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Research Paper

Antimicrobial activity of naturally occurring 20-epi cholanic acid derivatives

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Abstract: Naturally occurring synthesized 20-*epi* cholanic acid derivatives were evaluated *in vitro* for their antifungal and antibacterial activities. The compounds generally showed good to moderate antifungal and antibacterial activity against all the tested fungal and bacterial strains. This is the first report of screening the biological activity of naturally occurring 20-*epi* cholanic acid derivatives.

Introduction

The total synthesis of complex natural products remains one of the most fascinating and challenging endeavors in organic chemistry. The total synthesis of any steroid is an exacting intellectual and technical challenge that has been met with an extraordinary ingenuity and originality by several chemists. The number of novel steroids from marine sources is increasing dramatically due to new and more discriminating chromatographic and spectral techniques as well as a greater appreciation of older methods. In addition to novel structures, marine species have recently been shown to contain complex mixture of sterols.

Marine natural products have been the subject of chemical and pharmacological interest for several decades now and have established themselves as a diverse group of biomedically important compounds.[1] It is well known that steroids play an important biological role. They represent constituents of biomembranes and hormones, fulfill 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 tumor

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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.[2] The chemistry and biochemistry of these natural products were extensively studied and utilized in the development of various drugs, especially for hormonal imbalance, for the treatment of infections and cancer as well as inflammation.

The isolation, biological evaluation and synthesis of many important steroids with modified side chains, such as ecdysones,[3] metabolite of vitamin D_3,[4] brassinosteroids,[5] squalamine,[6] OSW-1,[7] contignasterol[8] and marine sterols[9] have stimulated much interest. Certonardosterol D_2, a polyhydroxysterol isolated from starfish *Certonardoa semiregularis* with exceptionally potent antitumor activity.[10]

Compounds with unnatural configuration at C-20 have attracted attention because of the interesting biological activities of these epimers.[11] It was reported that 20-isocholesterol (20-epicholestrol) **1** (Figure 1) with C(20*S*) unnatural stereochemistry showed significant *in vitro* inhibitory activity for the conversion of cholesterol to pregnenolone.[12] The 20-*epi* analogue of the metabolite of vitamin D_3 (calcitriol) **2** is more potent in regulating cell growth and cell differentiation than the corresponding compound with natural C-20 stereoisomer.[13] It is also interesting to mention that, the 20-*epi* analogue **2** exhibits immunosupressive properties[14] and that of 1α-fluoro-16,23-diene-20-*epi* hybrid deltanoid (Ro 26-9228) **3** is in human clinical trials for the treatment of osteoporosis.[15]

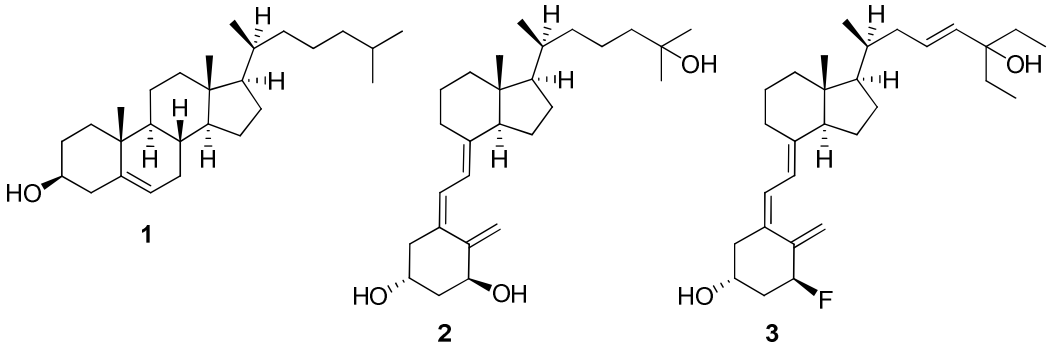

Figure 1. Isocholesterol (20-epicholesterol) **1**, vitamin D_3 **2** and deltanoid (Ro 26-9228) **3**.

Vanderah and Djerassi have isolated[16] four 20-*epi* cholanic acid derivatives **4-7** with unnatural configuration at C-20 from the marine invertebrate a sea pen, *Ptilosarcus guerneyi* (Figure 2).

Tomono and coworkers reported the isolation of four unknown steroids from an octocoral *Dendronephthya sp.* of the order Alcyonacea.[17] These compounds showed no antifouling activity against barnacle (*Balanus amphitrite*) larvae, but, instead, lethality to the barnacle larvae at a concentration of 100 μg/mL (LD_{100}). It was identified one of the new compound as methyl 3-oxochola-1,4,22-trien-24-oate without specifying the configuration at C(20). The synthesis of the same compound

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starting from the (20*S*)-3-oxo-23,24-dinorchol-4-en-22-al, both (20*S*) and (20*R*) diastereoisomeric methyl esters by a three-step procedure was achieved by Kreiser and coworkers.[18] Only the analytical data of the (20*S*)-compound was in good agreement with those reported for the natural product. Yu and coworkers reported the synthesis and antitumor activity of A,B-ring-truncated *epi*-OSW saponin analog against the growth of *HeLa* and *Jurkat T* cells (IC_{50} =0.8 and 21.1 μM respectively).[19]

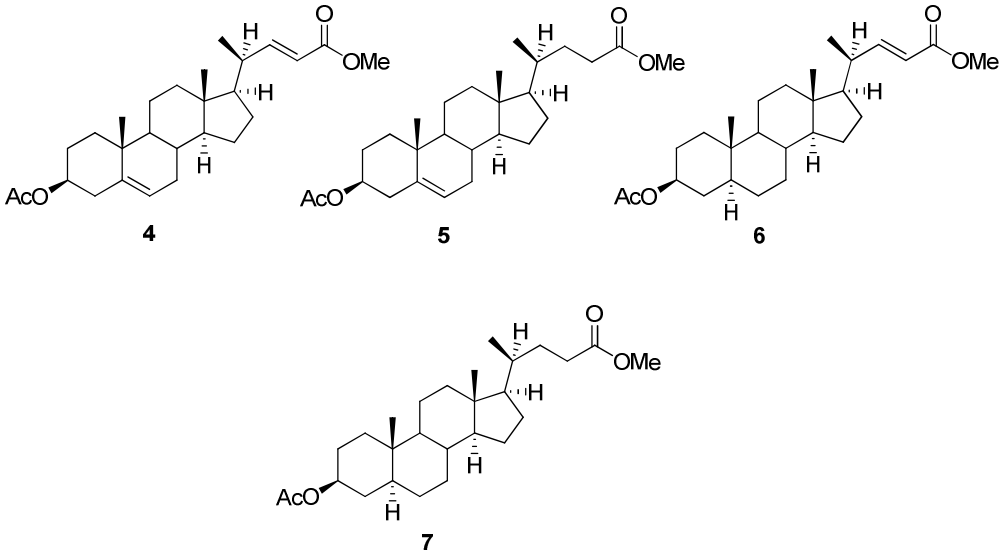

Figure 2. 20-*epi* cholanic acid derivatives **4-7**.

Vanderah and Djerassi have synthesized these 20-*epi* cholanic acid derivatives **4-7** (Figure 2) with unnatural configuration at C-20.[20] Again, syntheses of the 20-*epi* cholanic acid derivatives **4**, **5** and **7** have been carried out by Takano[21] and Dauben.[22] We have already reported the syntheses of the naturally occurring 20-*epi* cholanic acid derivatives **4-7** in less number of steps and high yields, starting from commercially available 16-dehydropregnenolone acetate *via* steroidal C(20*R*) aldehyde.[23] Till date, biological activity of these naturally occurring 20-*epi* cholanic acid derivatives **4-7** has not been reported. In continuation of our work[24] on bioevaluation of various steroid derivatives, herein we would like to report antimicrobial activity of naturally occurring synthesized 20-*epi* cholanic acid derivatives with various fungal as well as bacterial strains.

Antimicrobial activity of 20-epi cholanic acid derivatives 4-7

The synthesized compounds **4-7** were tested in *vitro* for antifungal and antibacterial activity. The antifungal activity was tested using NCL isolate fungal strains *Candida albicans*, *Cryptococcus neoformans* (human pathogen), *Benjaminiella poitrasii*, *Yarrowia lipolytica* (saprophytes) and *Fusarium oxysporum* (plant pathogen). Most of the pathogen fungi viz *C. albicans* are dimorphic in nature. However, their use as

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model faces a number of problems of slow growth rate and difficulties in getting synchronous growth.[25] Therefore non pathogenic dimorphic fungus *B. poitrasii* was used as a model which exhibits a rapid and simple one-step process of yeast-mycelium transition in response to temperature and/or glucose change.[26]

Table 1. In *vitro* antimicrobial activity of compounds **4-7**.

Compound	Minimum Inhibitory concentration (MIC)[a] (μg/mL)						
	Fungal Strains					Bacterial Strains	
	CA	CN	BP	YL	FO	EC	SA
4	>64	>64	32	>64	64	32	32
5	>64	>64	8	>64	>64	32	16
6	16	>64	16	>64	>64	16	32
7	>64	>64	16	>64	64	16	16
Ampho. B	2	16	16	16	16	-	-
Fluconazole	32	32	32	64	8	-	-
Tetracycline	-	-	-	-	-	8	16
Erythromycin	-	-	-	-	-	>64	32

CA**,** *Candida albicans* (NCL1); CN**,** *Cryptococcus neoformans* (NCL2); BP**,** *Benjaminiella poitrasii* (NCL3); YL**,** *Yarrowia lipolytica* (NCL4); FO**,** *Fusarium oxysporum* (NCL5); EC**,** *Escherichia coli* (NCIM No.2574); SA**,** *Staphylococcus aureus* (NCIM No.2122).

[a]MIC (Minimum inhibitory concentration) was determined as 90% inhibition of growth with respect to the growth control.

Negative control, DMSO and THF (50:50), No inhibition.

All the naturally occurring synthesized 20-*epi* cholanic acid derivatives **4-7** were tested in *vitro* for antifungal and antibacterial activity (Table 1). Most of the compounds **4-7** showed good to moderate antifungal and antibacterial activity against all the tested fungal and bacterial strains. The activity of compound **6** was better or comparable to that of fluconazole against *C. albicans* with MIC value of 16 μg/mL, however all the derivatives proved inactive against *C. neoformans*. In addition to this, all compounds were more potent with MIC value of 8-32 μg/mL than the reference drug amphotericin B and fluconazole against *B. poitrasii*. In particular, compound **5** showed better activity against *B. poitrasii* with MIC value of 8 μg/mL. *Y. lipolytica* and *F.*

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oxysporum were adversely affected by all the compounds. From table 1, it has been observed that, the presence or absence of double bond in a ring or side chain, did not affect the activity of the compounds against all fungal strains, except against *B. poitrasii*. Furthermore, all the synthesized compounds showed moderate antibacterial activity against *E. coli* having MIC value of 16 μg/mL. In addition to this, 20-*epi* cholanic acid derivatives **4**-**7** showed good to moderate antibacterial activity against *S. aureus* with MIC value of 16-32 μg/mL. In particular, compounds **5** and **7**, with saturated side chain showed good antibacterial activity with MIC value of 16 μg/mL against *S. aureus*.

Experimental

Antimicrobial Activity: Materials and Methods

Candida albicans, Cryptococcus neoformans (human pathogen), *Benjaminiella poitrasii* and *Yarrowia lipolytica* (non pathogen) strains were maintained on YPG (yeast extract, 0.3%, peptone, 0.5%, and glucose, 1%) agar slants. *Fusarium oxysporum* (plant pathogen) strain was maintained on PDA (potato, 20% dextrose, 2%) agar slants at 28 ^{o}C. *Escherichia coli* (NCIM No. 2574) and *Staphylococcus aureus* (NCIM No. 2122) were maintained on NA (beef extract, 0.3%, peptone, 0.5%, sodium chloride, 0.5%) slants. Strains of *C. albicans, C. neoformans, Y. lipolytica* were inoculated in YPG broth at 28 ^{o}C and *B. poitrasii* at 37 ^{o}C for 24 h respectively, *F. oxysporum* in potato dextrose at 28 ^{o}C for 48 h whereas bacterial strains *E. coli* and *S. aureus* in NA broth for 24 h. Compounds **4**-**7** were solubilized in DMSO and THF (50:50), and stock solutions of 1.28 mg/mL were prepared. Amphotericin B, Fluconazole, Tetracycline and Erythromycin were also dissolved in DMSO and THF (50:50), and were used as a positive control.

MIC determination

In *vitro* antifungal and antibacterial activity of newly synthesized compounds were studied against fungal strains viz., *C. albicans, C. neoformans, B. poitrasii, Y. lipolytica, F. oxysporum* and bacterial strains *E. coli* (NCIM No. 2574), and *S. aureus* (NCIM No. 2122) respectively to find out MIC (Minimum Inhibitory Concentration). All the experiments were done in triplicate under similar experimental conditions. MIC of the synthesized compounds was determined according to standard broth microdilution technique as per NCCLS guidelines.[27] Testing was performed in U bottom 96 well tissue culture plates in YPG, PDA for fungal strains and NA for bacterial strains. The concentration range of tested compounds and standard was 64-0.5 μg/mL. The plates were incubated at 28 °C for all the microorganisms except for *B. poitrasii* at 37 °C, absorbance at 600 nm was recorded to assess the inhibition of cell growth after 24 h for *B. poitrasii* and *Y. lipolytica*, 48 h for *C. albicans* and *F. oxysporum*, 72 h for *C. neoformans* and 24 h for bacterial cultures. MIC was determined as 90% inhibition of growth with respect to the growth control was observed.

Conclusions

We have screened naturally occurring synthesized 20-epi cholanic acid derivatives **4**-**7** and generally showed good to moderate antifungal and antibacterial activity against all the tested fungal and bacterial strains. This is the first report of screening the biological activity of naturally occurring 20-*epi* cholanic acid derivatives **4**-**7**.

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