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Original article

Stereoselective synthesis and antimicrobial activity of steroidal C-20 tertiary alcohols with thiazole/pyridine side chain

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ABSTRACT

Stereoselective synthesis of novel steroidal C-20 tertiary alcohols with thiazole and pyridine side chain using Grignard reaction of steroidal ketones and thiazole/pyridine magnesium bromide have been realized. These molecules were evaluated *in vitro* for their antifungal and antibacterial activities. Most of the compounds exhibited significant antifungal and antibacterial activity against all the tested strains.

1. Introduction

It is well known that steroids play an important biological role. They represent constituents of biomembranes and hormones, fulfil protective functions, stimulate plant growth, etc. Many representatives of this group are widely used in medicine as essentials of anti-inflammatory, anabolic and contraceptive drugs. Steroids isolated from various marine organisms (marine steroids) manifest diverse biological activities. Some of them are extremely toxic against tumour cells and show antimicrobial and other effects. It is therefore not surprising that marine steroids arouse considerable interest in not only chemists, but also pharmacologists and physicians. Steroids form a group of structurally related compounds that are widely distributed in animals and plants. Medicinal chemistry of steroids covers a large and interesting series of structures and biological activities [1–3].

Heterocyclic analogues of steroids, in particular the aza-, oxa-, and thia-analogues, are of interest for the study of structure activity relationship [4]. They have an important practical value in the development of novel pharmacological agents for regulating and maintaining biochemical homeostasis in man and domestic animals. Attention to steroid derivatives of oxazole is caused by the fact that these compounds proved to be interesting from the biological activity point of view and moreover are convenient intermediates in the synthesis of numerous polyfunctional compounds [4]. The thiazoles, furans, and thiophenes appended to the steroid nucleus were positioned on the α-face and the β-face of the steroid, and the conjugated with a 16,17-olefin, to test their ability to coordinate the heme iron of the P450 enzyme complex. The position of the heterocycle with respect to the steroid skeleton was determined to be important for optimum affinity, and in general, compounds with the heterocycle attached to a trigonal centre at C-17, had the best affinity for $C_{17(20)}$ lyase [5].

The investigation of new modified steroid derivatives condensed with various heterocyclic rings has been given great attention [6,7]. The addition of heterocyclic rings to steroids often leads to a change of their physiological activity and the appearance of new interesting pharmacological and biological properties [8], especially anti-inflammatory [9], antineoplastic [10] and antiandrogenic activity [11].

The treatment of infectious diseases still remains an important and challenging problem. Despite search of novel antimicrobial agents is fields of current and growing interest and many

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compounds have been synthesized to this aim. Their clinical use has been limited by their relatively high risk of toxicity, bacterial resistance and/or pharmacokinetic deficiencies. A major research emphasis to counter this growing problem is the development of antimicrobials structurally unrelated to the existing molecules. One possibility to achieve this goal is the combination of a steroid molecule with structural elements possessing appropriate biological activities [12–14]. The advantage of employing hydrophobic steroid units is their ability to interact with cell membranes and thus pave the way for biological activity of such hybrid molecules. Attention has been devoted in the literature to the synthesis of several steroidal heterocycles that exhibit valuable pharmacological activities [15–17].

There are several reports [18–26] on the synthesis and bioevaluation of steroidal heterocycles particularly pyridine, pyrimidine, imidazole, pyrazole, substituted at C-17 position of steroid backbone. Substituted thiazoles are often found in natural products as well as synthetic pharmaceuticals or agrochemicals with interesting biological activity [27–33].

Magaraci and coworkers described [34] the synthesis of some novel azasterols based on (20*R*,22ξ)-5α-pregnan-20-(piperidin-2-yl)-3β,20-diol, and are potential inhibitors of the enzyme sterol 24-methyltransferase (24-SMT), a vital enzyme in the biosynthesis of ergosterol and related 24-alkyl sterols.

The reaction of Grignard and other organometallic reagents with 20-ketones has been utilized by a number of investigators to construct the side chain in one- or in multistep sequences [35]. In these reactions, chiral centre at C-20 is created with mixture of epimers, the ratio of epimers depending greatly upon the structure of the steroids, particularly the nature of substituents near C-20 and the bulkiness of the reagent [35].

In continuation of our work [36–41] on the synthesis and bioevaluation of various steroid derivatives, herein, we report a highly stereoselective synthesis of steroidal C-20 tertiary alcohols with thiazole and pyridine side chain and their antimicrobial activity.

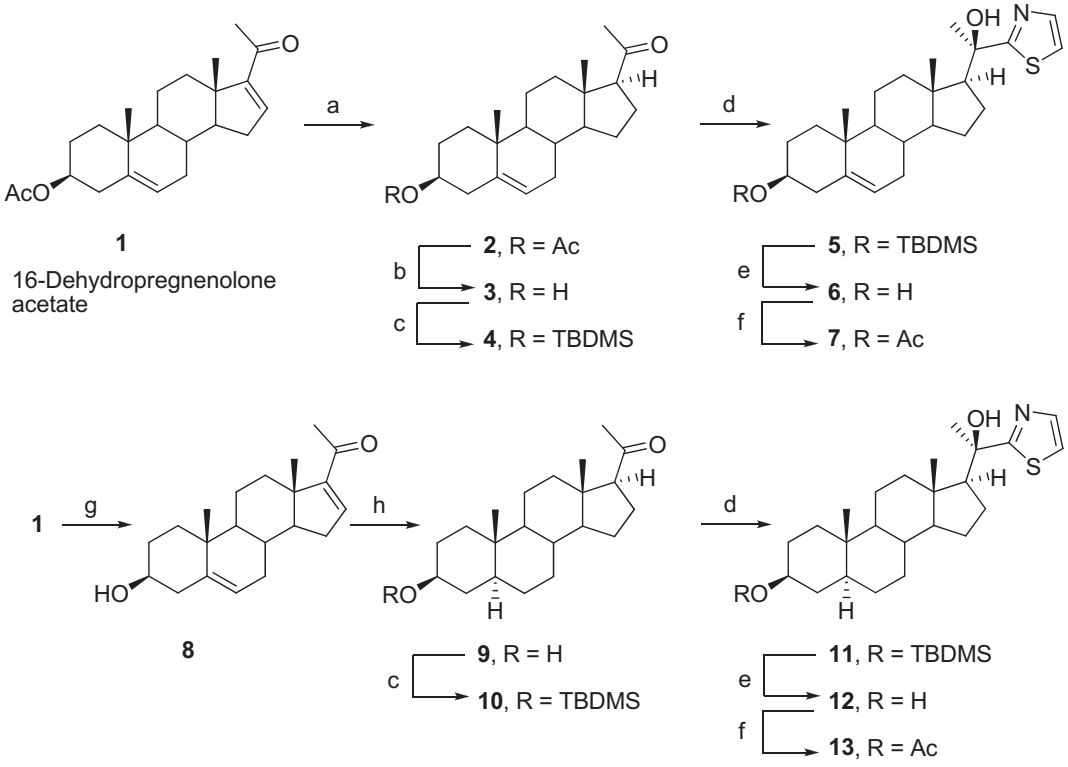
2. Results and discussion

2.1. Synthesis of steroidal C-20 tertiary alcohols with thiazole side chain

Commercially available 16-dehydropregnenolone acetate (**1**) [42], on chemoselective catalytic hydrogenation with 10% palladium on charcoal in ethyl acetate, hydrolysis of acetate **2** with potassium hydroxide in aqueous methanol, followed by protection of 3β-hydroxy group in compound **3** with *tert*-butyldimethylsilyl chloride (TBDMSCl) in the presence of imidazole in *N*,*N*-dimethylformamide (DMF) afforded [43,44] compound **4** with an overall yield of 87% in three steps (Scheme 1).

The synthesis of compound **10** was achieved from 16-dehydropregnenolone acetate **1**, which on treatment with KOH in *t*-butanol, yielded the 3β-hydroxy compound **8** in 96% yield. 3β-Hydroxy compound **8** on catalytic hydrogenation with 10% palladium on charcoal in ethanol afforded 5,6,16,17-tetrahydro 20-ketone **9** in 99% yield. The 3β-hydroxy group of **9** was transformed [45,46] to its TBDMS derivative **10** in excellent yield. The stereochemistry at C-5 and C-17 in compound **10** has been already reported by single X-ray crystal structure [46].

Grignard reaction of steroidal ketones **4** and **10** with thiazole-2-magnesium bromide [47] (prepared *in situ* from 2-bromothiazole and ethyl magnesium bromide) lead to C(20*R*) tertiary alcohols **5** and **11** in 98% and 96% yields respectively (Scheme 1). Literature survey reveals that, addition of Grignard reagents on steroidal C-20 ketones resulted into the one major isomer [48]. It can be mentioned here that, the products **5** and **11** are stable, can be purified by column chromatography over silica gel in excellent yield. The C(20*R*) tertiary



Scheme 1. Reagents and conditions: (a) 10% Pd/C, H_2, EtOAc, 45 psi, 30 °C, 12 h, 98%; (b) KOH, MeOH, H_2O, 30 °C, 2 h, 97%; (c) TBDMSCl, imidazole, DMF, 30 °C, 10 h, **4** (92%) and **10** (97%); (d) thiazole-2-magnesium bromide, THF, rt, 2 h, **5** (98%) and **11** (96%); (e) *n*-Bu_4NF, THF, 30 °C, 18 h, **6** (94%) and **12** (95%); (f) Ac_2O, Pyridine, DMAP, 25 °C, 2 h, **7** (95%) and **13** (94%); (g) KOH, *t*-BuOH, H_2O, 30 °C, 10 h, 96%; (h) 10% Pd/C, H_2, EtOH, 55 psi, 30 °C, 12 h, 99%.