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Stereoselective Synthesis of 7α- and 7β-Aminocholestanol as Potent Fungicidal Drugs

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Abstract—Potentially lymphotropic 7α- and 7β-aminocholestanol were stereoselectively synthesized. In vitro bioassay studies have shown that these fungicidal lipidic derivatives possess strong antifungal activity against *Candida* spp resistant strains to amphotericin B, 5-fluorocytosine and azoles. © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

Invasive fungal infections constitute, in the past two or three decades, a major cause of morbidity and mortality in immune-compromised patients as in cancer and in AIDS.[1,2] The frequency of deeply invasive candidiasis has increased nearly 10-fold during the past decade.[3,4] Many infections due to *Candida* spp are refractory to antifungal therapy. Isolates of *Candida albicans* and *Candida tropicalis*, the two most common causes of invasive candidiasis, were found to be significantly associated with increased mortality of bone marrow transplant recipients.[5] These findings may be particularly ominous for *C. tropicalis*, due to its increased virulence in granulocytopenic hosts in comparison to *C. albicans*.[6] Most antifungal agents inhibit the biosynthesis of ergosterol, the principal sterol in fungi, or interact directly with ergosterol in membranes. Amphotericin B (amp B), a polyene interacting with ergosterol in fungal cell membrane, is still the 'gold standard' for systemic fungal infections in spite of acute toxicity, nephrotoxicity, thrombophlebitis and erythroid suppression. Moreover, early observed diversity of pathogens and resistance of mycoses amp B therapy have posed new challenges for development of alternative antifungal therapy.

For a long time, amp B was the only drug that could be used against systemic mycoses, although 5-fluorocytosine (5-FC) became a useful combination partner.[7,8]

Most antifungal drugs are sterol biosynthesis inhibitors (SBIs), acting as site-specific inhibitors at different steps of ergosterol biosynthesis, the predominant sterol in most fungi.[9] Most SBIs, like azole and morpholine derivatives, are fungistatic rather than fungicidal and have low cerebrospinal fluid penetration.[10]

Transformation in the B ring of sterol is the target for many inhibitors such as morpholine derivatives. The $\triangle^8 \rightarrow \triangle^7$-sterol isomerase reaction is conducted with initial addition on the $\triangle^8$ double bond of a proton to the α face of C-9 giving a stabilized carbonium ion at C-8. This high energy intermediate is converted to the $\triangle^7$ alkene by removal of the 7β (mammals, plants) or the 7α (fungi) hydrogen. Morpholine derivatives—containing an amine function which is protonated in biological media—are analogues, of this carbonium ion (Scheme 1).[11]

Rahier[12] has shown that 8-aza-decalols are excellent inhibitors of $\triangle^8 \rightarrow \triangle^7$-sterol isomerase. These compounds are presumed to act due to their resemblance to the C-8 carbonium ion.

Oxysterols interfering with membranes lipids modified membrane fluidity,[13] membrane permeability for cations[14] and permeability to glucose.[15] By analogy to

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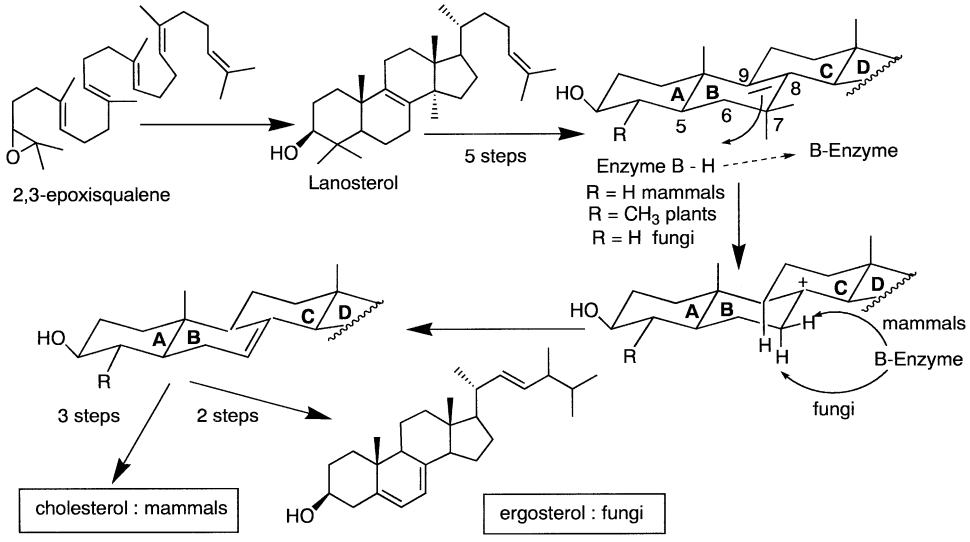
oxysterols, we have focused on a study of aminosterols and we have shown that 7-aminocholesterol, an epimeric mixture (7α/7β: 77/23) inhibits $\triangle^8 \rightarrow \triangle^7$-sterol isomerase and $\triangle^{14}$-sterol reductase as morpholine inhibitors, but this compound is fungicidal.[16]

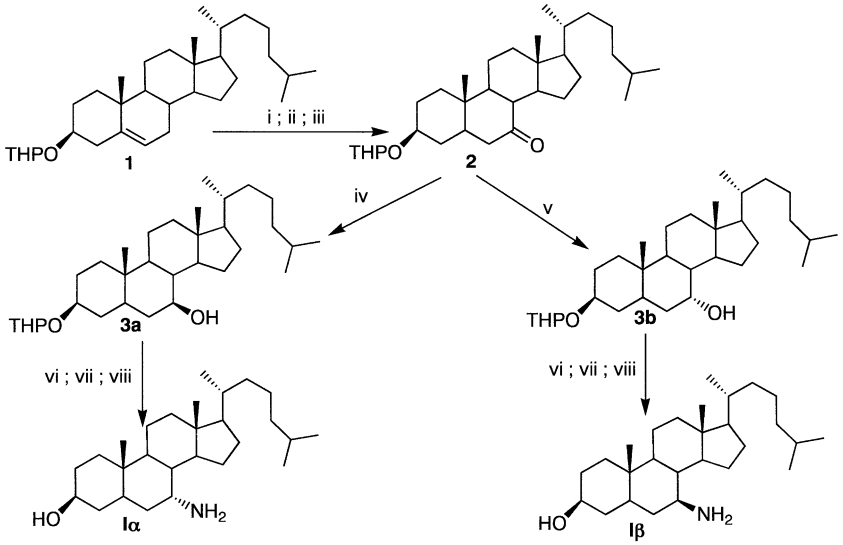
In the biosynthetic pathway, $\triangle^5$-unsaturation occurs only after $\triangle^8 \rightarrow \triangle^7$ isomerization. Our approach was then to synthesize the saturated 7α and 7β-amino derivatives (Scheme 2) so as to compare their efficiencies with amp B, 5-FC and bifonazole.

Chemistry

Hydroxyl functionality of cholesterol was protected with a tetrahydropyranyl group (**1**). Oxidation with chromium trioxide–pyridine complex afforded the allylic ketone. Selective reduction of the $\triangle^5$ double bond under Birch conditions, with lithium ammonia at −78 °C, gave the A/B *trans* fused steroid in 62% yield. However, the saturated and unsaturated ketones were not easily separated mixture of chromatographically methods. At −50 °C, the conjugated ketone gave (70% yield) an epimeric 7α- and 7β-hydroxycholestanol. This hydroxyl mixture was then oxidized to afford ketone **2**. Stereoselective reduction of this ketone with sodium borohydride in the presence of either cerium trichloride heptahydrate or L-selectride gave, respectively, 7β-hydroxyl **3a**[17] and 7α-hydroxyl **3b**.[18] These hydroxyl derivatives were converted into tosylates with an excess of tosyl chloride in pyridine. On heating at 60 °C tosylate with sodium azide in dimethylformamide, the required 7-azido derivatives[19,20] (47%) was obtained in low yield, probably due to steric hinderance. Azide reduction was achieved with lithium aluminium hydride and finally alcohol functionality was deprotected to give compound **Iα**[21] and **Iβ**[22] (Scheme 2).



Scheme 1. Pathways of sterol biosynthesis and mechanism proposed to $\triangle^8 \rightarrow \triangle^7$-sterol isomerase action, in fungi and mammals.



Scheme 2. Synthesis of 7α- and 7β-aminocholestanol. Reagents and conditions: (i) CrO_3–2py, CH_2Cl_2, rt; (ii) Li, NH_3, THF, −50 °C → rt; (iii) CrO_3, 2py, CH_2Cl_2, rt; (iv) L-selectride, THF, −78 °C; (v) $NaBH_4$, $CeCl_3$–$7H_2O$, MeOH/THF, −30 °C; (vi) (1) TsCl, py, rt; (2) NaN_3, DMF, 60 °C; (vii) $LiAlH_4$, THF, reflux; (viii) PPTS, ethanol, reflux.

Table 1. Determination of MIC (μg/mL) values after 48 h incubation

Compd	**Iα**	**Iβ**	**IIα,β**	amp B	5-FC	Bifonazole
S. cerevisiae ATCC 28 383	3.1	0.4	2.4	0.3	6.2	6.2
C. albicans CIP 1180-79	6.2	1.5	2.6	0.4	50	12.5
C. tropicalis CIP 1275-81 amp B resistant	1.5	1.5	1.5	12.5	—	—
C. tropicalis CIP 1745-88 5-FC resistant	3.1	3.1	3.1	—	>100	—
C. albicans CIP 1760-88 azole resistant	3.1	1.5	2.3	—	—	50

IIα,β: epimeric mixture of 7-aminocholesterol (7α/7β: 77/23).

Biological Results and Discussion

Minimum inhibitory concentration in μg/mL values after 48 h incubation[23] of **Iα** (6.2 μg/mL) and **Iβ** (1.5 μg/mL) were compared to 7-aminocholesterol **IIα,β** (epimeric mixture (7α/7β: 77/23); 2.6 μg/mL), amp B (0.4 μg/mL), bifonazole (12.5 μg/mL) and 5-FC (50 μg/mL) against *C. albicans*. These compounds were then tested against resistant strains such as *C. tropicalis* (amp B resistant): MIC for amp B was 12.5 μg/mL while **Iα**, **Iβ** and **IIα,β** gave lower identical value (1.5 μg/mL). On *C. tropicalis* (5-FC resistant), **Iα, Iβ** and **IIα,β** gave a value of 3.1 μg/mL while that for 5-FC was more than 100 μg/mL. Finally, MIC value for bifonazole was 50 μg/mL against *C. albicans* azole resistant strain though **Iα** (3.1 μg/mL) **Iβ** (1.5 μg/mL) and **IIα,β** (2.3) revealed a constant activity. **Iα** (3.1 μg/mL) and **Iβ** (0.8 μg/mL) were fungicidal[24] against *Saccharomyces cerevisiae* and **Iα, Iβ**, **IIα,β** at 6.2 μg/mL against *C. albicans* (Table 1).

Soustre[25] has proposed the hypothesis that 7-aminocholesterol is implicated either in the regulation of the sterol pathway in yeast or in the nutritional control of cell proliferation. This process could be also the basis of the cytotoxicity of 7-aminocholestanol instead of cell proliferation inhibition, as do the antifungal agents of the SBIs family generally.

Conclusion

Reduced fungi susceptibility to recent analogues of existing structural classes has arisen because fungi resistance mechanisms are so widely entrenched. *Candida* spp resistant strains to amp B, 5-FC and azole do not show increased resistance to 7-aminocholestanol. Moreover, unlike most SBIs, these drugs are fungicidal suggesting a new mechanism of action. Finally, the lipophilic 7-aminocholestanol derivatives are promising candidates for deeply invasive candidiasis.

Acknowledgement

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References and Notes

1. Powderly, W. G.; Kobayashi, G. S.; Herzip, G. P.; Medoff, G. *Am. J. Med.* **1988**, 826.
2. Lamb, D.; Corran, A.; Baldwin, B.; Kwonchung, J.; Kelly, S. *FEBS Lett.* **1995**, *368*, 326.
3. Rabodinirina, M.; Piens, M. A.; Monier, M. F.; Guého, E.; Fiere, D.; Mojon, M. *Eur. J. Clin. Microbiol. Infect. Dis.* **1994**, *13*, 152.
4. Hood, S.; Denning, D. W. *J. Antimicrob. Chemother.* **1996**, *37*, 71.
5. Hay, R.; Dupont, B.; Graybill, J. *Rev. Infct. Dis. (Suppl)* **1987**, *9*, 1.
6. Wingard, J. R.; Merz, W. G.; Saral, R. *Ann. Int. Med.* **1979**, *91*, 537.
7. Lyman, C. A.; Walsh, T. *J. Drugs* **1992**, 449.
8. White, M. H. *Curr. Opinon Inf. Dis.* **1993**, *6*, 737.
9. Oehlschlager A. C.; Czyzewska, E. In *Emerging Targets in Antibacterial and Antifungal Chemotherapy*; Sutcliffe J. A., Georgopapadakou N. H., Eds.; Chapman and Hall: New York, London, 1992; p 437.
10. Hartman, P. G.; Sanglard, D. *Current Pharm. Design* **1997**, *3*, 177.
11. Baloch, R. I.; Mercer, E. I. *Phytochemistry* **1987**, *26*, 663.
12. Rahier, A.; Genot, J. C.; Benveniste, P.; Place, P.; Anding, C. *Phytochemistry* **1985**, *24*, 1223.
13. Pannecoucke, X.; Starck, J. P.; Milon, A.; Ourisson, G.; Luu, B. *Bull. Soc. Chim. Fr.* **1994**, *131*, 393.
14. Holmes, R. P; Yoss, N. L. *Biochim. Biophys. Acta* **1984**, *770*, 15.
15. Theunissen, J. J. H.; Jackson, R. L.; Kempen, H. J. M.; Demel, R. A. *Biochim. Biophys. Acta* **1986**, *860*, 66.
16. El kihel, L.; Soustre, I.; Karst, F.; Letourneux, Y. *FEMS Microbiol.* **1994**, *120*, 163.
17. **3β-(tetrahydropyran-2-yl)oxycholestan-7β-ol** (**3a**): ^{1}H NMR δ_H (400 MHz; $CDCl_3$; Me_4Si): 0.65 (3H, s, 18-Me); 0.81 (3H, s, 19-Me); 0.83 (6H, dd, J=6.6 and 0.84 Hz, $CH(CH_3)_2$); 0.88 (3H, d, J=6.6 Hz; 21-Me), 3.29–3.35 (1H, m, H-7α); 3.42–3.46 (1H, m, H-6′ of THP), 3.51–3.60 (1H, m, H-3α); 3.86–3.90 (1H, m, H-6′ of THP); 4.68 (1H, s, H-2′ of THP). IR (KBr) ν (cm^{-1}) : 3508 (OH), 2933 (CH_2).
18. **3β-(tetrahydropyran-2-yl)oxycholestan-7α-ol** (**3b**): ^{1}H NMR δ_H (400 MHz; $CDCl_3$; Me_4Si): 0.65 (3H, s, 18-Me); 0.81 (3H, s, 19-Me); 0.85 (6H, dd, J=6.6 and 0.86 Hz, $CH(CH_3)_2$); 0.90 (3H, d, J=6.6 Hz; 21-Me), 3.44–3.48 (1H, m, H-6′ of THP), 3.57–3.66 (1H, m, H-3α); 3.82 (1H, large s, H-7β); 3.87–3.92 (1H, m, H-6′ of THP); 4.69 (1H, s, H-2′ of THP). IR (KBr) ν (cm^{-1}): 3480 (OH), 2935 (CH_2).
19. **7α-azido-3β-(tetrahydropyran-2-yl)oxycholestane:** ^{1}H NMR δ_H (400 MHz; $CDCl_3$; Me_4Si): 0.63 (3H, s, 18-Me); 0.80 (3H, s, 19-Me); 0.86 (6H, dd, J=6.4 and 0.86 Hz, $CH(CH_3)_2$); 0.90 (3H, d, J=6.4 Hz; 21-Me), 3.48–3.50 (1H, m, H-6′ of THP), 3.60–3.66 (1H, m, H-3α); 3.70 (1H, large s, H-7β); 3.90–3.93 (1H, m, H-6′ of THP); 4.71 (1H, s, H-2′ of THP). IR (KBr) ν (cm^{-1}): 2935 (CH_2); 2100 (N_3).
20. **7β-azido-3β-(tetrahydropyran-2-yl)oxycholestane:** ^{1}H NMR δ_H (400 MHz; $CDCl_3$; Me_4Si): 0.67 (3H, s, 18-Me); 0.81 (3H, s, 19-Me); 0.86 (6H, dd, J=6.6 and 0.84 Hz, $CH(CH_3)_2$); 0.90 (3H, d, J=6.6 Hz; 21-Me), 2.80–2.86 (1H, m, H-7α); 3.45–3.49 (1H, m, H-6′ of THP), 3.54–3.64 (1H, m, H-3α); 3.88–3.93 (1H, m, H-6′ of THP); 4.71 (1H, s, H-2′ of THP). IR (KBr) ν (cm^{-1}): 2929 (CH_2); 2093 (N_3).
21. **7α-aminocholestanol** (**Iα**): ^{1}H NMR δ_H (400 MHz; MeOD; Me_4Si): 0.73 (3H, s, 18-Me); 0.87 (3H, s, 19-Me); 0.88 (6H, dd, J=6.6 and 0.84 Hz, $CH(CH_3)_2$); 0.94 (3H, d, J=6.6 Hz; 21-Me); 3.52–3.60 (1H, m, H-3α); 3.64 (1H, m, H-7β). ^{13}C NMR δ_H (400 MHz; MeOD; Me_4Si): 11.6 (C18); 12.1 (C19); 19.1 (C21); 22.9 (C26); 23.1 (C27); 36.8 (C10); 37.9 (C7); 44.1 (C13); 71.3 (C3). IR (KBr) ν (cm^{-1}): 3408 (OH and NH_2);

2932 (CH_2). EI-SM, *m*/*z* (rel intensity %): 403 ($M^{+\cdot}$); 386 (100%); 371 (20%); 273 (13%); 152 (30%). Mp 220–222 °C.

22. **7β-aminocholestanol (Iβ)**: ^1H NMR δ_H (400 MHz; MeOD; Me_4Si): 0.74 (3H, s, 18-Me); 0.86 (3H, s, 19-Me); 0.88 (6H, dd, $J = 6.6$ and 0.84 Hz, $CH(CH_3)_2$); 0.94 (3H, d, $J = 6.6$ Hz; 21-Me), 2.97–3.04 (1H, m, H-7α); 3.49–3.55 (1H, m, H-3α). ^{13}C NMR δ_H (400 MHz; MeOD; Me_4Si): 12.4 (C18); 12.5 (C19); 19.2 (C21); 22.9 (C26); 23.1 (C27); 35.8 (C10); 43.1 (C7); 45.1 (C13); 71.2 (C3). IR (KBr) ν (cm^{-1}): 3340 (OH and NH_2); 2935 (CH_2). EI-SM, *m*/*z* (rel intensity %): 403 ($M^{+\cdot}$); 386 (100%); 371 (42%); 273 (26%); 161 (18%). Mp 228–231 °C.

23. The fungal growths were measured in vitro using a liquid-phase turbidimetric system (Bioscreen® from Labsystem, France) and automatically evaluated every 30 min for 48 h using various concentrations of drugs. Dei-Cas, E.; Dujardin, L.; Ribeiro Pinto, M. E.; Ajana, F.; Fruit, J.; Poulain, D.; Camus, D.; Vernes, A. *Mycoses* **1991**, *34*, 167.

24. After a 48 h growth period, incubation fluid was transferred into fresh broth, diluting the drug at least 100-fold, and then incubated for another 48 h. The drug was considered fungicidal when no growth was observed.

25. Soustre, I. Thesis, Université de Poitiers, France, Feb. 27, 1997.