; Boyer, P. D., Ed.; Academic Press: New York, 1970; Vol. 2, pp 1–65.

but were best described by a parabolic model in which the binding of the first inhibitor molecule changes the intrinsic dissociation constant of the second molecule by a factor α (see Data Analysis in the Experimental Section). As shown in Table I, the value of α determined for these analogues was very small, indicating that the second inhibitor molecule binds more tightly than the first. While the reason for this complicated kinetic behavior is not clear, one possible explanation is that the positively charged sterol derivatives bind not only as analogues of the sterol but also mimic the methylsulfonium moiety of SAM, subsequently binding to the SAM site as well.[15]

It is critical that any therapeutically useful Δ^{24}-sterol methyltransferase inhibitor avoid inhibition of the Δ^{24}-sterol reductase of the cholesterol biosynthetic pathway. Clinical experience with triparanol, an antihypercholesterolemic agent which inhibits both enzymes,[7] revealed a range of side effects of sufficient severity to require withdrawal of the drug from the market.[21-23] The Δ^{24}-sterol reductase is a poorly characterized microsomal enzyme which is capable of performing the NADPH-dependent reduction of a variety of Δ^{24} sterols.[24] Isotopic-labeling studies are consistent with a mechanism in which protonation at C-24 yields a C-25 carbocation intermediate which is subsequently reduced by hydride derived from NADPH.[25] This postulated carbocation intermediate is virtually identical to intermediate (i) (Scheme I) proposed for the methyltransferase reaction, suggesting that Δ^{24}-sterol methyltransferase inhibitors might also display high affinity for the Δ^{24}-sterol reductase.

The conversion of lanosterol to dihydrolanosterol by microsomal rat liver Δ^{24}-sterol reductase was monitored by gas chromatography.[26] The reaction was linear with time for 60 min and with enzyme over the range of 0.5–3 mg of microsomal protein per 1 mL assay. A K_m value for NADPH of 180 μM was determined at 70 μM lanosterol. Variation of lanosterol concentration at 2 mM NADPH revealed substrate inhibition which was described by a K_m of 40 μM and a K_i of 210 μM.

As displayed in Table I, there was no exploitable difference in inhibitory potency between the two enzymes, with the 9-fold selectivity of **10** for the methyltransferase providing the largest difference in activity. This lack of specificity supports the proposal that carbocation intermediates are formed in the reductase reaction. Surprisingly, C-24 and C-25 carbocation intermediate analogues were equally effective as inhibitors of the reductase, although its reaction is proposed to proceed only through a C-25 carbocation.

The in vitro antifungal activity of several sterol analogues was tested against strains of *C. albicans*, *C. tropicalis*, and *T. glabrata*. In general, a good correlation between methyltransferase inhibition and antifungal potency was observed. Sulfide **1**, which was a relatively poor methyltransferase inhibitor, was also ineffective against *C. albicans* (Table II). Methylsulfonium analogue **4** displayed impressive antifungal activity, in accord with its potent inhibition of the enzyme. The structurally similar ethyl analogue **6** was virtually inactive against *C. albicans*, as predicted by its reduced inhibitory activity relative to **4**. This result suggests that the efficacy of **4** is the result of methyltransferase inhibition and not due to a detergent effect, since disruption of the membrane by the methyl- and ethyl-substituted compounds should be comparable. The nitrogen-substituted sterol analogues were also highly potent inhibitors of fungal growth. While several of the compounds effectively inhibited growth at very low concentrations, the fungicidal activity of even the most potent compounds was modest (Table II).

Table II. In Vitro Antifungal Activity of Sterol Side-Chain Analogues

compound[b]	MIC/MLC[a] (µg/mL) *C. albicans*	*C. tropicalis*	*T. glabrata*
1	50/>100	>100/>100	>100/>100
4	0.2/50	0.2/6.3	1.6/50
6	100/>100	100/>100	>100/>100
7	0.2/12.5	0.4/12.5	3.1/50
9	1.6/>100	>100/>100	>100/>100
10	0.4/>100	12.5/>100	>100/>100
ketoconazole	0.1/12.5	1.6/6.3	3.1/12.5
amphotericin B	0.2/0.8	0.2/0.4	0.10/0.4

[a] Minimum inhibitory concentration/minimum lethal concentration, as defined in the Experimental Section. The results are the geometric mean of quadruplicate determinations. [b] See ref 13.

Imidazoles **9** and **10** were considerably less effective against *C. tropicalis* and *T. glabrata* than they were versus *C. albicans* (Table II). In contrast, the K_i values for inhibition of the *C. albicans* methyltransferase by these compounds are comparable to those obtained for inhibition of the *C. tropicalis* enzyme.[15,27] The lower susceptibility of these organisms must therefore be the result of differences in transport and metabolism of the compounds rather than the differential sensitivity of the methyltransferases.

In summary, a series of side chain modified analogues of lanosterol and cholesterol have been synthesized and examined as inhibitors of the *C. albicans* Δ^{24}-sterol methyltransferase. Substitution of C-24 or C-25 with a functional group which either is positively charged or becomes charged as a consequence of protonation yields compounds which are potent inhibitors of the methyltransferase. These results are consistent with the hypothesis that C-24 and C-25 carbocation intermediates are formed in the methyltransferase reaction. These compounds are effective in vitro antifungal agents, but they suffer from a lack of selectivity with respect to the mammalian Δ^{24}-sterol reductase.

Experimental Section

General Procedures. Anhydrous THF, CH_2Cl_2, and toluene were prepared by standard methods. All other solvents and reagents were reagent grade and used without purification. Melting points were determined in open capillary tubes on a Thomas-Hoover apparatus and are uncorrected. Capillary gas chromatography was performed on a Hewlett-Packard 5890A instrument equipped with an HP3392A digital integrator. All capillary GC analysis was carried out on a 10 m × 0.24 mm Chrompack fused silica WCOT CP Sil 5 column at an H_2 flow

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rate of 1 mL/min and an oven temperature of 270 °C. Infrared spectra ($CHCl_3$ thin films) were recorded on a Perkin-Elmer 727 spectrophotometer. ^1H NMR were recorded at 90 MHz (Varian EM 390) or 250 MHz (Brucker 350) with tetramethylsilane as the internal standard. Mass spectra were obtained on a Finnegan Model 3625 mass spectrometer equipped with chemical-ionization capability. Mass spectra and elemental analysis were performed in the SK&F Physical and Structural Chemistry Department. Analytical data were within 0.4% of the calculated values.

(3β,20*S*)-3-[(*tert*-Butyldimethylsilyl)oxy]pregn-5-ene-20-carboxylic Acid Methyl Ester (12). Bisnorcholenic acid methyl ester 11[12] (17 g, 47.2 mmol) in CH_2Cl_2 (100 mL) was cooled to −40 °C. 2,6-Lutidine (1 mL, 90.0 mmol) was added followed by the rapid addition of *tert*-butyldimethylsilyl triflate (14 g, 56 mmol). The reaction mixture was stirred for 15 min and warmed to 25 °C for 1 h. Standard workup and purification of the crude product by chromatography (50% CH_2Cl_2–hexane) gave ester 12 (98%): mp 152–153 °C; R_f 0.75 (20% hexane-CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 5.30 (m, 1 H, H-6), 3.65 (s, 3 H, OMe), 3.45 (m, 1 H, H-3), 0.05 and 0.03 (s, 3 H each, SiMe), 2.55–0.70 (m, remaining H); mass spectrum *m/e* (rel intensity) 475 (M + 1, 100), 459 (23), 417 (45).

(3β,20*S*)-3-[(*tert*-Butyldimethylsilyl)oxy]pregn-5-ene-20-carboxaldehyde (13). To a cooled suspension of LAH (1.5 g, 40 mmol) in anhydrous THF (100 mL) was added a solution of ester 12 (22 g, 40 mmol) in THF (50 mL) over a 5-min period. The reaction mixture was heated to reflux for 1 h and then cooled to 5 °C. Water (10 mL) was slowly added, followed by the addition of 2.5 N aqueous NaOH (10 mL) and finally water (30 mL). Insoluble solids were removed by filtration, and the solvent was removed in vacuo and replaced with CH_2Cl_2 (100 mL). The solution was washed with water and brine, and it was dried (Na_2SO_4). The solvent was evaporated and the alcohol (18 g, 87%) so obtained was used without further purification.

To a stirring solution of CH_2Cl_2 (300 mL) containing anhydrous DMSO (4.76 mL, 67 mmol) at −78 °C was added oxalyl chloride (2.94 mL, 33.5 mmol). After 5 min, the alcohol (10.0 g, 22.4 mol) obtained above was added, and the reaction mixture was stirred an additional 5 min. Triethylamine (27.8 mL) was then added, and the reaction mixture was stirred at 25 °C for 30 min. The reaction mixture was washed with water, dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was purified by chromatography on silica (20% CH_2Cl_2–petroleum ether) to give aldehyde 13 (8.8 g, 88%): mp 134–135 °C; R_f 0.8 (20% hexane–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 9.4 (d, 1 H, *J* = 2.0 Hz, CHO), 5.30 (m, 1 H, H-6), 3.45 (m, 1 H, H-3), 0.05 and 0.03 (s, 3 H each, SiMe), 2.55–0.70 (m, remaining H).

(3β,20*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-24-norchol-5-en-23-al (14). To a stirring solution of (methoxymethyl)triphenylphosphonium chloride (15.4 g, 45.0 mmol) in dry toluene (132 mL) was added potassium *tert*-amylate (26 mL of a 1.7 M solution in toluene). The reaction mixture was stirred for 1 h at room temperature. Aldehyde 13 (5.0 g, 11.2 mmol) in toluene (40 mL) was then added and the mixture was heated to 70 °C for 4 h. The reaction mixture was washed with water and dried (Na_2SO_4), and the solvent was removed in vacuo. The crude product was purified by chromatography (6% CH_2Cl_2–petroleum ether) to provide 4.0 g (75%) of a 1:2 mixture of cis–trans enol ethers: ^1H NMR ($CDCl_3$) δ 6.20 (d, 0.67 H, *J* = 14 Hz, C*H*CHOMe), 5.65 (d, 0.33 H, *J* = 8.0 Hz, C*H*CHOMe), 5.20 (m, 1 H, H-6), 4.44 (m, 1 H, CHC*H*OMe), 3.45 (s, 1 H, OMe), 3.43 (m, 1 H, H-3), 3.35 (s, 2 H, OMe), 0.05 and 0.03 (s, 3 H each, SiMe), 2.55–0.70 (m, remaining H). The mixture of enol ethers (2.35 g, 5.31 mmol), in THF (136 mL) and water (15 mL), was stirred with mercuric acetate (4.47 g, 7.9 mmol) for 1 h at room temperature. The reaction mixture was poured into 0.3 N aqueous potassium iodide (200 mL) and extracted with ethyl acetate (200 mL). The organic phase was removed, and it was washed successively with aqueous potassium iodide, water, and brine and dried (Na_2SO_4). Removal of the solvents in vacuo and purification of the residue by chromatography (hexane) furnished 1.5 g (66%) of aldehyde 14: mp 146–147 °C; R_f 0.45 (50% hexane–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 9.82 (t, 1 H, *J* = 2.0 Hz, CHO), 5.30 (m, 1 H, H-6), 4.75 (m, 1 H, H-3), 0.05 and 0.03 (s, 3 H each, SiMe), 2.55–0.70 (m, remaining H); mass spectrum, *m/e* (rel intensity) 459 (M + 1, 30), 443 (65), 401 (67), 367 (12), 355 (32), 327 (100).

(3β,20*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-24-norchol-5-en-23-ol *p*-Toluenesulfonate (15). To a solution of aldehyde 14 (200 mg, 0.46 mmol) in CH_2Cl_2 (10 mL) was added *tert*-butylamine–borane (100 mg, 1.15 mmol). The reaction mixture was stirred for 10 min at room temperature, and then it was washed with 1 M aqueous HCl, water, and brine and dried (Na_2SO_4). Removal of the solvent in vacuo gave 200 mg (100%) of the corresponding alcohol. To this alcohol (200 mg, 0.46 mmol) in pyridine (5 mL) was added tosyl chloride (265 mg, 1.4 mmol) in one portion. After stirring the reaction mixture for 1 h, the pyridine was evaporated, and the residue was purified by chromatography (40% CH_2Cl_2–hexane) to yield tosylate 15 (130 mg, 50%): mp 177–179 °C; R_f 0.9 (CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 7.80 and 7.40 (m, 4 H, Ar), 5.40 (m, 1 H, H-6), 4.20 (m, 2 H, H-22), 3.50 (m, 1 H, H-3), 2.55 (s, 3 H, $ArCH_3$), 0.05 and 0.03 (s, 3 H each, SiMe), 2.55–0.70 (m, remaining H); mass spectrum *m/e* (rel intensity) 613 (M − 1, 12) 483 (62), 443 (22), 311 (100).

(3β)-24-Thiacholest-5-en-3-ol (1). To 2-propanethiol (0.44 mL, 4.6 mmol) in dry DMF (21 mL) was added potassium hydride (92.4 mg, 2.31 mmol). The reaction mixture was heated to 60 °C for 10 min and then cooled to 0 °C. One drop of dicyclohexano-18-crown-6 was added, followed by the addition of tosylate 15 (472 mg, 0.77 mmol). The reaction mixture was stirred at room temperature for 1 h and then heated to 65 °C for 15 min. After removal of the DMF, the residue was dissolved in a mixture of 1:1:1.5 THF–H_2O–HOAc and stirred at 65 °C for 3 days. The solvents were removed in vacuo, and the residue was purified by chromatography (CH_2Cl_2) to give 171 mg (95%) of 1: mp 135–136 °C; R_f 0.5 (CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 5.30 (m, 1 H, H-6), 3.50 (m, 1 H, H-3), 2.75–0.70 (m, remaining H); mass spectrum *m/e* (rel intensity) 405 (M + 1, 100), 387 (92). Anal. ($C_{26}H_{44}OS$) C, H.

(3β,24*R*,*S*)-24-Thiacholest-5-en-3-ol *S*-Oxide (3). Sulfide 1 (50 mg, 0.12 mmol) was dissolved in CH_2Cl_2 (1 mL) and cooled to 0 °C, and *m*-chloroperbenzoic acid (15 mg, 0.12 mmol) was added. The reaction mixture was stirred for 24 h at room temperature. Removal of the solvent in vacuo and purification of the product by chromatography (5% MeOH–CH_2Cl_2) furnished 25 mg (50%) of 3 as a mixture of diasteromers: mp 179–181 °C; R_f 0.2 (CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 5.30 (m, 1 H, H-6), 3.50 (m, 1 H, H-3), 2.75–0.70 (m, remaining H); mass spectrum *m/e* (rel intensity) 421 (M + 1, 100), 403 (17). Anal. ($C_{26}H_{44}O_2S$) C, H.

(3β,24*R*,*S*)-24-Methyl-24-thioniacholest-5-en-3-ol Iodide (4). Sulfide 1 (50 mg, 0.12 mmol) and methyl iodide (170 mg, 1.2 mmol) in CH_2Cl_2 (1 mL) were stirred for 24 h at 25 °C. The deposited crystals of 4 (diastereomeric mixture) were isolated by filtration (42 mg, 60%): mp 179–180 °C; ^1H NMR ($CDCl_3$) δ 5.35 (m, 1 H, H-6), 4.65 (m, 1 H, $CH(CH_3)_2$), 3.75 (m, 1 H, H-22), 3.50 (m, 2 H, H-3,22), 2.75–0.70 (m, remaining H); mass spectrum *m/e* (rel intensity) 419 (M^+, 100). Anal. ($C_{27}H_{47}IOS$) C, H.

(3β,24*R*,*S*)-24-Ethyl-24-thioniacholest-5-en-3-ol Iodide (6). Ethyl iodide (187 mg, 95 mmol) and 1 (50 mg, 0.12 mmol) in CH_2Cl_2 (1 mL) were heated to 35 °C for 5 days. The reaction mixture was cooled to 0 °C, and the crystalline salt which deposited was collected by filtration to give 6 as a mixture of diastereomers (20 mg, 30%): mp 166–168 °C; ^1H NMR ($CDCl_3$) δ 5.35 (m, 1 H, H-6), 4.65 (m, 1 H, $CH(CH_3)_2$), 3.75 (m, 1 H, H-22), 3.50 (m, 2 H, H-3,22), 2.95–0.70 (m, remaining H); mass spectrum *m/e* (rel intensity) 433 (M^+, 100). Anal. ($C_{28}H_{49}IOS$) C, H.

(3β,5α,14α,20*R*)-3-(Trifluoroacetoxy)-4,4,14-trimethylchol-8-en-24-al (17). To a stirred solution of lanosterol 16 (30 g, 70 mmol; 60% pure, remainder 24,25-dihydrolanosterol; Sigma Chemical Co.) in CH_2Cl_2 (300 mL) at 0 °C containing pyridine (12 mL, 140 mmol) and DMAP (4.27 g, 35 mmol) was added trifluoroacetic anhydride (22.5 ml, 140 mmol). The reaction mixture was stirred for 20 min, and then it was washed with 1 N aqueous HCl and saturated aqueous $NaHCO_3$ and dried (Na_2SO_4). Removal of the solvent in vacuo gave the corresponding trifluoroacetate (36.7 g, 100%; R_f 0.3 (30% CH_2Cl_2–hexane)) which was used without further purification.

Ozone (0.035 mol) was passed into a solution of the trifluoroacetate in CH_2Cl_2 (3.5 L) at −78 °C over a 10-min period. Dimethyl sulfide (80 mL) was added, and the solution was allowed to warm to room temperature over 2 h. Following evaporation of the solvent, the product was isolated by chromatography (20% CH_2Cl_2–hexane), affording 13.6 g of analytically pure aldehyde

17 (45% from 16): mp 170–172 °C; R_f 0.7 (50% hexane–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 9.60 (t, 1 H, J = 1.5 Hz, CHO), 4.70 (m, 1 H, H-3), 2.75–0.70 (m, remaining H); mass spectrum m/e (rel intensity) 497 (M + 1, 90), 481 (55), 383 (100), 355 (20).

(3β,5α,14α,20R)-3-(Trifluoroacetoxy)-4,4,14-trimethyl-24-norchol-8-en-23-al (18). To an ice-cooled solution of 17 (2.0 g, 4.0 mmol) in $(CH_2)_2Cl_2$ (15 mL) containing triethylamine (1.5 mL) was added *tert*-butyldimethylsilyl triflate (1.37 mL, 6.0 mmol). The reaction mixture was warmed to room temperature for 1 h and then heated at 35 °C for 1 h. TLC indicated the complete formation of a cis/trans mixture of silyl enol ethers (R_f 0.8 (30% CH_2Cl_2–hexane)). The solvent was evaporated in vacuo, and the residue was triturated with dry hexane. The solution was filtered, and the hexane was evaporated and replaced with CH_2Cl_2. This solution was cooled to −78 °C and ozone (4 mmol) was added. Excess dimethyl sulfide (4 mL) was then added, and the solution was allowed to warm to room temperature over a 4-h period. Aldehyde 18 was purified by chromatography on deactivated silica (30% CH_2Cl_2–hexane) following standard workup to give 754 mg (45%) of product. Aldehyde 18 contained 2% of the higher homologue 17, as determined by gas chromatography: R_f 0.65 (50% hexane–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 9.71 (t, 1 H, J = 1.0 Hz, CHO), 4.70 (m, 1 H, H-3), 2.75–0.70 (m, remaining H); mass spectrum m/e (rel intensity) 481 (M − 1, 100).

(3β)-24-Thialanost-8-en-3-ol (2). To a solution of 18 (0.5 g, 1.0 mmol) in CH_2Cl_2 (100 mL) at 0 °C was added *tert*-butylamine–borate (45 mg, 0.52 mmol). After 15 min, the reaction mixture was allowed to warm to room temperature and 1 M aqueous HCl (3 mL) was added. Following an additional 30 min of stirring and standard workup, the crude product was purified by chromatography (50% hexane–CH_2Cl_2) to give the corresponding alcohol (380 mg, 76%): ^1H NMR ($CDCl_3$) δ 4.70 (m, 1 H, H-3), 3.20 (m, 2 H, CH_2OH), 2.75–0.70 (m, remaining H).

The alcohol (380 mg, 0.788 mmol), 2,6-lutidine (2.23 mL, 19.2 mmol), and DMAP (240 mg, 0.2 mmol) in anhydrous CH_2Cl_2 (35 mL) were cooled to −30 °C, and triflic anhydride (0.28 mL, 1.57 mmol) was added slowly by cannula. The reaction mixture was stirred for 15 min, washed with ice-cold 1 N aqueous HCl and brine, and dried (Na_2SO_4). Evaporation of the solvent gave a triflate (460 mg, 0.74 mmol, 95%). The triflate was dissolved in acetone (10 mL) containing NaI (440 mg, 2.96 mmol), and the solution was heated to 50 °C for 10 min. Following evaporation of the solvent, the product was dissolved in ethyl acetate, washed with 1 M aqueous sodium thiosulfate, and dried (Na_2SO_4). Removal of the solvent in vacuo and final purification of the residue by chromatography (hexane) furnished 402 mg (91%) of the iodide: R_f 0.8 (20% hexane–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 4.70 (m, 1 H, H-3), 3.15 (m, 2 H, CH_2I), 2.75–0.70 (m, remaining H).

The iodide (301 mg, 0.5 mmol) was added to a solution of dicyclohexano-18-crown-6 and sodium 2-propanethiolate in DMF (prepared from 40 mg of 18-crown-6, 1.2 mL of 2-propanethiol and 240 mg of 60% sodium hydride in 5 mL of DMF with warming for 15 min at 35 °C) and heated to 80 °C for 24 h. Solvents were removed in vacuo, and the residue was dissolved in ethyl acetate and washed with 1 N aqueous HCl and brine and dried (Na_2SO_4). Upon evaporation of the solvent, the residue was further dissolved in MeOH–CH_2Cl_2 (3:1) to which excess powdered K_2CO_3 was added. The heterogeneous solution was stirred at 25 °C for 24 h. The solution was then filtered, and the solvents were removed in vacuo. The residue was purified by chromatography (40% hexane–CH_2Cl_2) to yield sulfide 2 (87 mg, 30%): mp 135–136 °C; R_f 0.55 (CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 3.4 (m, 1 H, H-3), 2.90–0.65 (m, remaining H); mass spectrum m/e (rel intensity) 447 (M + 1, 95), 429 (100). Anal. ($C_{29}H_{50}OS$) C; H: calcd, 11.28; found, 10.71.

(3β,24R,S)-24-Methyl-24-thionialanost-8-en-3-ol Iodide (5). Sulfide 2 was stirred overnight in CH_2Cl_2 with an excess of methyl iodide. Crystals formed and were collected by filtration, yielding 5 as a mixture of diastereomers: mp 174–176 °C; NMR δ 4.70 (m, 1 H, $CH(CH_3)_2$, 4.15 and 3.7 (m, 1 H each, H-22), 3.35 (m, 1 H, H-3), 3.25 (s, 3 H, SMe), 2.55–0.68 (m, remaining H); mass spectrum m/e 461 (M^+). Anal. ($C_{30}H_{53}IOS$) C, H.

(3β,5α,14α,20R)-3-(Trifluoroacetoxy)-4,4,14-trimethylchol-8-ene-23-carboxamide (19). To a solution of 17 (2.0 g, 4.18 mmol) in acetone (120 mL) at 0 °C was added Jones reagent (3.5 mL of a 2.8 M aqueous solution) over a 2-min period. The reaction mixture was stirred at 25 °C for 30 min, 2-propanol (2 mL) was added, and then the mixture was stirred an additional 10 min. The solution was filtered through Celite and evaporated to dryness. The residue was dissolved in CH_2Cl_2 and washed with 1.5 N aqueous $NaHSO_3$ and water and dried (Na_2SO_4). The solvent was removed in vacuo, and the residue was purified by chromatography (CH_2Cl_2) to obtain 1.2 g (60%) of the corresponding carboxylic acid: R_f 0.8 (10:1:0.05 CH_2Cl_2–MeOH–HOAc); ^1H NMR ($CDCl_3$) δ 10.10 (br s, 1 H, COOH), 4.80 (m, 1 H, H-3), 2.75–0.70 (m, remaining H).

To a solution of the acid (5.0 g, 10 mmol) in toluene (25 mL) was added oxalyl chloride (7.0 mL, 80 mmol). The reaction mixture was stirred at ambient temperature for 1 h, and following the removal of solvent, THF (120 mL) previously saturated with ammonia was added. The reaction mixture was stirred at 25 °C for 2 h and the solvents were removed in vacuo. The proton NMR indicated that partial removal of the trifluoroacetate group had occurred. The crude amide was dissolved in CH_2Cl_2 and cooled to 0 °C, and trifluoroacetic anhydride (1.4 mL, 10 mmol) was added followed by 3–4 drops of triflic acid. The solution was warmed to room temperature and stirred for 20 min. The solution was warmed to room temperature and stirred for 20 min. The solution was washed with saturated aqueous $NaHCO_3$ and brine and dried (Na_2SO_4). Removal of the solvents in vacuo and purification of the residue by chromatography (10% MeOH–CH_2Cl_2) gave amide 19 (2.5 g, 95%): R_f 0.35 (20% MeOH–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 4.75 (m, 1 H, H-3), 2.75–0.70 (m, remaining H); mass spectrum m/e 510 (M − 1).

(3β,5α,14α,20R)-24-Amino-24-iminio-4,4,14-trimethylchol-8-en-3-ol Trifluoromethanesulfonate (7). To amide 19 (3.5 g, 7.0 mmol) in dry CH_2Cl_2 (400 mL) was added methyl triflate (3.0 mL, 26.5 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (0.3 g, 1.5 mmol). The reaction mixture was stirred for 24 h at room temperature. TLC (5% methanol–CH_2Cl_2) indicated that only partial reaction had occurred, and an additional aliquot of methyl triflate and base was added. The reaction mixture was stirred an additional 48 h, at which time TLC indicated the disappearance of most of the starting amide. The solvent was removed in vacuo, and imidate 20 so obtained was azeotroped with dry toluene and used immediately in the subsequent reaction without purification.

Imidate 20 was dissolved in ice-cold absolute ethanol (500 mL) which had been previously saturated with ammonia gas, and the solution was stirred at room temperature for 3 days. The residue obtained following evaporation of the solvent was eluted through a short column of silica (1% methanol–CH_2Cl_2) to remove unreacted amide and then further purified by chromatography (step gradient of CH_3Cl–MeOH–15 N NH_4OH: 90/10/1; 80/20/2; 70/30/3; 60/40/10) to afford amidine 7 (1.4 g, 35% from 19): mp 225–228 °C; R_f 0.4 (70:30:3 CH_3Cl–MeOH–15 N NH_4OH); ^1H NMR (DMSO) δ 8.70 and 8.30 (br s, 2 H, NH_2), 3.25 (m, 1 H, H-3), 2.90–0.65 (m, remaining H); mass spectrum m/e (rel intensity) 415 (M + 1, 100), 397 (10), 380 (23). Anal. ($C_{27}H_{46}N_2O{\cdot}CF_3SO_3H$) C, H, N.

(3β,5α,14α,20R)-24-Amino-24-(dimethyliminio)-4,4,14-trimethylchol-8-en-3-ol Trifluoromethanesulfonate (8). The dimethylamidine was prepared in the same manner as described for the amidine above, using dimethylamine in place of ammonia. From the amide 19 (3.5 g, 7.0 mmol) was obtained 2.0 g of 8 (85%): mp 280–282 °C dec; R_f 0.35 (70:30:3 CH_3Cl–MeOH–15 N NH_4OH); ^1H NMR (DMSO) δ 3.25 (m, 1 H, H-3), 2.90–0.65 (m, remaining H); mass spectrum m/e (rel intensity) 443 (M + 1, 85), 425 (100), 398 (50), 380 (55), 195 (32), 127 (10). Anal. ($C_{29}H_{50}N_2O{\cdot}CF_3SO_3H$) C, H, N.

(3β,5α,14α,20R)-23-(1H-Imidazol-2-yl)-4,4,14-trimethyl-24-norchol-8-en-3-ol (9). Imidate 20 (4.0 g, 5.3 mmol) was treated with 2-amino-2,2-dimethylacetaldehyde (2.78 g, 26.5 mmol) in ethanol as described for the preparation of 7. Removal of the solvent in vacuo and purification of the residue by chromatography (3% MeOH–CH_2Cl_2) furnished the corresponding amidine (2.0 g, 45%). A solution of the amidine (2.0 g, 2.37 mmol) in THF (30 mL) containing 4 N aqueous HCl (10 mL) was heated at 50 °C for 2 h. Evaporation of the solvent and purification of the residue by chromatography (10% MeOH–CH_2Cl_2 provided 9 (1.0 g, 50%): mp 270–272 °C; R_f 0.2 (10% MeOH–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 7.00 (s, 2 H, imidazole), 3.25 (m, 1 H, H-3), 2.95–0.65 (m, remaining H); mass spectrum m/e (rel intensity) 439 (M +

1, 90), 421 (100). Anal. ($C_{29}H_{46}N_2O$) C, N; H: calcd, 10.92; found, 10.32.

(3β,5α,14α,20*R*)-23-(1-Methyl-1*H*-imidazol-2-yl)-4,4,14-trimethyl-24-norchol-8-en-3-ol (10). To a solution of imidazole **9** (0.5 g, 1.1 mmol) in DMF (5 mL) was added sodium hydride (52 mg, 2.2 mmol). After the evolution of hydrogen ceased, methyl iodide (781 mg, 5.5 mmol) was added, and the solution was heated to 30 °C for 1 h. The product was isolated as described for **9** above to provide N-methylated imidazole **10** (413 mg, 80%): mp 220–222 °C; R_f 0.35 (10% MeOH–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 6.95 and 6.90 (s, 1 H each, imidazole), 3.60 (s, 3 H, NMe), 3.25 (m, 1 H, H-3), 2.85–0.65 (m, remaining H); mass spectrum *m/e* (rel intensity) 453 (M + 1, 100), 435 (65). Anal. ($C_{30}H_{48}N_2O$) C, H, N.

Assay of Δ^{24}-Sterol Methyltransferase. The fermentation of *C. albicans* strain ATCC 28367 and preparation of microsomes were accomplished as described previously.[15] Inhibition experiments were performed as described previously,[15] except that the SAM concentration was 25 μM and the amount of microsomal protein used per assay was 0.20 mg.

Assay of Δ^{24}-Sterol Reductase. Microsomes were prepared from the livers of cholestyramine-fed rats as previously described.[26] The Δ^{24}-sterol reductase assay was performed using modifications of a literature procedure.[26] In a 7-mL vial were combined 0.1 M potassium phosphate (pH 7.4), 4 mM $MgCl_2$, 5% glycerol, 10 mM glutathione, 0.15 M glucose, 10 mM nicotinamide, 20 units *Aspergillus niger* glucose oxidase, 100 μM lanosterol, various concentrations of sterol analogues, and 1.5 mg of rat liver microsomal protein in a final volume of 1.0 mL. The mixture was incubated at 37 °C to allow for enzymatic removal of dissolved oxygen, followed by addition of NADPH to a final concentration of 0.33 mM. After 30 min at 37 °C, the reaction was terminated by the addition of 1 mL of 20% ethanolic KOH. Sterols were extracted as described previously,[15] and the residue was redissolved in hexane. The reaction products were analyzed on a Hewlett-Packard HP 5980A gas chromatograph equipped with a flame-ionization detector using a 0.23 mm × 50 m Chrompack WCOT fused silica CP Sil 8 CB column. The oven temperature was maintained at 270 °C, and the injector and detector temperatures were 290 °C. Velocities were calculated from the percent conversion of lanosterol to dihydrolanosterol. Inhibition constants were calculated using the method of Dixon[28] assuming competitive inhibition.

Data Analysis. Data from inhibition experiments were analyzed using the COMP and NONCOMP programs of Cleland.[29] If satisfactory fits were not obtained, the data were fitted to eq 1, which describes parabolic competitive inhibition[30] using Superfit.[31] If a poor fit to the data was obtained using eq 1, the results were then analyzed using eq 2, which describes a kinetic model in which

$$v = V_m A / [K_m(1 + 2I/K_i + I^2/K_i 2) + A] \quad (1)$$

$$v = V_m A / [K_m(1 + 2I/K_i + I^2/\alpha K_i^2) + A] \quad (2)$$

two inhibitor molecules bind to the enzyme, with binding of the first inhibitor molecule changing the intrinsic dissociation constant of the second binding site by the factor α.[30] Kinetic parameters for the Δ^{24}-sterol reductase were determined using the programs HYPER and SUBIN.[29]

Antifungal Susceptibility Testing. One strain each of *C. albicans* (strain B311), *C. tropicalis* (clinical isolate), and *T. glabrata* (clinical isolate) were selected for susceptibility testing. Prior to testing, cells were grown for 18 h at 35 °C on a rotary shaker (125 rpm) in the same medium to be used for testing. Following incubation, the cells were washed three times in normal saline, resuspended in testing medium, and standardized by hemocytometer counting.

Minimum inhibitory and lethal concentrations (MICs and MLCs) were determined by a microbroth dilution assay utilizing visual and colony forming unit (CFU) reduction endpoints, respectively. Test compounds and ketoconazole (Janssen Pharmaceutica, Inc., Piscataway, N.J.) were dissolved in DMSO and serially diluted 2-fold in sterile distilled water containing 5% DMSO. Amphotericin B (Fungizone, E.R. Squibb & Sons, Princeton, N.J.) was reconstituted and serially diluted in sterile distilled water. Yeast inocula were adjusted to 5 × 10^4 cells/mL in yeast nitrogen base broth (YNB, Difco; supplemented with 0.15% asparagine and 2% glucose; pH 5.5).

Aliquots of inocula (0.08 mL) were added to the diluted compounds (0.02 mL) in 96-well microtiter plates resulting in final drug concentrations of 0.10 to 100 μg/mL (final DMSO = 1%) and 4 × 10^3 cells/mL in a final volume of 0.10 mL. Quadruplicate wells were prepared for each compound. Control wells contained organisms in medium/diluent with no compounds. The plates were incubated at 35 °C for 24 h at which time the MIC was recorded as the first concentration showing marked reduction in growth from the previous concentration. The MLC was recorded as the first concentration from which a subculture (48 h on Sabouraud dextrose agar at 35 °C) produced ≤50 CFU/mL.

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