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Novel and efficient synthesis and antifungal evaluation of 2,3-functionalized cholestane and androstane derivatives

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ABSTRACT

Synthetic modifications of cholesterol and other traditional steroid molecules have become a promising area for the exploration and development of novel antifungal agents, especially with respect to the development of fatty-acid esters of steroids. In addition, 2,3-functionalized steroids are also compounds with potentially interesting biological properties and proper functionalization of 2,3-steroids can lead to the development of efficient syntheses of building blocks for novel fatty-acid esters of steroids. In this Letter, we outline a novel and efficient approach to the synthesis of 2,3-functionalized cholestane and androstane derivatives and present their promising preliminary antifungal activities against a number of fungal species.

As opportunistic fungal infections continue to represent continuous obstacles to individuals with compromised immune systems,[1–3] the development of novel and efficient methods for large scale synthetic development of novel antifungal therapeutic agents that have the capability of broad spectrum activity becomes inevitable. Over the last decade, synthetic modifications of the cholesterol molecule have become a promising area for the exploration and development of novel antifungal agents. In vitro analyses of oxygenated cholesterol derivatives, cholesterol-hydrazone derivatives and 6,5-fused cholestane oxazoles have shown promising antifungal activities against one or more species of fungi.[4–6] In addition, a number of fatty-acid derivatives have been characterized as antimicrobials and antifungals,[7,8] yet fatty-acid esters of cholesterol have not been explored exhaustively as potent therapeutic antifungal agents.[8] Finally, 2,3-functionalized steroids are well known compounds with potentially interesting biological properties.[9–12] Furthermore, proper functionalization of 2,3-steroids can be an important step in the development of building blocks for novel fatty-acid esters of cholesterol and other oxygenated and hydrazone-cholesterol derivatives. In this Letter, we outline a novel approach to the synthesis of 2,3-functionalized cholesterol and androstane derivatives and present the preliminary antifungal activities of each derivative against four fungal species, including *Candida albicans*, *Cryptococcus neoformans*, *Candida glabrata*, and the filamentous fungus *Aspergillus fumigatus*.

Over the last few decades, many naturally occurring products have been deemed effective antimicrobial agents, including saponins, typically isolated from plant and marine species.[13–15] In fact, we recently explored synthesis and antifungal activity of a number of novel 2α,3β-spirostandiol steroid components against several species of yeasts and molds, and obtained some very promising results in the initial in vitro analyses, and have begun to develop a structure–activity relationship between functionalized 2,3-spirostandiols and antifungal activity (*manuscript submitted and under review*). Nonetheless, we hypothesize that the spiral structure of the spirostandiol is not a pharmacophore and therefore can be totally removed, as in the case of androstane derivatives, or replaced by a hydrocarbon chain, as in the case of cholestane derivatives, to yield novel 2,3-functionalized targets that maintain the potential to elicit antifungal activity. To test this hypothesis, we began with the preparations of 2α,3β-androstandiol and 2α,3β-cholestandiol esters, using commercially available and inexpensive starting reagents as our templates for the 2,3-functionalization of steroids. The synthesis of androstane derivatives began with 5-androsten-17-one-3β-yl acetate (**1**, Scheme 1) (Sigma–Aldrich), while the cholestane derivatives began with cholesterol (**10**, Scheme 2) (Sigma–Aldrich).

A Wolff–Kishner reduction of the carbonyl group of dehydroisoandrosterone 3β-acetate (**1**, Scheme 1) followed by the hydrolysis

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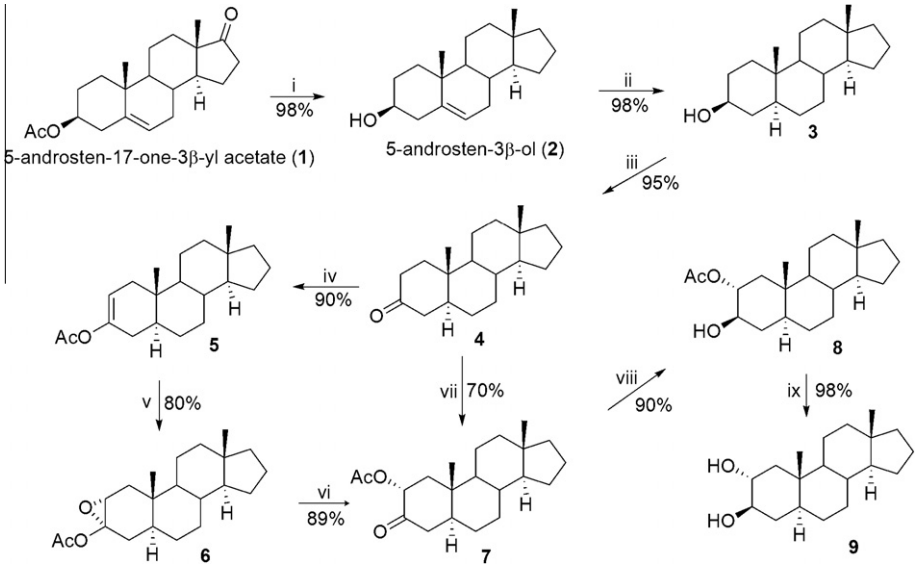
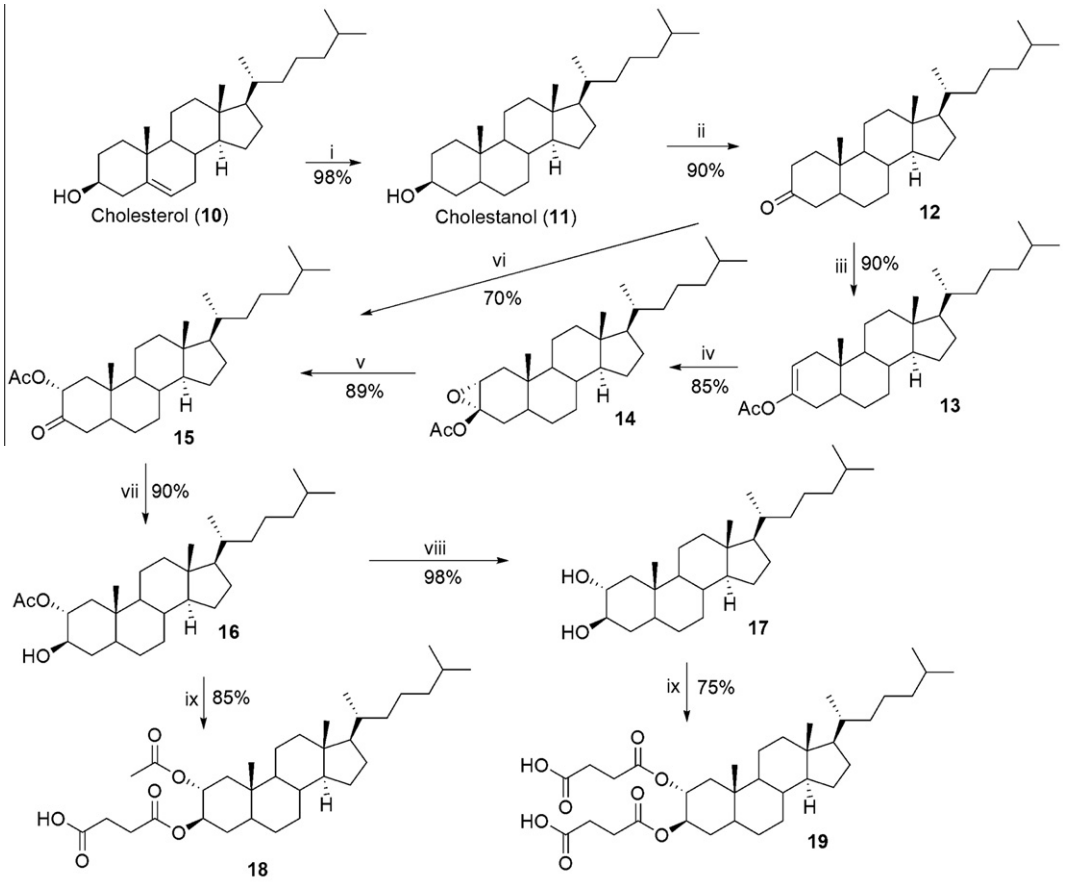


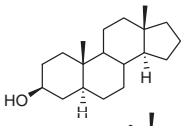
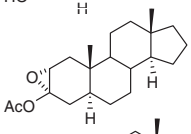
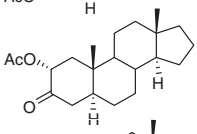
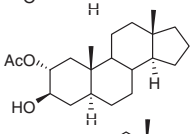
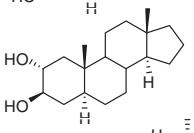
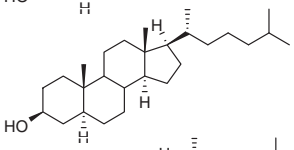
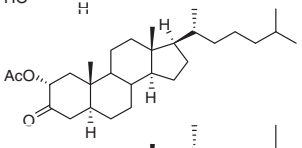
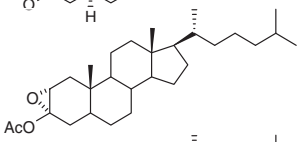
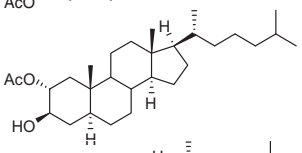
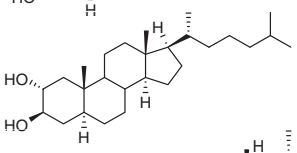
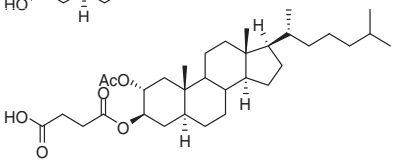
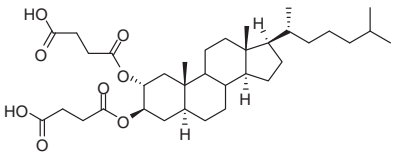
Scheme 1. Outline of general synthesis of 2,3-functionalized androstanes **3**–**9**. Reagents and conditions: (i) NH_2NH_2, 2-ethoxyethanol, KOH; (ii) PtO_2/CH_2Cl_2–CH_3CO_2H; (iii) CrO_3/H_2SO_4; (iv) $(CH_3CO)_2O$/montmorillonite clay; (v) *m*-chloroperbenzoic acid/CH_2Cl_2; (vi) $(CH_3CO)_2/(C_2H_5)_3N$; (vii) $Pb(O_2CCH_3)_4/(CH_3CO)_2O$–$CH_3CO_2H$; (vii) $CeCl_3 \times 7H_2O/CH_3OH$/tetrahydrofuran/$NaBH_4$; (ix) $NaOH/CH_2Cl_2/CH_3OH$, 98%.



Scheme 2. Outline of general synthesis of 2,3-functionalized cholestanes **11**–**19**. Reagents and conditions: (i) PtO_2/CH_2Cl_2–CH_3CO_2H; (ii) CrO_3/H_2SO_4; (iii) $(CH_3CO)_2O$/montmorillonite clay; (iv) *m*-chloroperbenzoic acid/CH_2Cl_2; (v) $(CH_3CO)_2/N(C_2H_5)_3$; (vi) $Pb(O_2CCH_3)_4/(CH_3CO)_2O$–$CH_3CO_2H$; (vii) $CeCl_3 \times 7H_2O/CH_3OH$/tetrahydrofuran/$NaBH_4$; (viii) $NaOH/CH_2Cl_2/CH_3OH$; (ix) succinic anhydride/pyridine.

of the acetate group was performed in one step by applying Reyes and coworkers' elegant steroid carbonyl reduction procedure.[16] This method was used to prepare 5-androsten-3β-ol (**2**, Scheme 1) in large quantities with very high yields. The double bond of **2** was then reduced by platinum dioxide catalyzed hydrogenation, which led to the formation of intermediate alcohol **3** (Scheme 1). The

Table 1
Antifungal activities of novel 2,3-functionalized steroid analogs (MIC in μg/mL)

Compound	Structure	MIC_{25} (μg/mL)[a,f]			
		C. albicans[b]	*C. glabrata*[c]	*C. neoformans*[d]	*A. fumigatus*[e]
3		NC[g]	NC[g]	NC[g]	NC[g]
6		4.0	NC[g]	NC[g]	NC[g]
7		0.125	NC[g]	NC[g]	1.0
8		NC[g]	NC[g]	NC[g]	NC[g]
9		NC[g]	NC[g]	NC[g]	NC[g]
11		NC[g]	NC[g]	NC[g]	NC[g]
14		NC[g]	NC[g]	NC[g]	128
15		128	NC[g]	NC[g]	8.0
16		NC[g]	NC[g]	4.0	NC[g]
17		NC[g]	NC[g]	32	NC[g]
18		NC[g]	NC[g]	8.0	2.0
19		NC[g]	32	NC[g]	8.0

[a] MIC values are reported only for compounds displaying (1) a prominent decrease in turbidity by visual comparison to the control wells containing no antifungal and (2) a >25% reduction in fungal growth compared to controls containing no antifungal, as measured spectroscopically by absorption at 530 nm.
[b] ATCC no. 10231.
[c] ATCC no. 48435.
[d] ATCC no. 36556.
[e] ATCC no. 16424.
[f] All values were determined after incubation at 30–35 °C for 48 h.
[g] Compounds that had (1) a slight reduction in turbidity to no change and (2) had less than a 10% reduction in growth compared to controls, as measured spectroscopically by absorption at 530 nm.

intermediate alcohol **3** was later oxidized by chromic acid by following standard procedures (Scheme 1).[17] From this point, we then branched the synthetic scheme into two different paths; the first involved the direct introduction of an acetyl group into the 2α position of **4** with lead tetraacetate to generate **7** (Scheme 1, step vii); while the second approach generated the same compound **7** using a three-step procedure (Scheme 1, steps iv–vi). The first step of the alternative preparation of 7 is the synthesis of vinyl acetate **5** (Scheme 1, step iv), followed by epoxidation of **5** with MCPBA to yield epoxide **6** (Scheme 1, step v). Finally, epoxide **6** ring opening occurs following treatment with acetic acid to yield the desired compound **7**. Although longer, the alternative route through the preparation of vinyl ester **5** and epoxide **6** has an added advantage with respect to the purification of the product, and this method gave us comparable yields of **7** (Scheme 1). Finally, to generate the 2,3-functionalized steroids, we followed a straightforward $NaBH_4/CeCl_3$ reduction of the carbonyl group of **7**, followed by the acetate ester hydrolysis to produce **8** and **9**, respectively (Scheme 1).

The preparation of 2,3-functionalized cholestane derivatives follows an almost identical synthetic route using cholesterol as the starting material (**10**, Scheme 2). Ketoacetate **15** and ester **16** were prepared by following a previously described procedure for $Pb(OAc)_4$ acetyl group introduction and $NaBH_4/CeCl_3$ keto group reduction.[18] One notable exception is the two additional steps that were taken to prepare succinic acid derivatives **18** and **19** from the corresponding alcohols **16** and **17**, respectively (Scheme 2). These reactions were adapted from the classical alcohol esterification by succinic acid anhydride. The antifungal activity of the synthesized stereoisomers of the 2,3-functionalized steroids evaluated in vitro.

The susceptibility studies and minimal inhibitory concentrations (MIC) values for *C. albicans* (ATCC no. 10231), *C. neoformans* (ATCC no. 36556), *C. glabrata* (ATCC no. 48435), and the filamentous fungus *A. fumigatus* (ATCC no. 16424) were determined by the broth dilution technique in accordance with NCCLS reference documents M27-A.[19] Dilution panels ranged from 0.01 to 128 μg/mL. Master stock concentrations of drugs (soluble in DMSO) were prepared to ensure that the maximum final concentration of DMSO in tested antifungal solutions was 1% or less. Subsequent twofold serial dilutions were made using RPMI 1640 broth or sterile water to a final concentration of 1280–0.1 μg/mL. A final 10-fold dilution of each drug was made by aliquotting 0.1 mL of each dilution to 0.9 mL of inoculating media, giving final drug concentrations tested in the range of 128–0.01 μg/mL. Antifungal drug controls used for these studies were Amphoteracin B and Itraconazole (Sigma–Aldrich). All controls used were diluted to 1.0 μg/mL concentrations, according to the manufacturer's instructions and run in parallel to each in vitro screening of the 2,3-functionalized steroid analogs. The results of these screenings are summarized in Table 1.

This present work describes the synthesis and preliminary antifungal evaluation of 2,3-functionalized androstane and cholestane derivatives, which were prepared by simple and efficient synthetic methods using readily available starting material. Several of the derivatives from the androstane and cholestane groups showed reasonable antifungal activity (as measured spectroscopically by a >25% reduction in fungal growth compared to control wells) against at least one species of fungus. Further, the 2,3-functionalized derivatives can further be used as building blocks for the synthesis of novel fatty-acid esters of steroids with potential antimicrobial activities.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.10.044.

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