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SYNTHESIS OF 25-AMINOSTEROLS, NEW ANTIFUNGAL AGENTS

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Abstract : 25-aminolanostenol **1** and 25-aminocholesterol **2** were hemisynthesized from natural sterols and tested *in vitro* against *Candida albicans*. The biological activity of compound **1** was rather weak, whereas **2** exhibited *in vitro* antifungal activity with MIC value of 4 µM.

Introduction : Since a few decades, there has been an increasing demand for new fungicidal agents, due especially to resistances to current azole antifungals, enhanced by immunodeficiencies, metabolic derangements, or suppression of competitor organisms.[1] Antifungal chemotherapy mainly implies systemic treatment with inhibitors of ergosterol, the dominant sterol in yeasts and fungi.[2] Azole derivatives inhibit the P450-dependent lanosterol 14α-demethylase and cause accumulation of 14-methylated sterols; as a result, the lack of ergosterol modifies the membrane's fluidity, creates vesicles, deformation of buds, and abnormal thickening which leads to destruction by the phagocytes[3]. The Δ^7-5-desaturase is another target of inhibition, widely studied[4] because of its specificity in the ergosterol biosynthesis pathway. We have already reported[5] potent activities of hemisynthesized aminosterol derivatives as potential transition state's mimics of desaturation.

In ergosterol biosynthesis, another specific step different from mammalian cholesterol synthesis, is the side chain C24-methylation by the sterol methyl transferase (24-SMT).[2] Thus, 24,25-epiminolanostenol was reported to inhibit the growth of *Gibberella fujikuroi*,[6] whereas azasterols (with nitrogen at C23, C24 or C25)[7] inhibited *Saccharomyces cerevisiae*. Few others lanosterol or cholesterol derivatives with nitrogen functionalities at C24 displayed fungistatic properties.[8] On the other hand, cytoxicity was noticed for 24,25-iminocholesterol.[9] In the scope of our studies on primary amine sterol derivatives, we performed the hemisynthesis of 25-aminolanostenol **1** and 25-aminocholesterol **2** (figure 1) as new potential 24-SMT inhibitors.

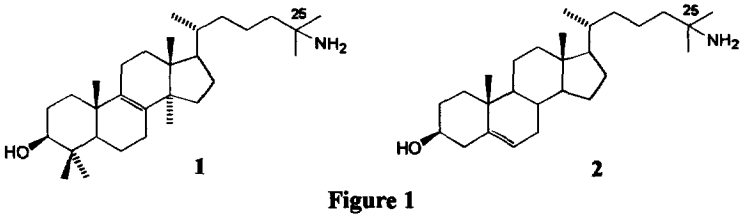

Figure 1

Chemistry : The synthetic route to **1** and **2** is outlined in scheme 1. Lanosterol and desmesterol of natural origin were acetylated at C3 by the usual method followed by acetoxymercuration/demercuration[10] to give respectively 25-hydroxylanostenyl acetate **5** and 25-hydroxycholesteryl acetate **6** in 90% yields. The tertiary alcohols were then treated with phosphorus tribromide in chloroform and led to the corresponding bromosteryl acetate derivatives (**7**,**8**) in 89-90% yield. Treatment with trimethylsilyl azide in excess and a catalytic amount of $SnCl_4$ in toluene[11] gave in 4 days 69 to 77 % of 25-azidosteryl acetate **9** and **10**. Saponification of **9** and **10** finally produced the 25-azidosterols **11** and **12** in quantity, or by reduction with $LiAlH_4$ in dry diethylether and subsequent acetate cleavage, afforded the title compounds[12] **1** and **2** in good yields. All compounds were fully described by IR, ^1H (400 MHz), ^{13}C (100 MHz) NMR, or MS spectroscopy experiments.

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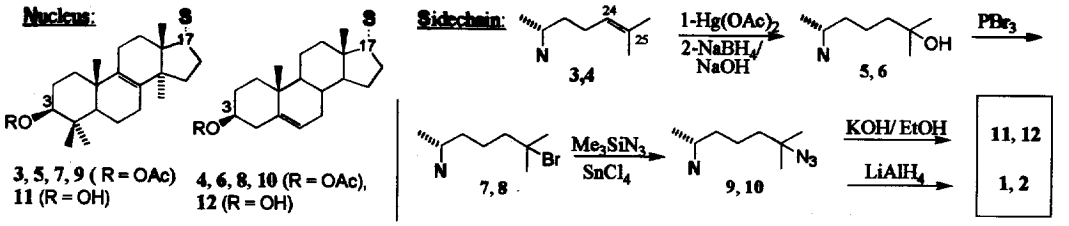

Scheme 1

Biological results and conclusion : Results of the antifungal *in vitro* screening[13] are summarized in table 1. No appreciable activity was found for **11** and **12**; conversely, **1** and **2** inhibited *Candida albicans*. Surprisingly, 25-aminocholesterol (MIC value = 4 μM) was found to be 15 times more potent than 25-aminolanostenol (MIC value = 60 μM). In addition, **2** at 4 μM was still inhibitory on *C. albicans* after 48h incubation. Bioassays on three bacterial strains (*E. hirae*, *S. aureus* and *E. coli*) displayed no activity on these microorganisms. These results are in perfect concordance with the reported fungicidal specific activity of 24-aminocholesterol and 24-aminolanostenol on *Candida sp.*[8] Antifungal activity is clearly in relation with the primary amine function and with the sterol tetracyclic structure. We postulate that the aminosterol **2** give rise to an ammonium form species that mimics the transition state (positive charge) of the methylation. In conclusion, we consider that *in vivo* bioevaluation should be performed in order to validate further development of **2**.

Table 1 : Growth inhibition* of *C. albicans* induced by 25-nitrogen substituted sterols

Fungus strain :	1	2	11	12
Candida albicans (IP 1180.79)	60	4	> 250	> 250

* MIC (inhibitory concentration in μM). *C. albicans* was cultured in liquid Sabouraud medium at 30°C containing 2% v/v of an appropriate solvent. Innoculum size : 2% v/v.

Acknowledgments : We acknowledge the Region Poitou Charentes Council for financial support.

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12. **25-Aminocholest-5-en-3β-ol (2)**: M.p=177 °C. IR (ν, cm^{-1}): 3500-3300 (OH); 3200-3250 (NH_2). ^1H NMR (δ, ppm): 0.68 (s, 3H, Me-18); 0.93 (d, 3H, Me-21, J=6.4 Hz); 1.01 (s, 3H, Me-19); 1.09 (s, 6H, Me-26 and Me-27); 3.48-3.56 (m, 1H, H-3α); 5.35 (d, 1H, H-6, J=6 Hz). ^{13}C NMR (δ, ppm): 11.8 (C-18); 18.7 (C-21); 19.4 (C-19); 20.9 and 21.1 (C-23 and C-11); 49.5 (C-25); 71.7 (C-3); 121.7 (C-6); 140.7 (C-5). MS, m/z (%; disconnection): 401[$M^{+\cdot}$]; 384 (54; M-NH_3); 369 (27; M-NH_3-CH_3); 366 (13; M-NH_3-H_2O); 351 (23; M-NH_3-CH_3-H_2O); 299 (35; M-NH_3-C_6H_{11}-2H); 271 (100; M-NH_3-sidechain); 253 (27; 271- H_2O); 213 (38; M-NH_3-H_2O-D cycle). **25-Aminolanost-8-en-3β-ol (1)**: IR (ν, cm^{-1}): 3500-3100 (OH); 3200-3250 (NH_2). ^1H NMR (δ, ppm): 0.66 (s, 3H, Me-18); 0.71 and 0.81 (2s, 6H, Me-4β et Me-14α); 0.89-1.00 (m, 9H, Me-4α, Me-21, Me-19); 1.16 (s, 6H Me-26 and Me-27); 3.18-3.39 (m, 1H, H-3α).
13. The fungal growth was measured *in vitro* using a liquid-phase turbimetric system (Bioscreen®, Labsystem) and automatically evaluated every 30 minutes for 16 hours using various concentrations of drugs. Dei-Cas, E.; Dujardin, L.; Ribeiro Pinto, M.E.; Fruit, J.; Poulain, D.; Camus, D.; Vernes, A. *Mycoses*, **1991**, 34, 167.