Synthesis and Evaluation of Demethoxyviridin Derivatives as Potential Antimicrobials

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The *in vitro* antibacterial and antifungal activities of demethoxyviridin and some synthetic analogues were evaluated by the agar diffusion method. The minimum inhibitory concentrations (MIC) of the active compounds were also determined by the agar dilution method. Demethoxyviridin (**1**) showed moderate antibacterial activity against most of the strains tested. 1α-Hydroxydemethoxyviridin (**3**) showed antibacterial activity and the most potent *in vitro* antifungal activity with MIC of 20 μg/ml (0.062 mM) against *Aspergillus niger*, *A. fumigatus*, *A. flavus*, *A. parasiticus*, *Fusarium solani*, *F. graminarum*, *Geotrichum candidum* whereas 5′-methylfuro-(4′,3′,2′-4,5,6)androst-5-ene-3,17-dione (**7**) exhibited very weak antifungal activity against *Candida albicans* only.

Key words: Demethoxyviridin, *Nodulisporium hinnuleum*, Antimicrobial Activity, MIC

Introduction

Demethoxyviridin belongs to a group of steroidal antibiotics which possess a modified androstane carbon skeleton with a fused furan ring between rings A and B of a steroid framework. They are systematically named as furanosteroids and produced by various fungi. Demethoxyviridin and its corresponding 3-alcohol demethoxyviridiol are produced by *Nodulisporium hinnuleum* ACC3199 and ACC24911 as well as *Apiospora camptospora* (Cole *et al.*, 1975; Hanson, 1995). They have attracted interest as potential candidates for biological activity due to their involvement in the inhibition of some specific steps in the cell signalling process (Cutler *et al.*, 1997). Demethoxyviridin and wortmannin have been shown to inhibit *N*-formyl-Met-Leu-Phe-stimulated superoxide production, phospholipase D activation in the human neutrophil and to block phosphatidylinositol 4,5-diphosphate phospholipase C (Dewald *et al.*, 1988; Wymann *et al.*, 1990; Bonser *et al.*, 1991; Downey *et al.*, 1996). Demethoxyviridiol showed antifungal and anti-inflammatory activities againts plant pathogens such as *Rhizoctonia solani* and *Pythium ultimum* but it did not exhibit significant antibacterial activity. It also exhibits both plant-growth regulating and phytotoxic effects in plant systems. Demethoxyviridin was discovered to be a specific inhibitor of phosphatidyl-inositol 3-kinase (PI 3-kinase) activity in intact cells at nanomolar concentrations (Woscholski *et al.*, 1994). However, it was later shown that it is not as completely specific or selective an inhibitor of PI 3-kinase as originally thought (Cross *et al.*, 1995).

The aim of the present study was to synthesize demethoxyviridin and some analogues and carry out a comparative evaluation of their antimicrobial activities against some fungi and Gram-positive and Gram-negative bacteria in the context of defining the importance of the furan ring and carbonyl groups.

Materials and Methods

General experimental procedures

Melting point data were determined on a Gallenkamp MPD350 melting point apparatus and are uncorrected. IR spectra were recorded using KBr pellets with a Perkin-Elmer 1910 infrared spectrophotometer. FABMS was recorded on a VG Autospec Fisons instrument. ^{1}H NMR and ^{13}C NMR spectra were determined by a Bruker DPX 300 instrument while DEPT spectra were measured at 75 MHz in deuteriochloroform with tetramethylsi-

lane as an internal standard. The solvents used for purification were distilled prior to use. Silica gel type 60 (Merck, 230–400 mesh) was used for column chromatography. Thin-layer chromatography (TLC) was carried out on a 0.25 mm thick silica gel plate (Merck silica gel 60 GF_{254}) in EtOAc/petroleum ether (60–80 °C) (1:1, v/v). Compounds were detected by spraying with methanol/sulfuric acid (1:1, v/v) followed by heating with a hot gun.

Microbial strains

Bacterial and yeast cultures used in the present study were obtained from USA Agriculture Research Service Culture Collection (NRRL), American Type Culture Collection (ATCC), Microbiology Department of Osmangazi University, and Biology Department and Pharmacy Faculty of Anadolu University, Eskişehir, Turkey.

The bacterial strains were *Bacillus cereus* NRRL B-3711, *B. mycoides* NRRL B-4379, *B. subtilis* NRRL B-209, *Micrococcus luteus* NRRL B-1018, *Staphylococcus aureus* ATCC 25923, *S. epidermidis* NRRL B-4268, *Streptococcus faecium* NRRL B-3502, *Escherichia coli* ATCC 25922, *Enterobacter aerogenes* NRRL B-3567, *Pseudomonas aeruginosa* ATCC 10145, *Klebsiella pneumoniae*, and *Yersinia enterocolitica.* Fungi used were *Fusarium graminarum* (wild type), *F. solani* (wild type), *Geotrichum candidum* (wild type), *Nodulisporium hinnuleum* IMI 214826, *Cephalosporium aphidicola* IMI 68689, *Candida albicans* NRRL Y-12983, *Aspergillus niger* ATCC 10549, *A. flavus* ATCC 9807, *A. fumigatus* NRRL 163, and *A. parasiticus* NRRL 465.

Bacterial and fungal cultures of test organisms were maintained on nutrient agar (Merck) and malt extract agar (Merck) slants at 4 °C, respectively, and were subcultured in petri dishes prior to use. Each combination of microorganisms and antibiotics was repeated three times.

In vitro antimicrobial activity

The agar diffusion method described below was used for determining the antimicrobial activity using penicillin G (Sigma), tetracycline (Sigma) and cephataxime (Fluka) as standard antibacterial agents and amphotericin B (Sigma) as a standard antifungal agent.

Agar diffusion method

Mueller Hinton agar medium (MHA) (Fluka) and Sabouraud 4% glucose medium (SGM) (Fluka) were poured in petri dishes to give a uniform depth of approx. 4 mm and were allowed to cool to room temperature after autoclaving. Test bacteria were transfered to tubes containing 4–5 ml of Mueller Hinton Broth (MHB) (Merck). The cultures were incubated at 35–37 °C until they were visibly turbid. The density of these cultures was adjusted to a turbidity equivalent to that of the 0.5 McFarland standard used to standardize the inoculum density (at 625 nm, 0.08–0.01 absorbance) with sterile saline. The bacterial cultures adjusted to this standard contained approx. 10^8 CFUs/ml. On the other hand, to induce spore formation, the molds were grown on potato dextrose agar (Merck) slants at 27 °C for 5 to 7 d. Spor concentration was adjusted to 10^6 CFU/ml with steril 0.1% tween 80 (Merck) for each mold. The density of the yeast culture was adjusted to that of the 0.5 McFarland standard with sterile saline and then diluted to 10^7 CFU/ml. The entire surface of the MHA and SGM plates was inoculated by streaking with a sterile swab dipped into the adjusted suspensions. The paper discs (Schleicher & Schuell, Ø 6 mm) impregnated with 10 μl of the test compounds [10 mg/ml in dimethyl sulphoxide (DMSO; Merck)] were placed on the surface of the agar plate inoculated. The plates were preincubated for 1 h at room temperature and incubated at 35 °C. After 16 to 18 h of incubation, each plate was examined and the diameters of the zones of complete inhibition were measured, including the diameter of the disc (NCCLS, 1990a).

Determination of minimum inhibitory concentration (MIC)

The agar dilution method was used to determine the minimum inhibitiory concentration (MIC) of the synthesized compounds. Compounds were incorporated into the agar medium, with each plate containing a different concentration (20 to 640 μg/ml) of the agent. The compound solutions were prepared by dissolving appropriate amounts in DMSO. Meanwhile, one control without the tested compounds and another with DMSO were prepared. The bacterial cultures containing approx. 10^8 CFUs/ml were diluted 1:10 to obtain an inoculum concentration of 10^7 CFUs/ml and then 2 μl were inoculated on an agar surface area of 5 to

8 mm by the use of standardized loops. After the spots were dried the plates were inverted and incubated at 35 °C (NCCLS, 1990b, 2002). The molds and yeast suspensions whose density was 10^7 CFUs/ml were inoculated on agar surface in the same way. The MIC was recorded as the lowest concentration of antimicrobial agent that completely inhibited growth.

*Biosynthesis of demethoxyviridin (**1**)*

Nodulisporium hinnuleum was grown on a medium composed of glucose (50 g), tartaric acid (4 g), potassium carbonate sesquihydrate (600 mg), ammonium dihydrogen orthophosphate (600 mg), magnesium carbonate (400 mg), ammonium sulfate (250 mg), zinc sulfate heptahydrate (100 mg) and ferrous sulfate heptahydrate (100 mg) in pure water (1 l) in twelve Roux bottles containing 150 ml of the medium mentioned above under sterile conditions. Growth was allowed to proceed for 35 d at 21 °C and *N. hinnuleum* was harvested by filtering off the mycelium which was dried over a Buchner funnel under suction. The broth (1.5 l) was discarded and the mycelium (210 g) was extracted in a Soxhlet funnel with chloroform (800 ml) for 5 h. The extracts were concentrated *in vacuo* to yield a dark gum (900 mg), which was triturated with petroleum ether (200 ml) and filtered. The solid product was dissolved in hot acetone (500 ml) and treated with decolouring charcoal (3 g), filtered and concentrated *in vacuo* to yield crude demethoxyviridin (**1**). Recrystallization from acetone yielded **1** (100 mg) as needles, m.p. 143–152 °C [lit. (Hanson *et al.*, 1985) 145–160 °C]. – IR (nujol): ν_{max} = 3416 (OH), 1704 (C=O), 1676 (C=O), 1583 cm^{-1} (C=C). – ^1H NMR ($CDCl_3$): δ = 1.60 (3H, singlet, 19-Me), 3.37 (2H, singlet, H_2O cry.), 4.38 (1H, multiplet, 1-H), 6.20 (1H, doublet, J = 5 Hz, 1-H), 7.92 (1H, doublet, J = 8 Hz, 11-H), 8.62 (1-H, doublet, J = 8 Hz, 12-H), and 8.86 (1H, singlet, 21-H). – EIMS: m/z = 322 [M^+] ($C_{19}H_{14}O_5$).

*Dehydroxydemethoxyviridin-1-ene (**2**)*

Demethoxyviridin (**1**) (100 mg) in pyridine (5 ml) was treated dropwise with methanesulfonyl chloride (1.5 ml) at room temperature over 10 min. The mixture was stirred for 4 h, and was poured into dilute hydrochloric acid and the steroid was recovered in ethyl acetate. The organic phase was washed with water and dried over sodium sulfate. The solvent was evaporated *in vacuo* and the residue was recrystallized from acetone to give dehydroxydemethoxyviridin-1-ene (**2**) (75 mg, 70%), m.p. 228–231 °C [lit. (Hanson *et al.*, 1985) 228–232 °C]. – IR (nujol): ν_{max} = 1712 (C=O), 1666 (C=O), 1590 cm^{-1} (C=C). – ^1H NMR ($CDCl_3$): δ = 1.78 (3H, s, 19-Me), 2.76 (2H, t, J = 5.9 Hz, 16-H), 3.66 (2H, m, 15-H), 6.40 (1H, d, J = 10.1 Hz, 1-H), 7.69 (1H, d, J = 10.1 Hz, 2-H), 7.81 (1H, d, J = 8.1 Hz, 11-H), 8.03 (1H, d, J = 8 Hz, 12-H), and 8.29 (1H, s, 21-H). – EIMS: m/z = 304 [M^+] ($C_{19}H_{12}O_4$).

*1α-Hydroxydemethoxyviridin (**3**)*

Dehydroxydemethoxyviridin-1-ene (**2**) (50 mg) in acetone was treated with dilute hydrochloric acid (2.5 ml) for 3 d at room temperature. The solution was concentrated *in vacuo* and poured into water. The product was recovered in ethyl acetate, washed with water, and dried over sodium sulfate. The solvent was evaporated *in vacuo* to afford 1α-hydroxydemethoxyviridin (**3**) (25 mg, 52%) which crystallized from acetone as needles, m.p. 225–230 °C [lit. (Hanson *et al.*, 1985) 238–240 °C]. – IR (nujol): ν_{max} = 3290 (OH), 1735 (C=O), 1700 (C=O), 1590 cm^{-1} (C=C). – ^1H NMR (DMSO): δ = 1.60 (3H, s, 19-CH_3), 5.02 (1H, brs, 1-*H*), 5.36 (1H, d, J = 3.6 Hz, 1-O*H*), 7.96 (2H, s, 11 and 12-*H*), and 8.86 (1H, s, 21-*H*). – EIMS: m/z = 322 [M^+] ($C_{19}H_{14}O_5$).

*6α-Acetoxypregn-4-ene-3,20-dione (**5**)*

Pregnenolone (**4**) (1 g) was dissolved in dichloromethane (80 ml) and mixed with potassium permanganate (8 g), copper sulfate pentahydrate (4 g), water (0.6 ml), and *tert*-butyl alcohol (2.0 ml). The mixture was heated under reflux for 10 min and subsequently left to stir at room temperature overnight. The mixture was filtered through a celite pad and the filtrate was washed with more dichloromethane. The solvent was removed *in vacuo* to afford a gum, which was dissolved in pyridine (10 ml) and acetic anhydride (5 ml). This was stirred at room temperature overnight. The mixture was poured into cold dilute hydrochloric acid and the product was extracted with ethyl acetate. The combined extracts were washed with dilute hydrochloric acid, aqueous sodium hydrogen carbonate and water. The extracts were dried over sodium sulfate and the solvent was re-

moved *in vacuo* to afford an oily mixture (250 mg) which was directly employed in the next step.

To the crude mixture dissolved in glacial acetic acid (7 ml), two drops of hydrobromic acid were added portionwise. The steroidal solution was stirred at room temperature for 2 d. The solution was neutralized with aqueous sodium hydrogen carbonate and the steroid was extracted with ethyl acetate. The organic phase was washed with aqueous sodium hydrogen carbonate and water and dried over sodium sulfate. The solvent was removed *in vacuo* to give a dark gum, which was adsorbed onto silica gel and chromatographed. Elution with 25% ethyl acetate in petroleum ether yielded 6α-acetoxypregn-4-ene-3,20-dione (**5**) (150 mg, 35% overall yield), which crystallized from ethyl acetate and petroleum ether as needles, m.p. 148–152 °C [lit. (Komeno *et al.*, 1969) 145–146 °C]. – IR (nujol): ν_{max} = 1733 (Me-*C*=*O*), 1668 (*C*=*O*), 1618 cm^{-1} (C=C). – ^{1}H NMR ($CDCl_3$): δ = 0.67 (3H, s, 18-Me), 1.20 (3H, s, 19-Me), 2.16 (3H, s, 21-Me), 5.35 (1H, dd, J = 4.8 ve 12 Hz, 6β-H) and 6.85 (1H, s, 4-H).

*5′-Methylfuro-(4′,3′,2′-4,5,6)pregn-5-ene-3,20-dione (**6**)*

A solution of 6α-acetoxypregn-4-ene-3,20-dione (**5**) (100 mg) in dry xylene (10 ml) was treated with sodium hydride (60% mineral oil dispersion) (100 mg) under reflux for 84 h. The mixture was cooled to room temperature and filtered. The filtrate was washed with toluene, and the combined filtrates were evaporated to dryness *in vacuo*. The residue was purified by chromatography. Elution with 15% ethyl acetate in petroleum ether afforded 5′-methylfuro-(4′,3′,2′-4,5,6)pregn-5-ene-3,20-dione (**6**) (45 mg, 30%), which crystallized from ethyl acetate and petroleum ether as needles, m.p. 159–161 °C [lit. (Komeno *et al.*, 1969) 161–162 °C]. – IR (nujol): ν_{max} = 1710 (Me-*C*=*O*), 1696 (*C*=*O*), 1610 cm^{-1} (C=C). – ^{1}H NMR ($CDCl_3$): δ = 0.71 (3H, s, 18-Me), 1.19 (3H, s, 19-Me), 2.17 (3H, s, 21-Me) and 2.55 (3H, s, furan-Me). – ^{13}C NMR ($CDCl_3$): δ = 209.35 (C-20), 195.63 (C-3), 155.82 (C-6), 145.75 (furan C), 128.24 (C-4), 116.20 (C-5), 63.47 (C-17), 56.68 (C-14), 49.36 (C-9), 44.20 (C-13), 38.30 (C-10), 36.80 (C-12), 36.80 (C-1), 33.78 (C-8), 32.39 (C-2), 31.56 (C-21), 28.39 (C-7), 24.63 (C-16), 22.62 (C-15), 21.11 (C-11), 20.25(C-19), 13.91 (furan Me) and 13.31 (C-18). – EIMS: m/z = 354 [M$^+$] ($C_{23}H_{30}O_3$).

Results and Discussion

There is a need to develope an antifungal agent with less side effects since serious fungal infections such as invasive aspergillosis increased dramatically in recent years. Amphotericin B and itraconazole are two of commonly used antifungal drugs to cure it, but their side effects and poor response rate (0–50%) limit their use. Different approaches are used for a search to find compounds with worthwhile antimicrobial action. These studies have revealed many compounds which exhibited good antimicrobial effects (Denning *et al.*, 1994; Denning, 1996; Rewankar and Patterson, 1997; Summers *et al.*, 1997; Iwai *et al.*, 2004).

We have initially found that demethoxyviridin (**1**) isolated as a fungal metabolite of *N. hinnuleum* showed good antimicrobial effects against a range of pathogenic microorganisms which led us to direct our research towards the structural element(s) required for the antimicrobial activity. For our purpose, three compounds in addition to demethoxyviridin were chosen as target compounds and synthesized as follows.

Synthesis of compounds

Demethoxyviridin (**1**) was biosynthesized from *N. hinnuleum* as described above. Treatment of **1** with methanesulfonyl chloride in pyridine at room temperature for 4 h afforded compound **2** which gave compound **3** on reacting with diluted hydrochloric acid in acetone at room temperature for 3 d (see Fig. 1). The synthesis of **6** is achieved by following a series of known literature methods applied for the first time in our laboratory and also presented in Fig. 1. Pregnenolone (**4**) was treated with a mixture of copper sulfate, potassium permanganate, water and *tert*-butyl alcohol in dichloromethane. The resulting mixture was acetylated by treatment with acetic anhydride in pyridine at room temperature overnight to yield an oily compound. It was then dissolved in glacial acetic acid and treated with a trace of hydrobromic acid at room temperature for 2 d to afford compound **5**. Aldol condensation of **5** was carried out using sodium hydride in xylene under reflux. The reaction had reached completion in 2 d and compound **6** was separated and purified by column chromatography. This methodology offers a good alternative way to those of literature methods used for the synthesis of the same compound. The prepara-

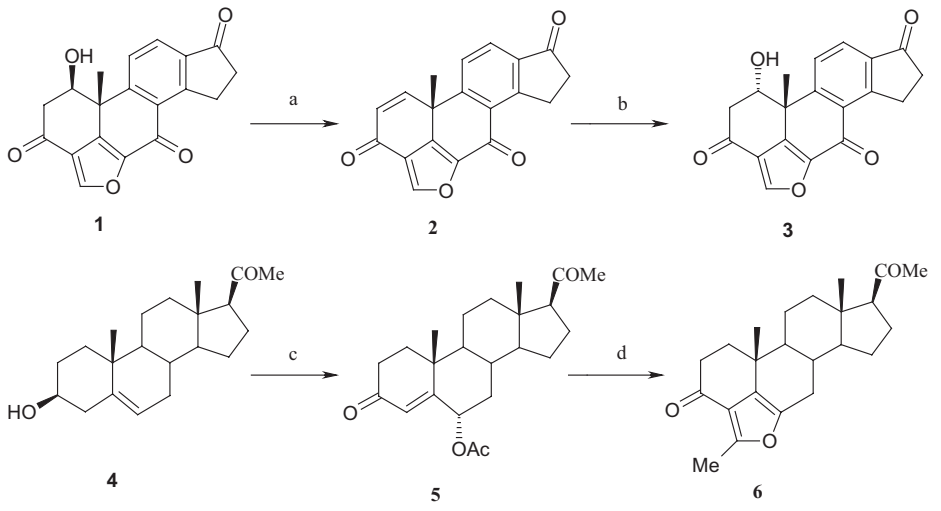

Fig. 1. The synthesis of 1α-hydroxydemethoxyviridin (**3**) and 5′-methylfuro-(4′,3′,2′–4,5,6)pregn-5-ene-3,20-dione (**6**). Conditions: a) $MeSO_2Cl$, C_5H_5N, 25 °C, 4 h; b) HCl (10%), $(CH_3)_2CO$, 25 °C, 72 h; c) (i) $CuSO_4 \cdot 5H_2O$, CH_2Cl_2, *t*-BuOH, H_2O, $KMnO_4$, 80 °C, 30 min, then 25 °C, 8 h; (ii) Ac_2O, C_5H_5N, 25 °C, 20 h; (iii) AcOH, HBr, 25 °C, 48 h; d) NaH (60% in mineral oil), *o*-xylene, reflux, 84 h.

tion of **7** (see Fig. 2) has been described in our previous study (Boynton *et al.*, 1999).

Biotransformation of **6** and **7** by *Cephalosporium aphidicola* was also carried out in the hope of introducing a hydroxyl group on the furanosteroid structures and the effects of it were studied. Unfortunately, no hydroxylated metabolite was obtained and only substrate molecules were recovered after biotransformation reactions.

All the spectral data of compounds synthesized in this study are in good agreement with the literature values.

Antimicrobial activity

The comparative activity of currently used antibacterial agents penicillin, tetracycline, cephataxime and antifungal agent amphotericin B and the compounds **1**, **3** on bacterial and fungal strains are summarized in Table I. The MIC results of the active compounds (**1** and **3**) are presented in Table II.

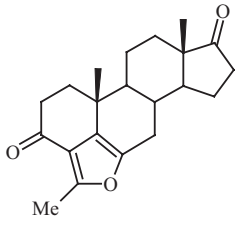

Fig. 2. The structure of 5′-methylfuro-(4′,3′,2′-4,5,6)androst-5-ene-3,17-dione (**7**).

Compound **1** exhibited very weak inhibition effects against *A. fumigatus* and *A. flavus* among the tested fungal strains. *M. luteus* was the most sensitive strain to the antibacterial effect of **1** followed by *S. epidermidis* and *P. aeruginosa* whereas *K. pneumoniae* and *S. faecium* were the least sensitive organisms.

Compound **3** showed a moderate degree of antibacterial activity against both *E. coli* and *E. aerogenes* with an inhibition zone of 11 mm i.d. at 100 μg/disc test concentration. On the other hand, compound **3** exhibited very good antifungal activity with zones of inhibition ranging from 9 to 15 mm i.d. *A. parasiticus* was the most sensitive strain with an inhibition zone of 15 mm i.d. at 100 μg/disc test concentration. It was followed by *A. niger*, *A. fumigatus*, *A. flavus* and *G. candidum* all having an inhibition zone of 10 mm i.d. at 100 μg/disc test concentration which is comparable with that of the antifungal agent, amphotericin B (see Table I) whereas *F. solani* and *F. graminarum* were not affected.

Compounds **6** and **7** did not exhibit any inhibitory effect against the test strains with the exception of *C. albicans* which was inhibited by compound **7** with 9 mm i.d. at 100 μg/disc.

Table I. Antibacterial and antifungal activities of compounds **1** and **3**.

	Zone of inhibition [mm]				
Bacterial strain	**1** (100 μg/disc)	**3** (100 μg/disc)	Penicillin (10 U/disc)	Tetracycline (30 μg/disc)	Cephataxime (30 μg/disc)
B. cereus	9	9	30	32	8
B. mycoides	9	10	11	33	10
B. subtilis	9	10	31	36	26
M. luteus	11	10	> 40	> 40	> 40
S. aureus	8	9	7	30	–
S. epidermidis	10	10	20	28	26
S. faecium	–	8.5	22	–	9
E. coli	7	11	–	30	32
E. aerogenes	8	11	–	30	35
P. aeruginosa	10	9	–	16	–
Y. enterocolitica	9	8	13	26	13
K. pneumoniae	–	9	–	25	32
Fungal strain				Amphotericin B (10 μg/disc)	
C. albicans	–	9		13	
A. niger	–	10		9	
A. fumigatus	7	10		–	
A. flavus	7	10		8	
A. parasiticus	–	15		7	
F. graminarum	–	–		7	
F. solani	–	–		9	
G. candidum	–	10		8	

–, No zone of inhibition.

Table II. Minimum inhibitory concentration (MIC) for **1** and **3** against bacterial and fungal strains.

	MIC [μg/ml (mM)]	
Bacterial strain	**1**	**3**
B. cereus	> 320 (0.994)	320 (0.994)
B. mycoides	320 (0.994)	160 (0.497)
B. subtilis	> 320 (0.994)	320 (0.994)
M. luteus	320 (0.994)	320 (0.994)
S. aureus	320 (0.994)	320 (0.994)
S. epidermidis	320 (0.994)	320 (0.994)
S. faecium	> 320 (0.994)	> 320 (0.994)
E. coli	320 (0.994)	320 (0.994)
E. aerogenes	320 (0.994)	320 (0.994)
P. aeruginosa	> 320 (0.994)	320 (0.994)
Y. enterocolitica	> 320 (0.994)	320 (0.994)
K. pneumoniae	> 320 (0.994)	> 320 (0.994)
Fungal strain		
C. albicans	> 320 (0.994)	80 (0.249)
A. niger	> 320 (0.994)	20 (0.062)
A. fumigatus	> 320 (0.994)	20 (0.062)
A. flavus	320 (0.994)	20 (0.062)
A. parasiticus	> 320 (0.994)	20 (0.062)
F. graminarum	320 (0.994)	20 (0.062)
F. solani	160 (0.497)	20 (0.062)

The MIC of compounds **1** and **3** against all bacterial strains tested was mostly ranged from 160 to 320 μg ml^{-1}. Compound **1** exhibited activity against *F. solani* with an MIC value of 160 μg ml^{-1} whereas compound **3** was active against most fungal strains with a range of MIC values of 20, 20, 20, 20, 20, 20 and 80 μg ml^{-1} against *A. niger*, *A. flavus*, *A. fumigatus*, *A. parasiticus*, *F. graminarum*, *F. solani* and *C. albicans*, respectively.

Two (**1** and **3**) out of four compounds showed reasonably good inhibitory action against bacterial and fungal strains *in vitro*. Compound **1** showed the highest antibacterial activity against *M. luteus* with an inhibition zone of 11 mm i.d. whereas compound **3** was the most active against *A. parasiticus*, *E. coli* and *E. aerogenes*. The MIC of **1** and **3** ranged from 20 to 320 μg ml^{-1} for most organisms. Compounds **6** and **7** had no antimicrobial activity.

Structure-activity relationships

The following conclusions can be drawn from this study of structure-activity relationships. First, when compared to demethoxyviridin (**1**), changing

the stereochemistry of the 1-hydroxyl group at alpha position leads to an increase in biological activity. Second, the presence of the furan ring in the molecule is important for biological activity which is in accordance with previous studies involved in the biological activities of furanosteroids (Norman *et al.*, 1995; Dodge *et al.*, 1995). Third, the introduction of a methyl group to the furan ring at C-20 and an acetyl group either at C-17 of androstane series or C-20 of pregn series might totally destroy the potency of this class of compounds as inhibitors of the pathogenic microorganisms tested in this study.

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