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Inhibitory effect of oxygenated cholestan-3β-ol derivatives on the growth of *Mycobacterium tuberculosis*

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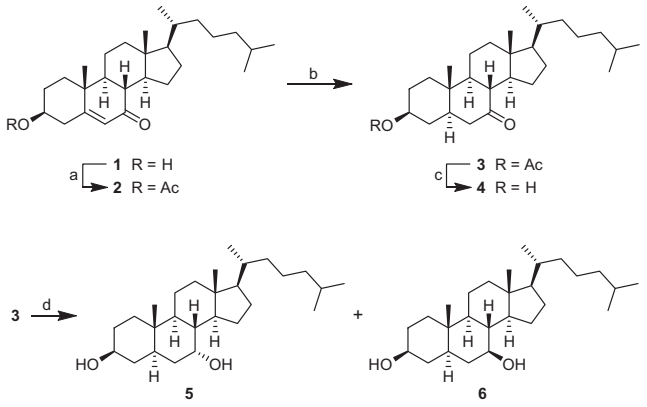
ABSTRACT

A variety of cholestan-3β-ol derivatives, which are oxygenated at different positions of the steroid ring system, were prepared and tested for their inhibition of the *Mycobacterium tuberculosis* $H_{37}Rv$ strain. Several compounds showed significant antitubercular activities with MIC_{90} values in the range 4–8 μM and low or non-detectable toxicity against mammalian cells.

Tuberculosis (TB) remains to represent a major global threat to the human population. For the year 2011, 8.7 million cases of new infections and 1.4 million deaths resulting from TB were estimated.[1] *Mycobacterium tuberculosis* (MTB), the pathogen causing TB, can develop efficient survival strategies and is becoming resistant towards key antibiotics currently in use to treat TB infections. Especially the global spreading of multidrug-resistant TB (MDR-TB) and the growing number of cases reported for extensively drug-resistant TB (XDR-TB) increased this problem dramatically in recent years.[1] Thus, it is an important challenge for medicinal chemistry to identify new lead structures which eventually may lead to more efficacious drugs for the treatment of TB. Over the past decade, an intense research activity, including screening of compound libraries and bioactivity-guided isolation of natural products, was directed towards the development of new potential drugs against TB.[1–6] Investigations of diverse natural sources led to a range of phytosterols and ergosterols which were identified to exhibit significant antitubercular (anti-TB) activities.[7] Previously, we had reported on the inhibition of *M. tuberculosis* by carbazole alkaloids which were obtained by means of total synthesis.[8] In another project, we studied substituted cholestan-3β-ols for their hormonal activity on the nematode *Caenorhabditis elegans*.[9–11] Herein, we describe the anti-TB activity of these synthetic cholestan-3β-ol derivatives which are oxygenated at different positions of the steroidal framework.

Starting from commercially available 7-ketocholesterol (**1**), the 7-oxygenated cholestan-3β-ol derivatives **2**–**6** are readily prepared (Scheme 1).[10] Acetylation of compound **1** afforded 7-ketocholesteryl acetate (**2**), which on catalytic hydrogenation gave 7-ketocholestan-3β-yl acetate (**3**). Cleavage of the ester led to 7-ketocholestan-3β-ol (**4**). Reduction of compound **3** using lithium aluminum hydride provided cholestane-3β,7α-diol (**5**) and cholestane-3β,7β-diol (**6**), which can be separated by chromatography.



Scheme 1. Synthesis of the cholestane-3β,7-diols **5** and **6**. Reagents and conditions: (a) Ac_2O, Et_3N, cat. DMAP, THF, rt, 20 h (99%); (b) 10% Pd/C, H_2, CH_2Cl_2, rt, 24 h (85%); (c) KOH, MeOH, reflux, 48 h (100%); (d) $LiAlH_4$, THF, −78 °C to rt, 24 h (57% **5**, 9% **6**).

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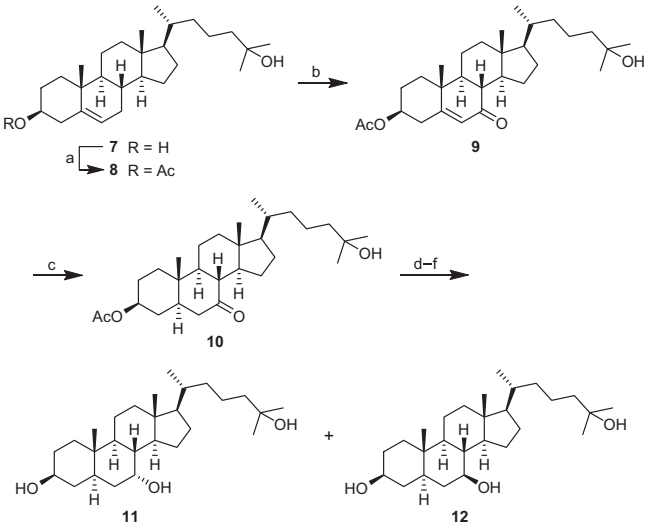
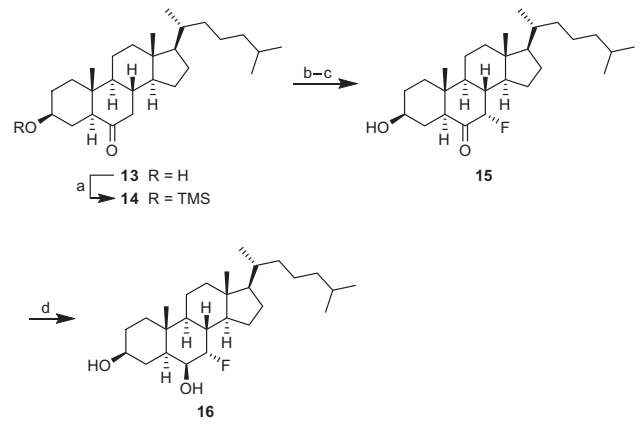
Commercial 25-hydroxycholesterol (**7**) served as starting material for the synthesis of the 7,25-dioxygenated cholestan-3β-ols **11** and **12** (Scheme 2).[10] Acetylation of **7** to compound **8** followed by allylic oxidation using Chandrasekaran's procedure[12] led to 25-hydroxy-7-ketocholesteryl acetate (**9**). Transfer hydrogenation of **9** using ammonium formate as hydrogen source[13] provided 25-hydroxy-7-ketocholestan-3β-yl acetate (**10**) in an improved yield of 90%.[10] Direct reduction of **10** with lithium aluminum hydride afforded an unseparable mixture of the triols **11** and **12**. As the diols **5** and **6** could be separated (see above), the 25-hydroxy group was protected first. Indeed, after protection of the tertiary hydroxy group of compound **10** by silylation and reduction of the 7-keto group with lithium aluminum hydride, the 7α-hydroxy and 7β-hydroxy diastereoisomers could be separated by chromatography. Cleavage of the trimethylsilyl ethers of the individual compounds provided cholestane-3β,7α,25-triol (**11**) and cholestane-3β,7β,25-triol (**12**).

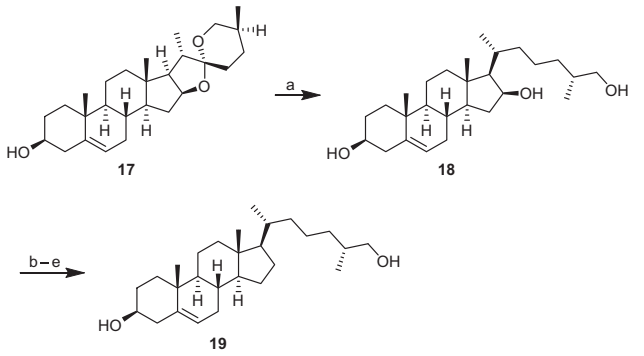
O-Silylation of 6-ketocholestan-3β-ol (**13**) gave compound **14** (Scheme 3).[10] Deprotonation of **14** with lithium diisopropylamide



Scheme 2. Synthesis of the cholestane-3β,7,25-triols **11** and **12**. Reagents and conditions: (a) Ac_2O, Et_3N, cat. DMAP, THF, rt, 20 h (100%); (b) PDC, Celite, *tert*-BuOOH, C_6H_6, 0 °C to rt, 48 h (85%); (c) 10% Pd/C, $HCOONH_4$, MeOH, reflux, 2 h (90%); (d) TMSCl, pyridine, rt, 1 h; (e) $LiAlH_4$, THF, −78 °C to rt, 16 h, separation of the 7α-hydroxy and 7β-hydroxy isomers; (f) 10% HCl, THF, rt, 30 min (63% **11**, 16% **12**, over three steps).



Scheme 3. Synthesis of 7α-fluorocholestane-3β,6β-diol (**16**). Reagents and conditions: (a) TMSCl, DMAP, THF, rt, 1 h (96%); (b) LDA, THF, −78 °C, 3 h, then TMSCl and warming to rt (72%); (c) Selectfluor, DMF, rt, 15 min, then TBAF, THF, rt, 5 min (71%); (d) $NaBH_4$, MeOH, rt, 2 h (100%).



Scheme 4. Synthesis of (25*R*)-cholest-5-ene-3β,26-diol (**19**). Reagents and conditions: (a) Zn dust, 19% HCl, EtOH, reflux, 4 h (85%); (b) TBSCl, DBU, THF, rt, 16 h (85%); (c) MsCl, pyridine, 0 °C to rt, 16 h; (d) $LiAlH_4$, Et_2O, reflux, 4 h (89%, two steps); (e) TBAF, THF, reflux, 20 h (89%).

Table 1
Antituberculosis activities, cytotoxicities, and selectivity indices of the oxygenated cholestan-3β-ol derivatives **1**–**19**

Compound	Ref.[a]	MIC_{90}[b] (μM)	IC_{50}[c] (μM)	SI[d]
1	–	>128	75	<0.6
2	10	>128	>128	–
3	10	>128	>128	–
4	10	8.0	62	7.8
5	10	>128	n.d.	–
6	10	>128	n.d.	–
7	–	>128	>128	–
8	10	>128	n.d.	–
9	10	>128	>128	–
10	10	>128	>128	–
11	10	>128	n.d.	–
12	10	24	>128	>5.3
13	–	6.0	92	15.3
14	10	3.5	38	10.9
15	10	4.6	40	8.7
16	10	>128	41	<0.4
17	–	>128	n.d.	–
18	11	6.0	>128	>21
19	11	>128	n.d.	–
INH[e]	–	0.4	>128	>320
RMP[e]	–	0.07	108	1543

[a] Reference for the synthesis.
[b] Minimum inhibitory concentration (90% growth inhibition) against MTB $H_{37}Rv$ in MABA assay.
[c] Concentration effecting a 50% decrease in tetrazolium dye reduction by Vero cells (African green monkey kidney cells). Both values are means of three replicate experiments; for experiments that gave a value higher than the maximum concentration used, >128 is denoted; n.d. = not determined.
[d] Selectivity index: $SI = IC_{50}/MIC_{90}$.
[e] INH = isoniazid and RMP = rifampicin (rifampin) used as positive controls; solvent used as negative control.

(LDA) followed by addition of trimethylsilyl chloride afforded the trimethylsilyl 6,7-enol ether, which on treatment with Selectfluor and subsequent desilylation provided 7α-fluoro-6-ketocholestan-3β-ol (**15**). Reduction of **15** using sodium borohydride stereoselectively afforded 7α-fluorocholestane-3β,6β-diol (**16**).

Clemmensen reduction of commercial diosgenin (**17**) afforded (25*R*)-cholest-5-ene-3β,16β,26-triol (**18**) (Scheme 4).[11,14] Silyl protection of the two hydroxy groups at C3 and C26, followed by mesylation of the hydroxy group at C16, removal of the mesylate by reduction with lithium aluminum hydride, and double desilylation led to (25*R*)-cholest-5-ene-3β,26-diol (**19**).

The oxygenated cholestan-3β-ols **1**–**19** described above were investigated for their potential anti-TB activity and several hits could be identified (Table 1). The minimum inhibitory concentrations (MIC_{90} values) against the MTB strain $H_{37}Rv$ were obtained

by the microplate alamar blue assay (MABA).[2,15] In vitro cytotoxicities (IC_{50} values) were determined using Vero cells.[2,16] The results of our initial screening demonstrate that cholestan-3β-ol derivatives which are oxygenated at specific positions of the steroidal framework exhibit a significant anti-TB activity. The following conclusions could be drawn from our initial screening of oxygenated cholestan-3β-ols. Among the 7-oxygenated cholestan-3β-ols shown in Schemes 1 and 2, only 7-ketocholestan-3β-ol (**4**) and cholestane-3β,7β,25-triol (**12**) showed anti-TB activity. Comparing compounds **5** and **6**, the orientation of the OH group at C-7 is not important as both are inactive; whereas in the presence of an OH group at C-25, the configutaion of OH at C-7 matters as compound **12** shows activity >5-fold than **11**. The 6-ketocholestan-3β-ols **13**–**15** exhibit substantial anti-TB activities with MIC_{90} values ranging from 3.5 to 6 μM. The trimethylsilyl ether **14** was presumably hydrolyzed to the corresponding alcohol **13** under the conditions of the assay. Compounds **13**–**16** show some level of cytotoxicity. A significant anti-TB activity (MIC_{90} = 6 μM) with no toxicity for the mammalian cell line has been found for (25*R*)-cholest-5-ene-3β,16β,26-triol (**18**). However, for the diol **19**, resulting from compound **18** by selective removal of the 16-hydroxy group, no anti-TB activity has been detected.

In summary, several oxygenated cholestan-3β-ols show significant inhibitory activity against MTB $H_{37}Rv$ with no or relatively low toxicity against the mammalian cell line (see compounds **4**, **13**, and **18**). Additional structural modification of cholestane derivatives directed towards improvement of their anti-TB potency may perhaps pave the way to find new lead structures for potential anti-TB drugs.

Acknowledgement

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