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Synthesis and cytotoxicity evaluation of 22,23-oxygenated stigmastane derivatives

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Abstract—Starting from (22*E*)-3α,5α-cyclo-6β-methoxystigmast-22-ene eighteen derivatives of (22*S*,23*S*)-22,23-oxidostigmastane, (22*R*,23*R*)-22,23-oxidostigmastane, and (22*R*,23*R*)-22,23-dihydroxystigmastane were synthesized and screened for cytotoxicity in human hepatoma Hep G2 cells and human breast carcinoma MCF-7 cells using MTT assay. Four compounds of this series exhibited high cytotoxicity in both cells; three compounds were selectively toxic in MCF-7 cells, one compound was toxic in Hep G2 cells, rather than in MCF-7 cells; four compounds at low concentrations increased MTT test values over the control.

1. Introduction

Oxygenated cholesterol derivatives (oxysterols) represent a class of potent regulatory molecules with remarkably diverse, important biological functions and significant potential for applications in medicine.[1] A number of oxysterols exhibit toxicity in mammalian cells. Investigations of oxysterol cytotoxicity and related effects on cell growth, proliferation, viability, differentiation, and apoptosis have been presented in numerous reviews.[1–6]

However, only a few studies have been performed concerning cytotoxicity of oxygenated derivatives of other sterols. It has been reported that ergosterol, exposed to oxidation in air, inhibited the growth and caused apoptosis in human breast cancer MCF-7 cells and MDA-231 cells in vitro.[7] (22*E*)-5α,8α-Epidioxyergosta-6,22-dien-3β-ol completely inhibited the growth and induced apoptosis in HL60 human leukemia cells.[8] (22*E*)-Ergosta-7,22-diene-3β,5α,6β-triol induced alkaline phosphatase activity and suppressed the estrogen-induced apoptosis in a mouse osteoblastic cell line MC3T3-E1.[9] Products of oxidation of campesterol and β-sitosterol caused cellular damage in cultured macrophages, though toxic effects of these oxides were weaker compared with related cholesterol oxides.[10] Products of β-sitosterol oxidation, as well as 3β-hydroxy-5α,6α-oxidostigmastane, 3β,7β-dihydroxystigmast-5-ene, 3β-hydroxystigmast-5-en-7-one, and 3β,5α,6β-trihydroxystigmastane, exhibited cytotoxicity in human monocytic U937 cells, colon adenocarcinoma CaCo-2 cells, and hepatoma Hep G2 cells.[11,12] Cytotoxicity of aforementioned oxygenated stigmastane derivatives was compared with those of related oxygenated cholestane derivatives (oxysterols). Oxysterols and oxygenated stigmastane derivatives indicated qualitatively similar toxic effects, however, higher concentrations of oxygenated stigmastane derivatives were required to elicit comparable levels of toxicity.[12]

Until now there were no reported data concerning cytotoxicity of stigmastane derivatives comprising oxygenated side chain. On the other hand, a number of side chain oxygenated sterols of natural origin are known to be toxic for mammalian cells, especially for fast proliferating tumor cells, and considered to be potent pharmacological agents.[13–17] The present study was undertaken to prepare a series of new 22,23-oxygenated stigmastane derivatives from easily available stigmasterol, (22*E*)-3β-hydroxystigmasta-5,22-diene, and evaluate cytotoxicity of synthesized compounds in two cell lines: human breast carcinoma MCF-7 cells and human hepatoma Hep G2 cells. Both these cell lines are widely used for screening of new biological active compounds, particularly cytotoxics.

Keywords: Sterols; Stigmastane; Cytotoxicity; MTT assay.
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2. Results and discussion

2.1. Transformation of the side chain

Transformation of 22(23) double bond in stigmasterol derivatives performed in this study is shown in Scheme 1. The known (22*E*)-3α,5α-cyclo-6β-methoxystigmast-22-ene **1** prepared from stigmasterol according to[18] was chosen as the starting compound. The reaction of cyclosterol **1** with CPBA excess in boiling $CHCl_3$ in the presence of Na_2CO_3 led to mixture of isomeric epoxides **2** and **3** (1:1) in 80% overall yield. Compounds **2** and **3** were satisfactorily separated by silica gel column chromatography in linear gradient hexane/$CHCl_3$ (3:2)/$CHCl_3$; the assignment of stereochemical configuration of C-22 and C-23 atoms in these compounds is given below. Both isomers **2** and **3** were converted into steryl acetates **4** and **5** by boiling in glacial AcOH, followed by deacetylation to obtain (22*S*,23*S*)-22,23-oxido-3β-hydroxystigmast-5-ene **6** and (22*R*,23*R*)-22,23-oxido-3β-hydroxystigmast-5-ene **7**.

The reaction of cyclosterol **1** with I_2 in the presence of AgOAc in 95% AcOH at 20 °C was found to be regio- and stereoselective. Though reaction led to mixture of products, the major one, 22-iodo-23-acetoxy derivative **8**, was successfully isolated by silica gel flash chromatography in 65% yield. Heating of iodoacetate **8** with AgOAc in glacial AcOH led to simultaneous transformation of 6β-methoxy-3,5-cyclosterol fragment to Δ5,3β-acetoxysterol fragment, and intramolecular substitution of 22-iodine resulting in equimolar mixture of two products **9** and **10** which was isolated by silica gel flash chromatography in 66% yield. Attempted preparative separation of these products was unsuccessful. According to ^{1}H NMR spectrum of the mixture of compounds **9** and **10**, 3β-acetoxy group (2.02, s; 4.59, m), one acetoxy group attached to either C-22 or C-23 (2.05, s and 2.10, s; 4.91, m and 5.06, m), and one hydroxy group either at C-22 or at C-23 (3.66, m and 3.72, m) were present in both products. Therefore, compounds **9** and **10** were identified as 3β,22-diacetoxy-23-hydroxystigmast-5-ene and 3β,23-diacetoxy-22-hydroxystigmast-5-ene. The heating of the above mixture with K_2CO_3 in aqueous MeOH gave the single triol **11** in 91% yield. The simple procedure was elaborated for one-pot synthesis of triol **11** from stigmasterol.

Acetylation of triol **11** (or the mixture of compounds **9** and **10**) with excess of Ac_2O in boiling pyridine led to triacetate 12 in quantitative yield. The treatment of triol **11** with 2,2-dimethoxypropane in the presence of TsOH led to acetonide **13** in 90% yield.

2.2. Assignment of stereochemical configuration of C-22 and C-23 atoms

Epoxidation of 22(23)-double bond in cyclosterol **1** with CPBA led to two isomers with configuration (22*S*,23*S*) and (22*R*,23*R*). Assignment of stereochemical configuration of C-22 and C-23 atoms in resulting epoxides (**2**–**7**) was carried out on the basis of experimental ^{1}H NMR spectra and calculation of the low energy conformers for (22*S*,23*S*)-3β-hydroxy-22,23-oxidostigmast-5-ene and (22*R*,23*R*)-3β-hydroxy-22,23-oxidostigmast-5-ene. Calculation was carried out by semi-empirical AM1 method using HyperChem computer program.

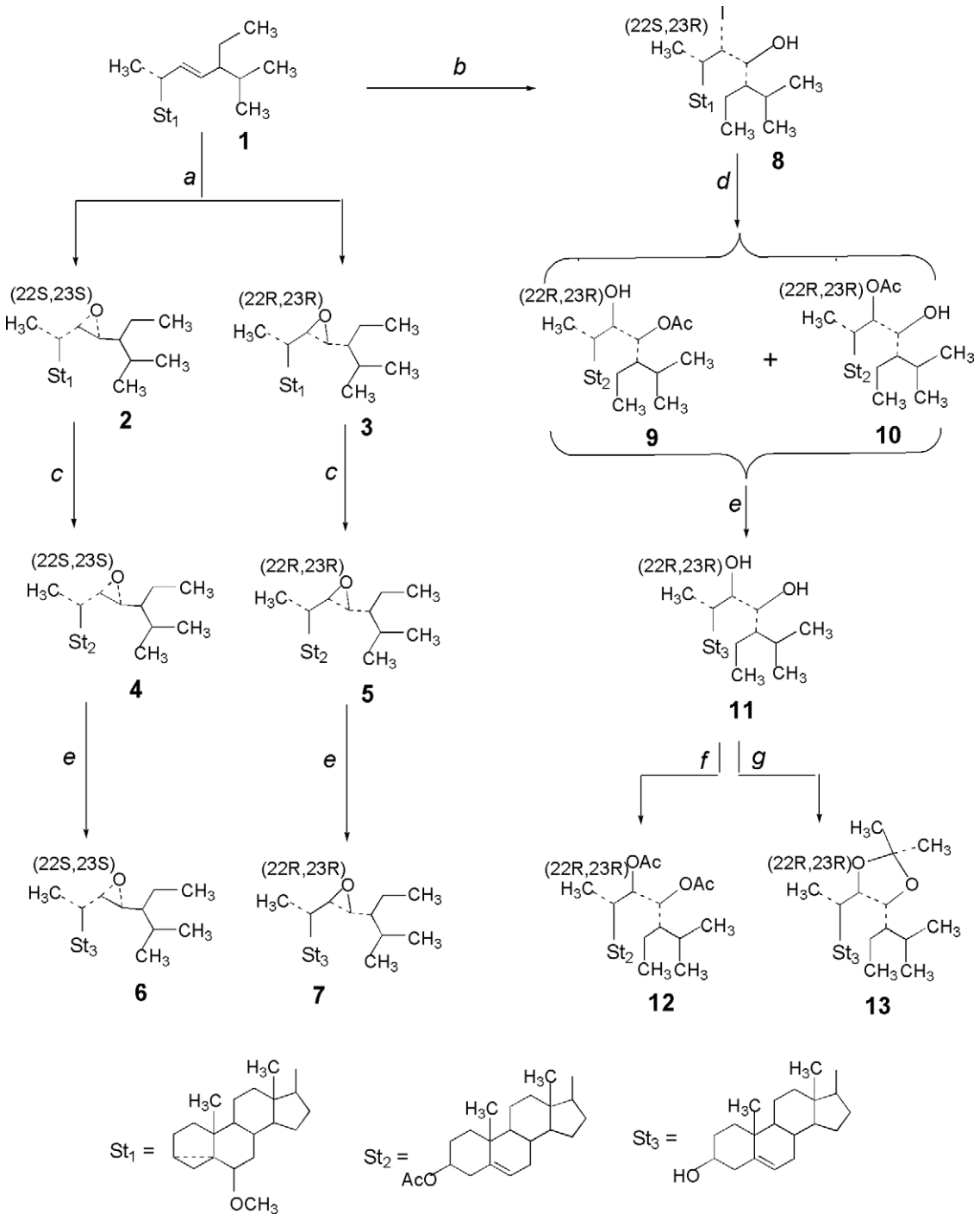
According to calculation both (22*S*,23*S*)-3β-hydroxy-22,23-oxidostigmast-5-ene and (22*R*,23*R*)-3β-hydroxy-22,23-oxidostigmast-5-ene exist as a pair of low energy conformers. Calculated preferable conformations of side chain are shown in Figure 1. Both low energy conformers of (22*S*,23*S*)-3β-hydroxy-22,23-oxidostigmast-5-ene have a completely extended side chain, whereas both low energy conformers of (22*R*,23*R*)-3β-hydroxy-22,23-oxidostigmast-5-ene have H-23 atom shielded with the terminal 29- and 26- (27-) methyl groups. The calculated distances between H-23 atom and carbon atoms of terminal methyl groups in both low energy conformers of (22*R*,23*R*)-epoxide are approximately equal to the distances between H-22 atom and C-21 and C-17, whereas these distances are reliably greater in both low energy conformers of the (22*S*,23*S*)-epoxide. Experimental values of ^{1}H NMR chemical shifts for selected protons in compounds **2**, **4**, **6** and in compounds **3**, **5**, **7** are shown in Table 1. The values of chemical shifts of H-23, H-21, and H-29 in compounds **2**, **4**, **6** were shifted downfield compared with those in compounds **3**, **5**, **7**, that was in agreement with calculation and allowed to assign stereochemical configuration of compounds **2**, **4**, **6** as (22*S*,23*S*) and configuration of compounds **3**, **5**, **7** as (22*R*,23*R*).

Stereochemical configuration of C-22 in compound **8** was suggested to be (22*S*) on the basis of comparison of H-22 resonance in ^{1}H NMR spectrum (5.43, dd, J = 10.5 and 1.0 Hz) with those of reported 22-substituted sterols[19–22]; and configuration of C-23 was suggested to be (23*R*), since reaction of olefins with iodine and AgOAc under used conditions is known to be a trans-addition. The (22*S*,23*R*)-configuration of **8** was unequivocally confirmed by its transformation to a single (22*R*,23*R*)-22,23-oxido-3α,5α-cyclo-6β-methoxystigmastane **3** by treatment with K_2CO_3 in aqueous MeOH (pathway e, Scheme 2).

Transformation of (22*S*,23*R*)-22-iodo-23-acetate fragment in compound **8** by treatment with AcOAg in AcOH requires intramolecular nucleophilic attack of carbonyl oxygen of 23-acetoxy group on C-22, and the formation of positively charged acylium intermediate (pathway d, Scheme 2), which is cleaved by nucleophile to give the mixture of products **9** + **10**. The proposed mechanism requires the inversion at C-22 and retention at C-23 in compound **8**. Therefore, the configuration of compounds **9** and **10** was assigned as (22*R*,23*R*).

2.3. Transformation of the steroid backbone

Preparation of 22,23-oxygenated stigmastane derivatives containing modified steroid backbone from compounds **4**–**13** was performed using parallel synthesis strategy and known methods elaborated earlier for transformation of cholesterol and related derivatives to common oxysterols (Scheme 3).



Scheme 1. Transformation of 22(23) double bond in stigmasterol derivatives. Reagents and conditions: (a) CPBA, $NaHCO_3/CHCl_3$, reflux; (b) I_2, AgOAc/95% AcOH; (c) AcOH, reflux; (d) AgOAc/AcOH, reflux; (e) $K_2CO_3/MeOH/H_2O$, reflux; (f) Ac_2O/Py, reflux; (g) $(CH_3)_2C(OCH_3)_2$, H^+.

Transformation of Δ5-3β-hydroxysterols **6**, **7**, **11** into corresponding Δ4-3-ketosterols **14**, **15**, **16** was performed by enzymatic oxidation according to the method[23] used earlier for the conversion of 25-hydroxycholesterol, (25*R*)-26-hydroxycholesterol and (24*R*)-24-hydroxycholesterol to related Δ4-3-ketosterols. Incubation of compounds **6**, **7**, **11** with mixture of cholesterol oxidase and peroxidase at pH 7.5 in the presence of sodium cholate resulted in Δ4-3-ketosterols **14**, **15**, **16** which were isolated by preparative TLC in about 80% yields. Structural peculiarities of side chains did not significantly affect the reaction catalyzed by cholesterol oxidase, although the rate of conversion of stigmastane derivatives **6**, **7**, **11** under our experimental conditions was lower than that of cholesterol oxidation to cholest-4-en-3-one.

For the preparation of Δ4-3,6-diketosterols **17**, **18**, **19**, the Δ5-3β-hydroxysterols (epoxides **6**, **7**, and acetonide **13**) were oxidized with a complex CrO_3*2Py in CH_2Cl_2 to obtain resulting products in 80–83% yields. Application of complex CrO_3*2Py is thought to have such advantages as simplicity and high rate compared with

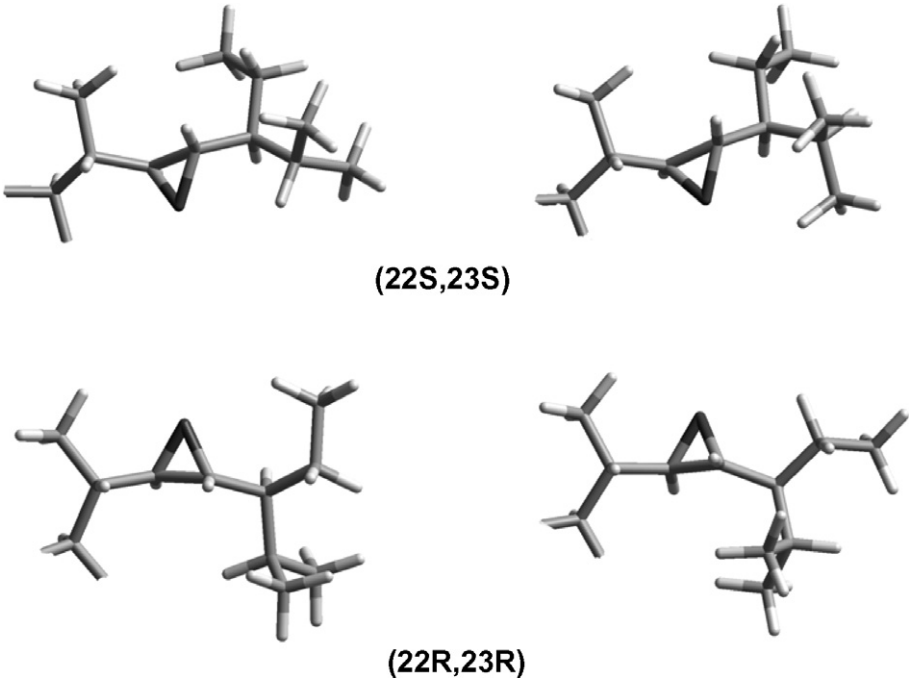
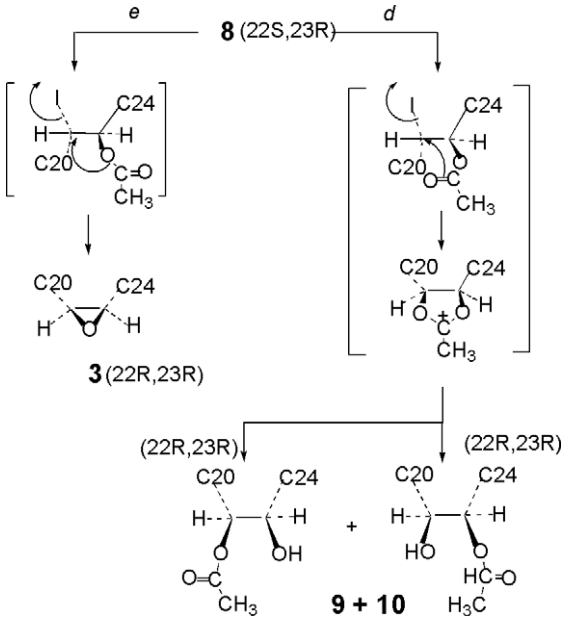


Figure 1. Calculated low energy conformations of C-20–C-29 fragments for (22*S*,23*S*)-3β-hydroxy-22,23-oxidostigmast-5-ene (upper) and (22*R*,23*R*)-3β-hydroxy-22,23-oxidostigmast-5-ene (bottom).

Table 1. Chemical shifts of selected protons in compounds **2**–**7**

Compound	Configuration	Chemical shift, δ, ppm, (J, Hz)		
		H-21	H-23	H-29
2	(22*S*,23*S*)	1.01, d (J = 6.8)	2.73, dd (J = 2.2; 7.2)	0.95, t (J = 7.5)
4	(22*S*,23*S*)	1.02, d (J = 6.8)	2.73, dd (J = 2.2; 7.2)	0.95, t (J = 7.5)
6	(22*S*,23*S*)	1.02, d (J = 6.8)	2.74, dd (J = 2.2; 7.2)	0.95, t (J = 7.5)
3	(22*R*,23*R*)	0.99, d (J = 6.8)	2.50, m	0.91, t (J = 7.5)
5	(22*R*,23*R*)	0.99, d (J = 6.8)	2.50, m	0.92, t (J = 7.5)
7	(22*R*,23*R*)	0.99, d (J = 6.8)	2.50, m	0.92, t (J = 7.5)



Scheme 2. Assignment of stereochemical configuration of C-22 and C-23 atoms in compounds **8**, **9**, **10**. Reagents and conditions: (d) AgOAc/AcOH, reflux; (e) K_2CO_3/MeOH/H_2O, reflux.

methods reported earlier,[24,25] and was as efficient as application of Jones reagent.[26] The removal of isopropylidene group in compound **19** (heating in 80% AcOH containing traces of TsOH) led to (22*R*,23*R*)-22,23-dihydroxystigmast-4-ene-3,6-dione **20** in 89% yield.

An attempt to prepare (22*R*,23*R*)-3β,22,23-trihydroxy-5α,6α-oxidostigmastane **22** by direct epoxidation of (22*R*,23*R*)-3β,22,23-trihydroxystigmast-5-ene **11** with CPBA in $CHCl_3$ resulted in mixture of isomeric 5α,6α- and 5β,6β-epoxides in a 4:1 ratio (^{1}H NMR spectrum of crude product: 2.89, d, J = 4.0 Hz, H-6 in 5α,6α-epoxide; and 3.05, d, J = 1.8 Hz, H-6 in 5β,6β-epoxide) which we failed to separate one from another by chromatography or crystallization. The pure 5α,6α-epoxide **22** was prepared from (22*R*,23*R*)-3β,22,23-triacetoxystigmast-5-ene **12** in overall 60% yield by the treatment with CPBA in CH_2Cl_2, followed by purification of resulting (22*R*,23*R*)-3β,22,23-triacetoxy-5α,6α-oxidostigmastane **21** by preparative TLC in $CHCl_3$/acetone (49:1), and removal of the acetate protecting groups with $LiAlH_4$.

(22*R*,23*R*)-3β,5α,6β,22,23- Pentahydroxystigmastane **23** was prepared from (22*R*,23*R*)-3β,22,23-triacetoxystig-

mast-5-en **12** according to method,[27] elaborated for the synthesis of 3β,5α,6β-cholestanetriol from cholesterol. Triacetate **12** was treated with H_2O_2 in formic acid for 30 min at room temperature, and the resulting product was heated with K_2CO_3 in aqueous MeOH, that led to the removal of all protecting groups. The yield of target product **23** was 79% (based on starting triacetate **12**).

Allylic oxidation of acetates **4**, **5**, and **12** with $K_2Cr_2O_7$ in the mixture AcOH–Ac_2O according to general procedure[28] resulted in 7-ketosteryl acetates **24**, **25**, and **26** in about 60% yields. Deacetylation of compounds **24**, **25**, **26** by heating with K_2CO_3 in aqueous MeOH gave 7-ketosterols **27**–**29** in 90–92% yields.

Reduction of 7-ketosteryl acetate **24** with suspension of $LiAlH_4$ in Et_2O resulted in a mixture of 7α-hydroxysterol **30** and 7β-hydroxysitosterol **32** (at a ratio of ~2:3) which were successfully separated one from another by column chromatography on silica gel in known triple system Et_2O/benzene/cyclohexane (90:9:1).[29] (22*R*,23*R*)-3β,7α-dihydroxy-22,23-oxidostigmast-5-ene **31** and (22*R*,23*R*)-3β,7β-dihydroxy-22,23-oxidostigmast-5-ene **33** (at a ratio ~1:2) were obtained from 7-ketosteryl acetate **25** by the same procedure. The assignment of stereochemical configuration of C-7 atom in compounds **30**–**33** was estimated by comparison of ^{1}H NMR spectra of compounds **30**–**33** with those of 7α-hydroxy and 7β-hydroxy derivatives of cholesterol and β-sitosterol.[30,31]

2.4. Cytotoxicity evaluation

Cytotoxicity of compounds **6**, **7**, **11**, **14**–**18**, **20**, **22**, **23**, **27**–**33** in human hepatoma Hep G2 cells and human breast carcinoma MCF-7 cells was evaluated by MTT assay[32] based on mitochondrial reduction of the yellow MTT tetrazolium dye to a highly colored blue formazan product. This assay usually shows high correlation with number of living cells, cell proliferation and release of mitochondrial matrix enzymes.[32–37]

The values of MTT test in Hep G2 cells and MCF-7 cells incubated for 48 h in serum-free media with oxygenated stigmastane derivatives at various concentrations are given in Figures 2–5. According to MTT test, all synthesized compounds were subdivided in four groups. Group 1 (toxic compounds): **11**, **23**, **29**, **31** were toxic both in Hep G2 and in MCF-7 cells, though effects displayed in MCF-7 cells more powerfully (Fig. 2). Group 2 (selectively toxic compounds) was consisted of **16**, **20**, **22**, **31**. Compounds **16**, **20**, **22** were toxic in MCF-7 cells without significant effects on Hep G2 cell viability, whereas compound **31** was moderately toxic in Hep G2 cells, rather than in MCF-7 cells (Fig. 3) Group 3 (non toxic or slightly toxic compounds): **6**, **7**, **18**, **28**, **32**, **33** did not significantly change MTT test values in both cells compared with control (Fig. 4). Four compounds of group 4: **14**, **15**, **17**, **27** exhibited complex dependency of MTT test values on concentration in MCF-7 cells and did not significantly affect MTT test values in Hep G2 cells (Fig. 5).

Among the compounds primarily tested in this study, the highest toxicity was exhibited by (22*R*,23*R*)-3β,22,23-trihydroxystigmast-5-ene **11** and (22*R*,23*R*)-3β,22,23-trihydroxystigmast-5-en-7-one **29**; (22*R*,23*R*)-22,23-dihydroxystigmast-4-ene-3,6-dione **20** was selectively toxic in MCF-7 cells. In general, cytotoxicity of (22*R*,23*R*)-22,23-dihydroxystigmastane derivatives exceeded that of (22*S*,23*S*)-oxidostigmastane derivatives. There was no cytotoxic compound found in this study among the (22*R*,23*R*)-22,23-oxidostigmastane derivatives.

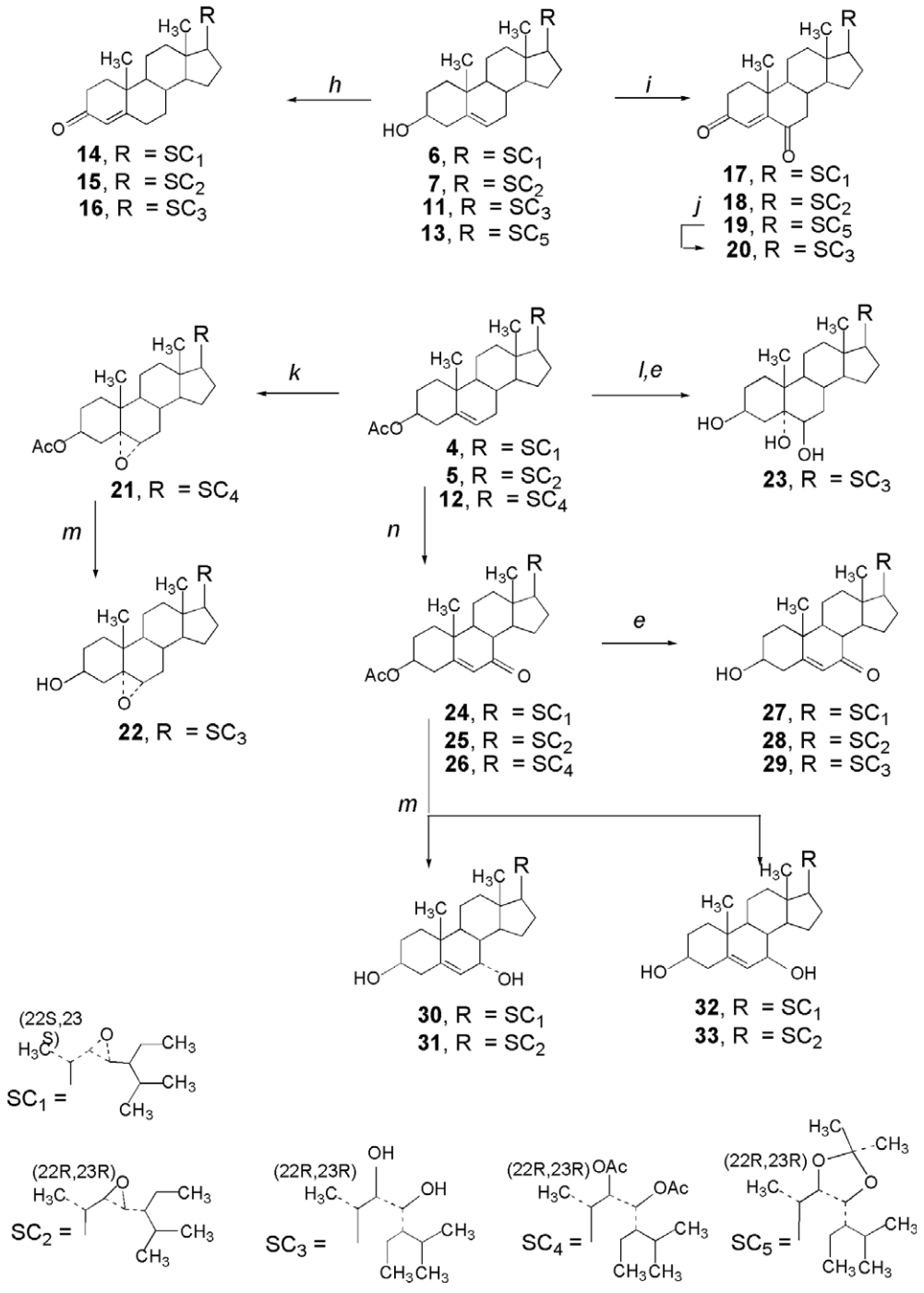
Cytotoxicity of 22,23-oxygenated stigmastane derivatives was dependent on the number and structure of additional steroid backbone substituents. 3β-Hydroxy-5-ene derivatives were more toxic compared with related 3-keto-4-enes and 3,6-diketo-4-enes. Cytotoxicity of (22*R*,23*R*)-3β,22,23-trihydroxy-5α,6α-oxidostigmastane **22** and (22*R*,23*R*)-3β,5α,6β,22,23-pentahydroxystigmastane **23** in both cells was significantly lower than that of triol **11**. 7β-Hydroxycholesterol is known to be the most toxic compound among the common oxysterols,[38–41] however the only 7β-hydroxystigmastane derivative **31** exhibited cytotoxicity among the compounds tested in this study.

Compounds of group 4 at low concentrations exhibited increased MTT test values over the control, that could indicate stimulating effects of compounds on cell proliferation and mitochondrial hydrogenase activity. There have been reported data that compounds stimulating cell proliferation in cultured cells, such as some cytokines,[33] phosphodiesterase inhibitors,[34] glucocorticoids,[35,36] and androgens,[37] could increase MTT test values over the control. However, such effects have not been shown earlier for oxygenated cholestane and stigmastane derivatives.[10–12,38–41] Effects of increasing MTT test values over the control by compounds of group 4 (**14**, **15**, **17**, **27**) were displayed only in MCF-7 cells.

3. Conclusion

Eighteen 22,23-oxygenated stigmastane derivatives were synthesized and screened for cytotoxicity in human hepatoma Hep G2 cells and human breast carcinoma MCF-7 cells using MTT assay. Four compounds of this series exhibited high cytotoxicity in both cells, three compounds were selectively toxic in MCF-7 cells, and one was selectively toxic in Hep G2 cells. (22*R*,23*R*)-22,23-Dihydroxystigmastane derivatives were the most toxic. There was no cytotoxic compound found in this study among the (22*R*,23*R*)-22,23-oxidostigmastane derivatives. Three (22*S*,23*S*)-22,23-oxidostigmastane derivatives and one (22*R*,23*R*)-22,23-oxidostigmastane derivative increased MTT test values in MCF-7 cells over the control, which indicated stimulation of cell proliferation, or alterations in mitochondria.

One can presume that some 22,23-oxygenated stigmastane derivatives may claim attention as potential cytotoxics, regulators of cell viability and proliferation; and further study of these compounds is of interest for



Scheme 3. Synthesis of compounds **14**–**33**. Reagents and conditions: (e) K_2CO_3/MeOH/H_2O, reflux; (h) cholesterol oxidase + peroxidase/20 mM Na cholate, pH 7.5; (i) CrO_3*2Py/CH_2Cl_2; (j) 80% AcOH, H^+, reflux; (k) CPBA/CH_2Cl_2; (l) H_2O_2/HCOOH; (m) $LiAlH_4$/Et_2O; (n) CrO_3/Ac_2O/AcOH, reflux.

understanding molecular mechanisms that underlie the biological activity of oxygenated sterols and related compounds in mammalian cells.

4. Experimental

4.1. Materials and general methods

Chemical reagents and solvents were purchased from 'Aldrich', 'Merck', and 'MedKhimLab'; culture plastics from 'Greiner', 'Costar', and 'Corning'; culture media and fetal calf serum (FCS) from 'Gibco BRL' and 'HyClone'; phosphate-buffered saline (PBS) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) from 'Sigma'; (22*E*)-6β-methoxy-3α,5α-cyclostigmast-22-ene **1** was synthesized from stigmasterol (purchased from ICN) according to method[18] with slight modifications.

Melting points (mp) of crystalline compounds were measured in glass capillaries. Column chromatography was carried out on 'Woelm' silica gel (100–200 μm) and Silasorb 600 (30 μm); TLC—on precoated HPTLC and PSC

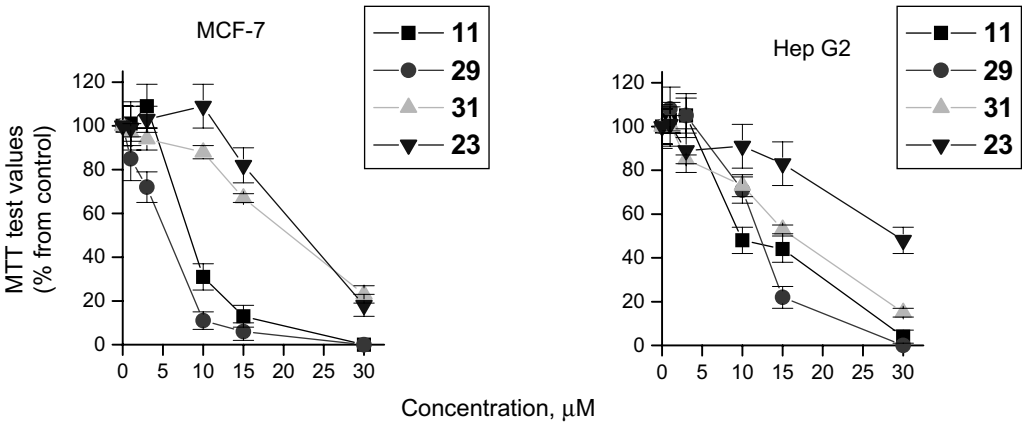


Figure 2. Effects of toxic compounds (group 1: **11**, **23**, **29**, **31**) on MCF-7 and Hep G2 cell viability according to MTT assay (details are given in Section 4).

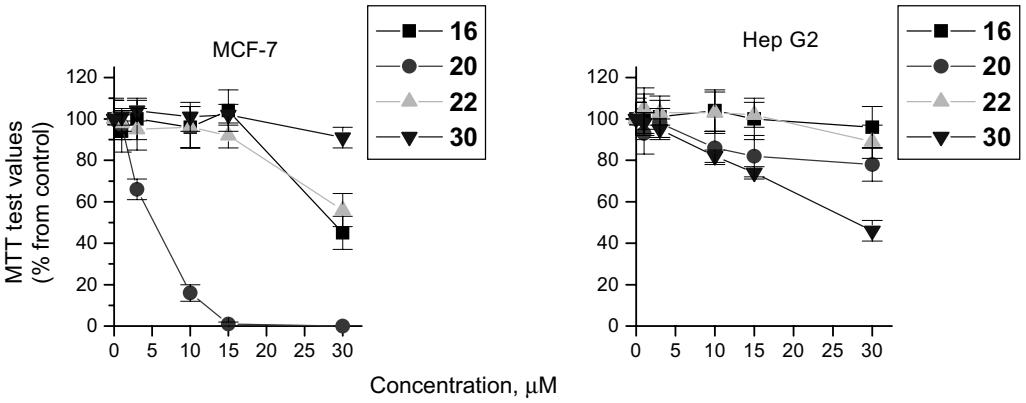


Figure 3. Effects of selectively toxic compounds (group 2: **16**, **20**, **22**, **30**) on MCF-7 and Hep G2 cell viability according to MTT assay (details are given in Section 4).

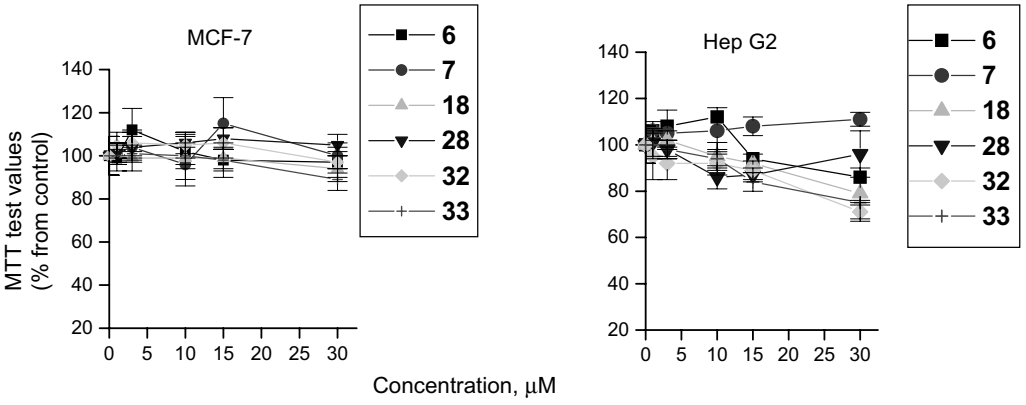


Figure 4. Effects of non toxic and slightly toxic compounds (group 3: **6**, **7**, **18**, **28**, **32**, **33**) on MCF-7 and Hep G2 cell viability according to MTT assay (details are given in Section 4).

Kieselgel plates from 'Merck'(detection of spots was performed by spraying plates with 3% $(NH_4)_2Mo_2O_7$ solution in 5% aqueous H_2SO_4 and/or with 5% $SbCl_3$ solution in dry $CHCl_3$, followed by heating).

^1H NMR and ^{13}C NMR spectra were registered on an AMX-III instrument (Bruker, 400 MHz) in $CDCl_3$; the values of δ $CHCl_3$ in ^1H NMR and ^{13}C NMR spectra were 7.25 ppm and 77.16 ppm, respectively. IR spectra were registered on a Pye Unicum SP 1000 instrument; UV spectra were registered on a Thermospectronic Helios α spectrophotometer in EtOH. Electron impact mass spectra (EIMS) were registered on a Kratos MS-890 instrument at the ionization energy of 70 eV. Trimethyl silyl (TMS) derivatives were prepared by treatment of 1 mg of sterol with $(CH_3)_3SiCl$/

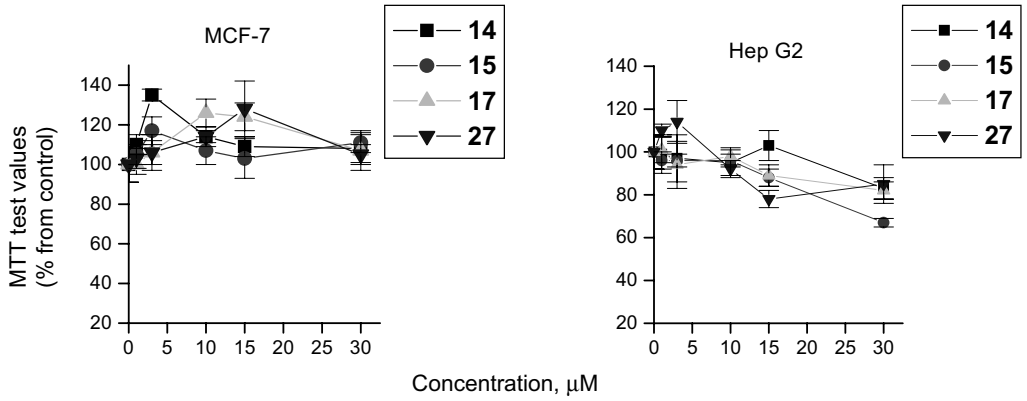


Figure 5. Effects of compounds increasing MTT values over the control (group 4: **14**, **15**, **17**, **27**) on MCF-7 and Hep G2 cell viability according to MTT assay (details are given in Section 4).

$(CH_3)_3SiNH/NHSi(CH_3)_3$/pyridine (1:2:10) mixture for 20 min at room temperature followed by passing of reaction mixture through silica gel microcolumn (1 cm^3) using hexane/EtOAc (7:1) mixture as eluent, and concentrating samples in a nitrogen flow. Electrospray ionization mass spectra (ESI-MS) were registered on a Agilent 1100 instrument in Nano-ESI off-line mode. High resolution electrospray ionization mass spectra (HR-ESI-MS) were obtained on a FT ICR MS Bruker 'Apex Qe' instrument.

The minimal energy conformers were calculated by semi-empirical method AM1 using a HyperChem 6.0 program.

4.1.1. (22*S*,23*S*)-22,23-Oxido-3α,5α-cyclo-6β-methoxystigmastane (2) and (22*R*,23*R*)-22,23-oxido-3α,5α-cyclo-6β-methoxystigmastane (3). The mixture of cyclosterol **1** (2.13 g, 5 mmol), $NaHCO_3$ (6.0 g) CPBA (2.60 g of 70% CPBA, 12 mmol), and $CHCl_3$ (50 ml) was heated under reflux for 90 min. After cooling toluene (200 ml) and 10% Na_2SO_3 solution (100 ml) were added, and the mixture was stirred until the complete dissolution. Aqueous layer was separated and extracted with toluene (2× 50 ml). The combined toluene extract was washed with water, dried over Na_2SO_4, evaporated, and the residue was applied onto a silica gel column (3.5 × 35 cm) equilibrated with hexane/$CHCl_3$ mixture (3:2). The column was washed with 100 ml of the same mixture, epoxides **2** and **3** were eluted one after another with linear gradient hexane/$CHCl_3$ (3:2)/$CHCl_3$ (300 ml). After evaporation of solvent both compounds were obtained as white waxy films homogeneous according to TLC. (22*S*, 23*S*)-22,23-Oxido-3α,5α-cyclo-6β-methoxystigmastane **2** (0.86 g, 1.95 mmol, 35%); ^{1}H NMR: 0.43 (1H, m, H-3), 0.65 (2H, m, H-4), 0.71 (3H, s, H-18), 0.92 (6H, d, J = 6.8 Hz, H-26 and H-27), 0.95 (3H, t, J = 7.5 Hz, H-29), 1.01 (3H, d, J = 6.8 Hz, H-21), 1.01 (3H, s, H-19), 2.49 (1H, dd, J = 2.2 and 5.9 Hz, H-22), 2.73 (1H, dd, J = 2.2 and 7.2 Hz, H-23), 2.76 (1H, m, H-6), 3.31 (3H, s, OCH_3); ^{13}C NMR: 12.40, 12.59, 13.26, 16.25, 19.41, 19.79, 20.32, 21.08, 21.63. 22.89, 24.59, 25.11, 28.18, 29.32, 30.70, 33.54, 35.31, 38.75, 40.27, 43.26, 43.57, 48.23, 48.48, 53.84, 56.34, 56.71, 58.68, 62.11, 62.31, 82.56; EIMS, *m*/*z* (I, %): 442 [M]+ (7), 427(41), 387(70), 341(11), 297(36), 253(59), 227(47); 213 (100). (22*R*,23*R*)-22,23-Oxido-3α,5α-cyclo-6β-methoxystigmastane **3** (0.80 g, 1.81 mmol, 32%); ^{1}H NMR: 0.42 (1H, m, H-3), 0.63 (2H, m, H-4), 0.70 (3H, s, H-18), 0.91 (3H, t, J = 7.5 Hz, H-29), 0.92 and 0.93 (each: 3H, d, J = 6.8 Hz, H-26 and H-27), 0.99 (3H, d, J = 6.8 Hz, H-21), 1.01 (3H, s, H-19), 2.50 (2H, m, H-22 and H-23), 2.76 (1H, m, H-6), 3.31 (3H, s, OCH_3); ^{13}C NMR: 12.53, 13.20, 16.38, 19.44, 19.55, 21.14, 21.74. 22.93, 24.59, 25.14, 27.36, 29.52, 30.55, 30.65, 33.55, 35.15, 38.94, 39.03, 40.38, 43.30, 43.58, 48.29, 48.96, 56.30, 56.40, 56.69, 58.69, 63.24, 68.33, 82.58; EIMS, *m*/*z* (I, %): 442 [M]+ (4), 427(9), 387(22), 341(19), 297(42), 253(39), 227(76), 213(100).

4.1.2. (22*S*,23*S*)-3β-Acetoxy-22,23-oxidostigmast-5-ene (4) and (22*R*,23*R*)-3β-acetoxy-22,23-oxidostigmast-5-ene (5). Epoxide **2** (440 mg, 1.0 mmol) was refluxed in glacial AcOH (10 ml) for 40 min, the solution was evaporated to dryness, the residue was dissolved in $CHCl_3$, washed with a saturated $NaHCO_3$ solution, dried over Na_2SO_4, evaporated, and the residue was recrystallized from CH_3CN to give compound **4** (420 mg, 0.9 mmol, 90%) as white needles (mp 95–97 °C) ^{1}H NMR: 0.67 (3H, s, H-18), 0.92 (6H, d, J = 6.8 Hz, H-26 and H-27), 0.95 (3H, t, J = 7.5 Hz, H-29), 1.01 (3H, s, H-19), 1.02 (3H, d, J = 6.8 Hz, H-21), 2.02 (3H, s, acetyl), 2.47 (1H, dd, J = 2.2 and 5.9 Hz, H-22), 2.73 (1H, dd, J = 2.2 and 7.2 Hz, H-23), 4.59 (1H, m, H-3), 5.36 (1H, m, H-6); ^{13}C NMR: 12.02, 12.64, 16.37, 19.46, 19.73, 20.34, 21.04, 21.16, 21.55, 24.71, 27.94, 28.12, 29.34, 32.06, 36.77, 37.17, 38.29, 38.88, 39.71, 42.82, 48.48, 50.20, 53.64, 56.49, 62.29, 63.37, 63.95, 74.11, 122.65, 139.91, 170.65. (22*R*,23*R*)-3β-Acetoxy-22,23-oxidostigmast-5-ene **5** was prepared from epoxide **3** by the same procedure. Compound **5**: yield 90%; mp 124–126 °C (from acetone); ^{1}H NMR: 0.66 (3H, s, H-18), 0.92 (3H, t, J = 7.5 Hz, H-29), 0.93 and 0.94 (each: 3H, d, J = 6.8 Hz, H-26 and H-27), 0.99 (3H, d, J = 6.8 Hz, H-21), 1.01 (3H, s, H-19), 2.02 (3H, s, acetyl), 2.50 (2H, m, H-22 and H-23), 4.59 (1H, m, H-3), 5.36 (1H, m, H-6); ^{13}C NMR: 12.14, 12.53, 14.35, 16.42, 19.55, 19.60, 20.41, 21.04, 21.18, 24.69, 27.24, 29.52, 29.85, 32.03, 36.80, 37.12, 38.29, 39.01, 39.83, 42.85, 48.96,

50.30, 53.82, 56.22, 58.70, 60.52, 62.26, 74.20, 122.77, 139.79, 170.66.

4.1.3. (22*S*,23*S*)-3β-Hydroxy-22,23-oxidostigmast-5-ene (6) and (22*R*,23*R*)-3β-hydroxy-22,23-oxidostigmast-5-ene (7). The mixture of compound **4** (235 mg, 0.5 mmol), K_2CO_3 (1.0 g) MeOH (5 ml), and water (3 ml) was heated under reflux for 30 min. After cooling the mixture was diluted with $CHCl_3$ (20 ml) and water (10 ml), chloroform extract was separated, dried over Na_2SO_4, evaporated, and the residue was recrystallized from acetone to give compound **6** (192 mg, 0.45 mmol, 90%) as white needles, mp 186–188 °C. Found (%): C-81.60; H-11.10. Calculated for $C_{29}H_{48}O_2$ (%): C-81.25; H-11.29. ^{1}H NMR: 67 (3H, s, H-18), 0.92 (6H, d, J = 6.8 Hz, H-26 and H-27), 0.95 (3H, t, J = 7.5 Hz, H-29), 1.00 (3H, s, H-19), 1.02 (3H, d, J = 6.8 Hz, H-21), 2.48 (1H, dd, J = 2.2 and 5.9 Hz, H-22), 2.74 (1H, dd, J = 2.2 and 7.2 Hz, H-23), 3.51 (1H, m, H-3), 5.34 (1H, m, H-6); ^{13}C NMR: 10.77, 11.38, 15.09, 18.30, 18.51, 19.09, 19.82, 19.97, 23.47, 26.87, 28.08, 30.59, 30.85, 30.79, 35.43, 36.19, 37.57, 38.53, 41.23, 41.57, 42.56, 49.06, 55.05, 55.33, 61.02, 61.05, 70.68, 120.46, 139.76; EIMS (TMS-derivative, *m*/*z*, I, %): 500 [M]+ (56), 485(3), 483(1), 482(2), 415(6), 410(40), 155(19), 127(100). (22*R*,23*R*)-3β-Hydroxy-22,23-oxidostigmast-5-ene **7** was prepared from compound **5** by the same procedure. Compound **7**: yield 92%; mp 147–149 °C (from CH_3CN). Found (%): C-81.40; H-11.45. Calculated for $C_{29}H_{48}O_2$ (%): C-81.25; H-11.29. ^{1}H NMR: 0.66 (3H, s, H-18), 0.92 and 0.93 (each: 3H, d, J = 6.8 Hz, H-26 and H-27), 0.92 (3H, t, J = 7.5 Hz, H-29), 0.99 (3H, s, H-19), 0.99 (3H, d, J = 6.8 Hz, H-21), 2.50 (2H, m, H-22 and H-23), 3.52 (1H, m, H-3), 5.34 (1H, m, H-6); ^{13}C NMR: 10.91, 11.29, 15.20, 18.30, 18.36, 19.15, 19.89, 20.02, 23.45, 26.01, 28.28, 30.61, 30.81, 30.85, 30.79, 35.44, 36.22, 37.78, 38.64, 41.26, 41.62, 42.59, 49.16, 55.00, 55.30, 61.97, 61.98, 70.71, 120.59, 139.65; EIMS (TMS-derivative, *m*/*z*, I, %): 500 [M]+ (30), 485(2), 483(1), 482(2), 415(3), 410(18), 155(24), 127(100).

4.1.4. (22*S*,23*R*)-3α,5α-Cyclo-6β-methoxy-22-iodo-23-acetoxystigmastane (8). Cyclosterol **1** (213 mg, 0.5 mmol) was dissolved in 20 ml of AcOH, then AcOAg (220 mg, 1.3 mmol) and water (1 ml) were added under vigorous stirring. Iodine (130 mg, 1.1 mmol) was then added in small portions during 20 min, and the mixture was stirred for 40 min more. The mixture was filtered, the residue was washed with toluene (3× 50 ml). The toluene extract was combined with filtrate, 30 ml of water was added. The toluene layer was separated, washed with saturated $NaHCO_3$ solution, then with water, dried over Na_2SO_4, and evaporated. Compound **8** (215 mg, 0.35 mmol, 65%) was isolated as a colorless glass-like film by flash chromatography on silica gel in hexane/EtOAc (9:1). ^{1}H NMR: 0.43 (1H, m, H-3), 0.64 (2H, m, H-4), 0.74 (3H, s, H-18), 0.84 and 0.92 (each: 3H, d, J = 6.8 Hz, H-26 and H-27), 0.96 (3H, d, J = 6.8 Hz, H-21), 0.96 (3H, t, J = 7.5 Hz, H-29), 1.01 (3H, s, H-19), 2.05 (3H, s, acetyl), 2.76 (1H, t, J = 1.5 Hz, H-6), 3.32 (3H, s, OCH_3), 4.36 (1H, dd, J = 7.5 and 1.0 Hz, H-23), 5.43 (1H, dd, J = 10.5 and 1.0 Hz, H-22); ^{13}C NMR: 12.61, 13.30, 13.38, 17.85, 18.38, 18.62, 19.38, 20.92, 21.56, 22.94, 23.48, 24.11, 25.11, 26.36, 27.57, 30.76, 33.54, 35.32, 36.81, 40.30, 42.84, 43.52, 47.86, 47.95, 48.12, 56.27, 56.52, 56.76, 74.24, 82.55, 169.90; EIMS, *m*/*z* (I, %): 612(4) [M]+; 597(15), 580(18), 557(34), 443(15), 393(100).

4.1.5. (22*R*,23*R*)-3β,22-Diacetoxystigmast-5-en-23-ol and (22*R*,23*R*)-3β,23-diacetoxystigmast-5-en-22-ol (mixture 9 + 10). The mixture of iodoacetate **8** (185 mg, 0.3 mmol), AcOAg (86 mg, 0.5 mmol), and glacial AcOH (10 ml) was heated under reflux for 1 h, evaporated, extracted with toluene, and filtered. The toluene extract was washed with saturated $NaHCO_3$, then with water, dried over Na_2SO_4, and evaporated. The mixture of compounds **9** and **10** (1:1, 105 mg, 0.2 mmol, 66%) was isolated as a colorless glass-like film by flash chromatography on silica gel in hexane/EtOAc (3:1). ^{1}H NMR: 0.68 (3H, s), 0.81 (3H, d, J = 6.6 Hz), 0.85 (3H, d, J = 6.6 Hz), 0.87 (3H, t, J = 7.5 Hz), 0.92 (3H, d, J = 6.8 Hz), 1.00 (3H, s), 1.08 (3H, d, J = 6.8 Hz), 2.02 (3H, s), 2.05 and 2.10 (both s, 3H), 3.66 and 4.91 (both m, 1H), 3.72 and 5.06 (both m, 1H), 4.59 (1H, m), 5.36 (1H, m).

4.1.6. (22*R*,23*R*)-3β,22,23-Trihydroxystigmast-5-ene (11). A mixture of compounds **9** and **10** (212 mg, 0.4 mmol) was refluxed with tenfold molar excess of K_2CO_3 in a MeOH/H_2O (2:1) mixture for 30 min. After cooling the mixture was extracted with $CHCl_3$, extract was dried over Na_2SO_4, evaporated, and the residue was recrystallized from CH_3CN to give triol **11** (162 mg 0.36 mmol, 91%) as white needles, mp 182–184 °C. Found (%): C-78.10; H-11.50. Calculated for $C_{29}H_{50}O_3$ (%): C-77.97; H-11.28; ^{1}H NMR: 0.71 (3H, s, H-18), 0.86 and 0.93 (each: 3H, d, J = 6.8 Hz, H-26 and H-27), 0.95 (3H, t, J = 7.5 Hz, H-29), 1.00 (3H, s, H-19), 1.02 (3H, d, J = 6.8 Hz, H-21), 3.51 (1H, m, H-3), 3.55–3.68 (2H, overlapped m, H-22 and H-23), 5.34 (1H, m, H-6); ^{13}C NMR: 11.70, 13.96, 14.10, 14.45, 17.72, 18.54, 19.30, 21.03, 21.67, 24.48, 26.87, 27.96, 29.61, 31.62, 31.79, 31.88, 37.21, 39.71, 42.25, 42.35, 49.60, 50.06, 52.64, 56.36, 70.65, 71.72, 72.30, 121.48, 140.76.

4.1.7. One-pot synthesis of (22*R*,23*R*)-3β,22,23-trihydroxysitost-5-ene (11) from stigmasterol. Tosyl chloride (8.64 g, 40 mmol) was added to a solution of stigmasterol (8.24 g, 20 mmol) in anhydrous pyridine (50 ml). The mixture was stirred for 14 h, poured into a mixture of saturated $NaHCO_3$ (500 ml) and ice (100 g), stirred for 2 h, and the precipitate was separated. The aqueous solution was extracted with toluene (2× 200 ml). The precipitate and toluene extract were combined, the resulting solution was washed with saturated Na_2SO_4, dried with Na_2SO_4, evaporated to a volume of 40 ml, and slowly poured into a boiled solution of AcONa (20 g) in MeOH (250 ml). The mixture was heated under reflux for 1 h and then evaporated. The residue was treated with toluene (200 ml) and water (50 ml), toluene solution was washed with water (2× 50 ml), dried over Na_2SO_4, evaporated, the residue was dissolved in 95% AcOH (40 ml) and AcOAg (6.72 g, 40 mmol) was added. Iodine powder (5.04 g, 40 mmol) was added to the mix-

ture in portions for 20 min during vigorous stirring. The mixture was stirred at room temperature for 40 min, then Ac_2O (15 ml) and AcOAg (3.36 g, 20 mmol) were added, and the mixture was refluxed under stirring for 1 h. After cooling the mixture was filtered, the filtrate was evaporated to dryness. The residue was dissolved in MeOH (100 ml), then K_2CO_3 and water (40 ml) were added, and the mixture was refluxed under stirring for 40 min. After cooling, $CHCl_3$ (200 ml) and water (50 ml) were added, the chloroform layer was separated, and the aqueous layer was extracted with $CHCl_3$/MeOH (9:1) mixture, The combined extract was dried over Na_2SO_4 and evaporated. The residue was treated with boiling light petroleum (40 ml) and stored for 14 h at room temperature. The resulting precipitate was twice recrystallized from a minimal volume of acetone to give 2.45 g (5.5 mmol, 28%) of compound **11**, completely identical to authentic sample (prepared in Section 4.1.6) according to TLC, mp, and ^1H and ^{13}C NMR.

4.1.8. (22*R*,23*R*)-3β,22,23-Triacetoxystigmast-5-ene (12). A mixture of compound **11** (446 mg, 1.0 mmol), Ac_2O (2 ml), and anhydrous pyridine (5 ml) was refluxed for 1 h, cooled, diluted with pyridine (10 ml) and methanol (5 ml), stored for 10 min, evaporated with adding of water, and the residue was dried in vacuo to give triacetate **12** (570 mg, 1.0 mmol, quantitative); ^1H NMR: 0.66 (3H, s, H-18), 0.91 (6H, d, J = 6.8 Hz, H-26 and H-27), 0.92 (3H, t, J = 7.5 Hz, H-29), 0.99 (3H, s, H-21), 2.02, 2.03 and 2.08 (each: 3H, s, acetyl), 4.58 (1H, m, H-3), 5.03 and 5.24 (each: 1H, m, H-22 and H-23), 5.36 (1H, m, H-6); ^{13}C NMR: 11.80, 14.44, 17.92, 18.85, 19.44, 21.15, 21.41, 21.55, 22.52, 24.64, 27.37, 27.76, 27.93, 29.84, 31.93, 32.02, 36.71, 37.14, 38.28, 39.21, 39.67, 43.03, 47.86, 50.09, 52.15, 56.21, 68.34, 72.36, 74.11, 75.49, 128.95, 139.78, 170.50, 170.53, 170.64.

4.1.9. (22*R*,23*R*)-3β-Hydroxy-22,23-isopropylidenedioxystigmast-5-en (13). A mixture of triol **12** (134 mg, 0.3 mmol), 2,2-dimethoxypropane (3 ml), and TsOH (5 mg) was stirred for 10 min, diluted with $CHCl_3$ (20 ml), then the solution was washed with saturated $NaHCO_3$ (2× 5 ml), dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography on a silica gel column in hexane/acetone (3:1) to obtain acetonide **13** (132 mg, 0.27 mmol, 90%) as colorless solid film. ^1H NMR: 0.69 (3H, s, H-18), 0.94 and 0.94 (each: 3H, d, J = 6.8 Hz, H-26 and H-27), 0.95 (3H, t, J = 7.2 Hz, H-29), 0.99 (3H, s, H-19), 1.02 (3H, d, J = 6.8 Hz, H-21), 1.36 (6H, s, isopropylidene), 3.51 (1H, m, H-3), 3.91 (1H, dd, J = 8.7 and 3.4 Hz) and 3.98 (1H, dd, J = 8.7 and 2.1 Hz, H-22 and H-23), 5.34 (1H, m, H-6); ^{13}C NMR: 11.57, 13.41, 14.24, 18.80, 19.53, 19.80, 21.25, 22.84, 23.49, 24.71, 26.97, 27.45, 27.72, 28.46, 29.85, 31.84, 32.00, 32.12, 37.45, 38.63, 39.95, 42.48, 43.22, 46.66, 50.27, 53.01, 56.64, 71.96, 80.02, 106.77, 121.75, 140.94.

4.1.10. (22*S*,23*S*)-22,23-Oxidostigmast-4-en-3-one (14), (22*R*,23*R*)-22,23-oxidostigmast-4-en-3-one (15), and (22*R*,23*R*)-22,23-dihydroxystigmast-4-en-3-one (16). Compound **6** (4.5 mg, 10 μmol) in 40 μl of *i*-PrOH was added to the mixture of cholesterol oxidase (1.2 U) and peroxidase (9.1 U) in 10 ml of 0.17 M sodium phosphate buffer (pH 7.5) containing 20 mM NaCl and 6 mM sodium cholate. The mixture was incubated at 37 °C for 6 h, thereafter MeOH (10 ml) and $CHCl_3$ (20 ml) were added. Chloroform layer was separated, dried over Na_2SO_4, evaporated, and the product **14** (3.4 mg, 8 μmol, 80%) was isolated by TLC in hexane/EtOAc (7:1) as a colorless transparent film. HR-ESI-MS calculated for $C_{29}H_{45}O_2^+$: 427.3576, found 427.3559. UV (λ_{max}, ε): 240 nm (6 100); 1H NMR: 0.70 (3H, s, H-18), 0.92 (6H, d, J = 6.8 Hz, H-26 and H-27), 0.95 (3H, t, J = 7.5 Hz, H-29), 1.02 (3H, d, J = 6.6 Hz, H-21), 1.17 (3H, s, H-19), 2.47 (1H, dd, J = 5.4 and 2.2 Hz, H-22), 2.73 (1H, dd, J = 2.2 and 7.5 Hz, H-23); 5.72 (1H, s, H-4); ^{13}C NMR: 12.11, 12.57, 16.21, 19.79, 20.29, 21.10, 21.19, 22.81, 24.59, 28.06, 29.34, 29.83, 32.20, 33.04, 34.12, 35.89, 38.72, 39.65, 42.90, 48.49, 53.67, 53.99, 55.74, 62.07, 62.17, 124.00, 129.96, 199.52; ESI-MS (*m*/*z*, I, %) 427.2 [M+1] (100), 381.1(19), 353.1(57), 301.0(56). (22*R*,23*R*)-22,23-Oxidostigmast-4-en-3-one **15** was prepared from compound **7** by the same procedure. Compound **15**: yield 80%. HR-ESI-MS calculated for $C_{29}H_{45}O_2^+$: 427.3576, found 427.3562. UV (λ_{max}, ε): 240 nm (6 100); 1H NMR: 0.70 (3H, s, H-18), 0.92 (3H, t, J = 7.5 Hz, H-29), 0.94 (6H, d, J = 6.8 Hz, H-26 and H-27), 1.00 (3H, d, J = 6.6 Hz, H-21), 1.17 (3H, s, H-19), 2.50 (2H, m, H-22 and H-23), 5.73 (1H, s, H-4); ^{13}C NMR: 12.25, 12.52, 16.42, 17.54, 19.55, 19.57, 21.13, 21.19, 24.55, 27.16, 29.51, 29.84, 32.19, 33.10, 34.10, 35.80, 35.86, 39.00, 39.69, 42.91, 48.94, 54.03, 55.65, 56.20, 58.80, 63.16, 123.92, 129.94, 195.30; ESI-MS (*m*/*z*, I, %) 427.2 [M+1] (46), 381.1(47), 353.1(100), 301.0(96). (22*R*,23*R*)-22,23-Dihydroxystigmast-4-en-3-one **16** was prepared from compound **11** by the same procedure, except isolation of product was carried out by TLC in hexane/acetone (4:1). Compound **16**: yield 80%; HR-ESI-MS calculated for $C_{29}H_{49}O_3^+$: 445.3682, found 445.3670. UV (λ_{max}, ε): 240 nm (6 000); 1H NMR: 0.71 (3H, s, H-18), 0.86 and 0.93 (each: 3H, d, J = 6.8 Hz, H-26 and H-27), 0.95 (3H, t, J = 7.5 Hz, H-29), 1.02 (3H, d, J = 6.8 Hz, H-21), 1.18 (3H, s, H-19), 3.55–3.64 (2H, overlaped m, H-22 and H-23), 5.72 (1H, s, H-4); ^{13}C NMR: 9.30, 11.56, 14.83, 16.06, 18.50, 19.16, 20.39, 21.23, 21.86, 24.38, 25.42, 26.38, 27.85, 29.47, 31.40, 33.11, 33.18, 36.24, 37.08, 39.78, 50.15, 51.23, 53.01, 65.63, 68.16, 69.77, 126.24, 128.26, 196.89; ESI-MS (*m*/*z*, I, %) 441.2 [M+1] (2), 423.1(62), 413.1(100).

4.1.11. (22*S*,23*S*)-22,23-Oxidostigmast-4-en-3,6-dione (17), (22*R*,23*R*)-22,23-oxidostigmast-4-en-3,6-dione (18), and (22*R*,23*R*)-22,23-isopropylidenedioxystigmast-4-en-3,6-dione (19). Compound **6** (89 mg, 0.2 mmol) in 5 ml of CH_2Cl_2 was added to the complex CrO_3*2Py (1.2 mmol CrO_3) in 12 ml of CH_2Cl_2 and the mixture was stirred at room temperature for 1 h. Thereafter EtOH (7 ml) was added, and the mixture was stirred for 10 min more. Silica gel (3 g) was added and the mixture was evaporated to dryness. The residue was applied onto a top of silica gel column, product **6** was eluted with a hexane/EtOAc (5:1) mixture, concentrated, and purified by preparative

TLC in a hexane/EtOAc (9:1) mixture to obtain dione **17** (52 mg, 0.14 mmol, 80%) as a colorless oil which was slowly crystallized during the storage. Compound **17** HR-ESI-MS calculated for $C_{29}H_{45}O_3^+$: 441.3369, found 441.3350. IR (KBr, cm^{-1}): 1685 (C=O); UV (λ_{max}, ε): 249 nm (7400); ^1H NMR: 0.73 (3H, s, H-18), 0.84 and 0.85 (each: 3H, d, J = 6.8 Hz, H-26 and H-27), 0.88 (3H, t, J = 7.5 Hz, H-29), 0.93 (3H, d, J = 6.8 Hz, H-21), 1.16 (3H, s, H-19), 2.50 (1H, dd, J = 2.2 and 5.9 Hz, H-22), 2.75 (1H, dd, J = 2.2 and 7.2 Hz, H-23); 6.18 (1H, s, H-4); ^{13}C NMR: 12.04, 12.56, 16.17, 17.70, 19.81, 20.29, 24.37, 27.25, 27.90, 29.36, 30.20, 30.58, 32.94, 34.10, 34.37, 35.74, 37.28, 38.60, 39.16, 46.86, 48.49, 51.14, 53.52, 56.39, 62.04, 129.01, 131.00, 199.36, 202.05; ESI-MS (*m*/*z*, I, %) 441.2 [M+1] (45), 381.1(42), 353.1(100), 301.0(43). (22*R*,23*R*)-22,23-Oxidostigmast-4-en-3,6-dione **18** was prepared from compound **7** by the same procedure. Compound **18**: yield 82%. HR-ESI-MS calculated for $C_{29}H_{45}O_3^+$: 441.3369, found 441.3365. IR (KBr, cm^{-1}): 1685 (C=O); UV (λ_{max}, ε): 250 nm (7600); ^1H NMR: 0.71 (3H, s, H-18), 0.84 and 0.94 (each: 3H, d, J = 6.8 Hz, H-26 and H-27), 0.87 (3H, t, J = 7.5 Hz, H-29), 1.02 (3H, d, J = 6.8 Hz, H-21); 1.16 (3H, s, H-21); 2.50 (2H, m, H-22 and H-23); 6.17 (1H, s, H-4); ^{13}C NMR: 12.20, 14.15, 16.48, 17.66, 19.57, 21.08, 21.19, 23.97, 24.36, 26.98, 29.11, 30.58, 34.12, 34.37, 35.76, 38.95, 39.24, 46.87, 48.94, 51.25, 56.11, 56.36, 58.79, 62.94, 66.34, 125.67, 130.97, 199.36, 202.04; ESI-MS (*m*/*z*, I, %) 441.2 [M+1] (19), 381.1(51), 353.1(100), 301.0(49). (22*R*,23*R*)-22,23-Isopropilidenedioxystigmast-4-en-3,6-dione **19** was prepared from compound **13** by the same procedure, except the oxidation was carried out during 4 h. Compound **19**: yield 83%, mp 136–139 °C (from acetone/hexane, 1:5), ^1H NMR: 0.75 (3H, s, H-18), 0.95 (6H, d, J = 6.8 Hz, H-26 and H-27), 0.96 (3H, t, J = 7.5 Hz, H-29), 1.03 (3H, d, J = 6.8 Hz, H-21), 1.16 (3H, s, H-19), 1.36 (6H, s, isopropylidene), 3.89 (1H, dd, J = 8.7 and 3.4 Hz) and 3.97 (1H, dd, J = 8.7 and 1.8 Hz, H-22 and H-23), 6.17 (1H, s, H-4).

4.1.12. (22*R*,23*R*)-22,23-Dihydroxystigmast-4-en-3,6-dione (20). The mixture of acetonide **19** (50 mg, 0.1 mmol), AcOH (4 ml), water (1 ml), and TsOH (2 mg) was heated under reflux for 90 min, diluted with $CHCl_3$ (20 ml) and water (10 ml). Chloroform layer was separated, washed with saturated $NaHCO_3$ solution, dried over Na_2SO_4, and evaporated. The residue was purified by silica gel flash chromatography in hexane/acetone (3:1) to give compound **20** (41 mg, 0.89 mmol, 89%) as colorless glass. HR-ESI-MS calculated for $C_{29}H_{49}O_3^+$: 459.3474, found 459.3482. IR (KBr, cm^{-1}): 1680 (C=O); UV (λ_{max}, ε): 249 nm (7600); ^1H NMR: 0.76 (3H, s, H-18), 0.88 and 0.94 (each: 3H, d, J = 6.8 Hz, H-26 and H-27), 0.96 (3H, t, J = 7.2 Hz), 1.04 (3H, d, J = 6.8 Hz, H-21); 1.16 (3H, s, H-19); 3.60 (2H, m, H-22 and H-23); 6.17 (1H, s, H-4); ^{13}C NMR: 11.96, 14.39, 14.41, 17.69, 17.97, 18.85, 21.08, 21.85, 24.38, 27.13, 27.96, 29.82, 34.10, 34.38, 35.76, 39.33, 42.33, 43.27, 46.83, 49.84, 51.16, 52.77, 56.40, 70.98, 72.54, 126.95, 130.95, 199.33, 202.00.

4.1.13. (22*R*,23*R*)-3β,22,23-Triacetoxy-5α,6α-oxidostigmastane (21) and (22*R*,23*R*)-3β,22,23-trihydroxy-5α,6α-oxidostigmastane (22). Triacetate **12** (106 mg, 0.2 mmol) was dissolved in CH_2Cl_2 (20 ml), 70% mCPBA (65 mg, 0.25 mmol) was added, and the mixture was stirred for 30 min. The resulting solution was washed with saturated Na_2SO_3 solution (5 ml), then with saturated $NaHCO_3$ solution (10 ml), dried over Na_2SO_4, evaporated, and the residue was separated by preparative TLC in $CHCl_3$/acetone (49:1). The resulting compound **21** was dissolved in Et_2O (5 ml) and added dropwise to stirred suspension of $LiAlH_4$ (40 mg, 1 mmol) in Et_2O (10 ml) at 0 °C. The mixture was stirred for 5 min at 0 °C, the excess of $LiAlH_4$ was decomposed by water, and the residue was extracted with Et_2O (4× 5 ml). The combined ethereal solution was dried over Na_2SO_4 and evaporated. The residue was purified by preparative TLC in hexane/acetone (3:1) to obtain compound **22** (50 mg, 0.12 mmol, 60% based on **12**) as colorless glass. Found (%): C-75.50; H-11.00. Calculated for $C_{29}H_{52}O_4$ (%): C-75.28; H-10.89. ^1H NMR: 0.65 (3H, s, H-18), 0.87 and 0.94 (each: 3H, d, J = 6.8 Hz, H-26 and H-27), 0.95 (3H, t, J = 7.5 Hz, H-29), 1.00 (3H, d, J = 6.8 Hz, H-21), 1.05 (3H, s, H-19), 2.89 (1H, d, J = 4.0 Hz, H-6), 3.56-3.63 (2H, m, H-22 and H-23), 3.90 (1H, m, H-3); ^{13}C NMR: 11.11, 11.94, 14.24, 17.97, 18.76, 20.84, 21.93, 22.84, 23.95, 27.25, 28.05, 29.51, 32.08, 32.60, 37.27, 38.95, 39.59, 40.06, 42.53, 42.76, 46.66, 49.84, 52.62, 56.72, 59.33, 68.35, 68.91, 70.83, 72.50.

4.1.14. (22*R*,23*R*)-3β,5α,6β,22,23-Pentahydroxystigmastane (23). The mixture of triacetate **12** (106 mg, 0.2 mmol), HCOOH (1 ml), and 30% H_2O_2 (1 ml) was stirred for 30 min at room temperature, evaporated to the volume of 3 ml, and diluted with $CHCl_3$ (30 ml). Then water (5 ml) and $NaHCO_3$ (2.5 g) were added, the chloroform layer was separated, and the aqueous layer was extracted with $CHCl_3$/MeOH (9:1) (3× 30 ml). The combined chloroform extract was washed with saturated $NaHCO_3$ (10 ml), then with saturated Na_2SO_4 (10 ml), dried over Na_2SO_4, and evaporated. The residue was dissolved in 2 ml of MeOH, then K_2CO_3 (400 mg) and water (1.2 ml) were added to the solution and the mixture was heated under reflux for 40 min. After cooling $CHCl_3$ (4 ml) and water (4 ml) were added, chloroform layer was separated, and aqueous layer was extracted with $CHCl_3$/MeOH (9:1) (3× 10 ml). The combined chloroform solution was dried over Na_2SO_4, evaporated, and the residue was purified by silica gel flash chromatography in $CHCl_3$/MeOH (15:1) mixture. After the recrystallization from mixture hexane/acetone (3:1) compound **23** (71 mg, 0.16 mmol, 79%) was obtained. Found (%): C-72.55; H-11.05. Calculated for $C_{29}H_{52}O_5$ (%): C-72.46; H-10.90; mp 200–201 °C (from hexane/acetone, 3:1); ^1H NMR: 0.72 (3H, s, H-18), 0.87 and 0.94 (each: 3H, d, J = 6.8 Hz, H-26 and H-27), 0.95 (3H, t, J = 7.5 Hz, H-29), 1.02 (3H, d, J = 6.8 Hz, H-21), 1.18 (3H, s, H-19), 3.53 (1H, broad t, J = 2.5 Hz, H-6), 3.59-3.63 (2H, m, H-22 and H-23), 4.08 (1H, m, H-3); ^{13}C NMR: 12.20, 14.25, 14.48, 16.96, 17.99, 18.77, 21.34, 21.91, 24.53, 27.09, 28.19, 29.81, 30.41, 31.04, 32.52, 34.71, 40.13, 40.98, 42.55, 43.46, 46.00, 49.88, 52.96, 55.82, 67.75, 68.34, 70.85, 72.56, 76.25.

4.1.15. (22*S*,23*S*)-3β-Acetoxy-22,23-oxidostigmast-5-en-7-one (24), (22*R*,23*R*)-3β-acetoxy-22,23-oxidostigmast-5-en-7-one (25), and (22*R*,23*R*)-3β,22,23-triacetoxystigmast-5-en-7-one (26). Finely ground $K_2Cr_2O_7$ (0.36 g, 1.20 mmol) was added to a solution of (22*S*,23*S*)-3β-acetoxy-22,23-oxidostigmast-5-ene **4** (0.5 g, 1.05 mmol) in a mixture of AcOH (10 ml) and Ac_2O (10 ml). The mixture was stirred at 50 °C for 50 min, then poured in toluene (150 ml) and shaken for 5 min. Then the toluene extract was filtered through paper into vigorously stirred saturated $NaHCO_3$ solution (100 ml). The toluene layer was separated, the aqueous layer was extracted with toluene (100 ml), the combined toluene extract was washed with water, dried over Na_2SO_4, and evaporated. The residue was dissolved in boiled $CHCl_3$ (5 ml), diluted with boiled hexane (40 ml), and stored for 24 h at −5 °C to give 220 mg of compound **24**. The solution was concentrated and the residue was separated by silica gel flash chromatography in hexane/EtOAc (5:1) to obtain additional 120 mg of compound **24**. The combined product was recrystallized from hexane to give (22*S*,23*S*)-3β-acetoxy-22,23-oxidostigmast-5-en-7-one **24** (0.32 g, 0.68 mmol, 64%); mp 132–134 °C; IR (KBr, cm^{-1}): 1735, 1680 (C=O); ^{1}H NMR 0.67 (3H, s, H-18), 0.91 (6H, d, *J* = 6.8 Hz, H-26 and H-27), 0.95 (3H, t, *J* = 7.5 Hz, H-29), 1.04 (3H, d, *J* = 6.8 Hz, H-21), 1.20 (3H, s, H-19), 2.05 (3H, s, acetyl), 2.47 (1H, dd, *J* = 2.2 and 5.9 Hz, H-22), 2.74 (1H, dd, *J* = 2.2 and 7.2 Hz, H-23); 4.50 (1H, m, H-3), 5.70 (1H, d, *J* = 1.2 Hz, H-6). (22*R*,23*R*)-3β-Acetoxy-22,23-oxidostigmast-5-en-7-one **25** was prepared from (22*R*,23*R*)-3β-acetoxy-22,23-oxidostigmast-5-ene **5** by the same procedure. Compound **25**: yield 58%; mp 144–146 °C; IR (KBr, cm^{-1}): 1735, 1680 (C=O); ^{1}H NMR: 0.67 (3H, s, H-18), 0.91 (6H, d, *J* = 6.8 Hz, H-26 and H-27), 0.93 (3H, t, *J* = 7.5 Hz, H-29), 0.99 (3H, d, *J* = 6.8 Hz, H-21); 1.20 (3H, s, H-19); 2.04 (3H, s, acetyl); 2.45–2.60 (2H, m, H-22 and H-23), 4.70 (1H, m, H-3), 5.69 (1H, d, *J* = 1.2 Hz, H-6). (22*R*,23*R*)-3β,22,23-Triacetoxystigmast-5-en-7-one **26** was prepared from (22*R*,23*R*)-triacetoxystigmast-5-ene **12** by the same procedure. Compound **26**: yield 65%; IR (KBr, cm^{-1}): 1735, 1680 (C=O); ^{1}H NMR: 0.67 (3H, s, H-18), 0.83 and 0.92 (each: 3H, d, *J* = 6.8 Hz, H-26 and H-27), 0.92 (3H, t, *J* = 7.5 Hz, H-29), 0.95 (3H, d, *J* = 6.8 Hz, H-21), 1.19 (3H, s, H-19), 2.03, 2.04 and 2.07 (each: 3H, s, acetyl), 4.71, 5.05 and 5.24 (each: ^{1}H, m, H-3, H-22, H-23), 5.70 (1H, br s, H-6); ^{13}C NMR: 11.93, 14.07, 14.35, 17.41, 17.90, 18.81, 21.29, 21.37, 21.42, 22.51, 26.58, 27.37, 27.51, 28.15, 29.84, 36.17, 37.91, 38.64, 39.27, 43.81, 45.50, 48.00, 49.64, 49.89, 50.09, 50.87, 72.18, 72.34, 75.31, 126.83, 163.95, 170.46, 170.48, 170.49, 201.63.

4.1.16. (22*S*,23*S*)-3β-Hydroxy-22,23-oxidostigmast-5-en-7-one (27), (22*R*,23*R*)-3β-hydroxy-22,23-oxidostigmast-5-en-7-one (28), and (22*R*,23*R*)-3β,22,23-trihydroxystigmast-5-en-7-one (29). Acetates **24**, **25**, and **26** (0.2 mmol each) were refluxed with a tenfold molar excess of K_2CO_3 in MeOH/water (2:1) mixture for 30 min. Products were extracted with $CHCl_3$/MeOH mixture (9:1), extracts were dried over Na_2SO_4 and evaporated. (22*S*,23*S*)-3β-hydroxy-22,23-oxidostigmast-5-en-7-one **27** was isolated by silica gel flash chromatography in hexane/EtOAc (3:2) as white solid in 92% yield. HR-ESI-MS calculated for $C_{29}H_{47}O_3^+$: 443.3525, found 443.3540. ^{1}H NMR: 0.68 (3H, s, H-18), 0.92 (6H, d, *J* = 6.8 Hz, H-26 and H-27), 0.95 (3H, t, *J* = 7.5 Hz, H-29), 1.04 (3H, d, *J* = 6.8 Hz, H-29), 1.19 (3H, s, H-19), 2.48 (1H, dd, *J* = 2.2 and 5.7 Hz, H-22), 2.74 (1H, dd, *J* = 2.2 and 7.2 Hz, H-23), 3.67 (1H, m, H-3), 5.69 (1H, d, *J* = 1.2 Hz, H-6); ^{13}C NMR: 10.88, 11.40, 16.24, 18.33, 18.47, 19.50, 19.75, 20.12, 25.46, 27.24, 28.11, 30.13, 35.29, 35.92, 37.18, 37.47, 37.60, 42.35, 43.75, 44.31, 48.86, 55.75, 56.44, 61.01, 61.16, 69.44, 124.95, 164.02, 200.79; EIMS (TMS-derivative, *m*/*z*, I, %): 514 [M]+ (100), 499(3), 497(4), 496(2), 429(61), 155(5), 127(15). (22*R*,23*R*)-3β-Hydroxy-22,23-oxidostigmast-5-en-7-one **28** was isolated by silica gel flash chromatography in hexane/EtOAc (3:2) as white solid in 90% yield. HR-ESI-MS calculated for $C_{29}H_{47}O_3^+$: 443.3525, found 443.3507. ^{1}H NMR: 0.67 (3H, s, H-18), 0.91 (3H, t, *J* = 7.5 Hz, H-29), 0.92 and 0.93 (each: 3H, d, *J* = 6.8 Hz, H-26 and H-27), 0.99 (3H, d, *J* = 6.8 Hz), 1.19 (3H, s, H-19), 2.49 (2H, m, H-22 and H-23), 3.67 (1H, m, H-3), 5.69 (1H, d, *J* = 1.2 Hz, H-6); ^{13}C NMR: 11.05, 11.29, 16.22, 18.31, 18.34, 19.58, 19.90, 20.14, 25.44, 26.18, 28.28, 30.08, 35.31, 37.25, 37.44, 35.96, 37.52, 42.36, 43.72, 44.36, 48.93, 55.89, 56.58, 61.64, 61.66, 69.45, 125.02, 163.87, 200.86; EIMS (TMS-derivative, *m*/*z*, I, %): 514 [M]+ (45), 499(2), 497(2), 496(2), 429(47), 155(23), 127(100). (22*R*,23*R*)-3β,22,23-Trihydroxystigmast-5-en-7-one **29** was isolated by recrystallization from EtOAc/hexane (1:2) in 90% yield. Mp 136 °C. Found (%): C-76.05; H-10.39. Calculated for $C_{29}H_{48}O_4$ (%): C-75.61; H-10.50; ^{1}H NMR: 0.72 (3H, s, H-18), 0.93 and 0.95 (each: 3H, d, *J* = 6.8 Hz, H-26 and H-27), 0.95 (3H, t, *J* = 7.5 Hz, H-29), 1.03 (3H, d, *J* = 6.8 Hz, H-21), 1.20 (3H, s, H-19), 3.55–3.72 (3H, m, H-3, H-22 and H-23), 5.68 (1H, d, *J* = 1.2 Hz, H-6); ^{13}C NMR: 12.05, 14.25, 14.37, 14.60, 17.44, 17.93, 18.73, 21.37, 21.92, 26.66, 27.09, 28.50, 31.50, 36.54, 37.44, 38.83, 42.00, 42.59, 43.75, 45.57, 49.78, 50.70, 51.56, 70.66, 70.76, 72.29, 126.15, 165.39, 202.28.

4.1.17. (22*S*,23*S*)-3β,7α-Dihydroxy-22,23-oxidostigmast-5-ene (30), (22*R*,23*R*)-3β,7α-dihydroxy-22,23-oxidostigmast-5-ene (31), (22*S*,23*S*)-3β,7β-dihydroxy-22,23-oxidostigmast-5-ene (32), and (22*R*,23*R*)-3β,7β-dihydroxy-22,23-oxidostigmast-5-ene (33). The solution of ketosteryl acetate **24** (50 mg, 0.1 mmol) in anhydrous Et_2O (8 ml) was added dropwise to stirred suspension of $LiAlH_4$ (100 mg) in anhydrous Et_2O (20 ml) at 0 °C. After 5 min excess of $LiAlH_4$ was decomposed by adding ice, the ethereal solution was separated, and the residue was extracted with Et_2O containing 5% MeOH (3× 15 ml). The combined ethereal solution was evaporated, and the residue was dissolved in 1 ml of Et_2O/benzene/cyclohexane mixture (90:9:1). The solution was applied onto column packed with Silasorb 600 (30 μm) equilibrated with the same mixture, and compounds **30** and **32** were eluted one after another by the same mixture. After evaporation, the products were obtained as white wax-like films. (22*S*,23*S*)-3β,7α-Dihydroxy-22,23-oxidostigmast-5-ene **30** (27 mg, 0.06 mmol, 60%): HR-ESI-

MS calculated for $C_{29}H_{49}O_3^+$: 445.3682; found 445.3659. ^{1}H NMR: 0.68 (3H, s, H-18); 0.91 (6H, d, J = 6.8 Hz, H-26 and H-27); 0.95 (3H, t, J = 7.5 Hz, H-29); 0.98 (3H, s, H-19); 1.05 (3H, d, J = 6.8 Hz, H-21); 2.47 (1H, dd, J = 2.2 and 5.7 Hz, H-22); 2.73 (1H, dd, J = 2.2 and 7.5 Hz); 3.57 (1H, m, H-3); 3.84 (1H, m H-7); 5.60 (1H, dd, J = 1.3 and 5.4 Hz, H-6); ^{13}C NMR: 11.96, 12.56, 16.30, 19.27, 19.72, 20.30, 21.07, 21.18, 26.74, 28.37, 29.29, 29.81, 31.72, 36.60, 37.09, 38.63, 39.52, 41.08, 41.86, 43.37, 48.49, 55.60, 55.76, 62.20, 62.24, 71.56, 73.44, 125.60, 143.67; EIMS (TMS-derivative, *m/z*, I, %): 588 [M]+ (31), 573(3), 571(1), 570(1), 498(100), 408(47), 155(3), 127(8). (22*S*,23*S*)-3β,7β-Dihydroxy-22,23-oxidostigmast-5-ene **32** (18 mg, 0.04 mmol, 40%): HR-ESI-MS calculated for $C_{29}H_{49}O_3^+$: 445.3682; found 445.3669. ^{1}H NMR: 0.69 (3H, s, H-18); 0.91 (6H, d, J = 6.8 Hz, H-26 and H-27); 0.95 (3H, t, J = 7.5 Hz, H-29); 1.02 (3H, s, H-21); 2.49 (1H, dd, J = 2.2 and 5.7 Hz, H-22); 2.74 (1H, dd, J = 2.2 and 7.5 Hz, H-23); 3.54 (1H, m, H-3); 3.83 (1H, dt, J = 1.3 and 7.8 Hz, H-7); 5.28 (1H, t, J = 1.3 Hz, H-6); ^{13}C NMR: 12.09, 12.50, 16.51, 19.26, 19.53, 20.24, 21.10, 21.21, 26.69, 27.45, 29.48, 29.88, 31.71, 36.60, 37.12, 38.91, 39.64, 41.05, 41.87, 43.41, 48.71, 55.59, 55.72, 63.15, 63.16, 71.54, 73.43, 125.65, 143.55; EIMS (TMS-derivative, *m/z*, I, %): 588 [M]+ (41), 573(3), 571(2), 570(1), 498(68), 408(100), 155(82), 127(37). (22*R*,23*R*)-3β,7α-Dihydroxy-22,23-oxidostigmast-5-ene **31** and (22*R*,23*R*)-3β,7β-dihydroxy-22,23-oxidostigmast-5-ene **33** were prepared from compound **25** (50 mg, 0.1 mmol) using the same procedure. (22*R*,23*R*)-3β,7α-Dihydroxy-22,23-oxidostigmast-5-ene **31** (26 mg, 0.06 mmol, 60%). HR-ESI-MS calculated for $C_{29}H_{49}O_3^+$: 445.3682; found 445.3661. ^{1}H NMR: 0.67 (3H, s, H-18); 0.92 (3H, t, J = 7.5 Hz, H-29); 0.93 (6H, d, J = 6.8 Hz, H-26 and H-27); 0.98 (3H, s, H-19); 1.01 (3H, d, J = 6.8 Hz, H-21); 2.50 (2H, m, H-22 and H-23); 3.54 (1H, m, 3-H); 3.85 (1H, m, H-7); 5.60 (1H, dd, J = 1.3 and 5.4 Hz, H-6); ^{13}C NMR: 11.76, 12.65, 16.48, 18.37, 19.68, 20.33, 20.82, 20.92, 24.67, 28.20, 29.34, 30.20, 31.53, 37.17, 37.67, 39.08, 39.16, 42.15, 42.47, 42.64, 48.86, 55.73, 56.01, 62.27, 62.28, 65.42, 71.48, 123.99, 146.42; EIMS (TMS-derivative, *m/z*, I, %): 588 [M]+ (41), 573(3), 571(2), 570(2), 498(58), 408(52), 155(83), 127(100). (22*R*,23*R*)-3β,7β-Dihydroxy-22,23-oxidostigmast-5-ene **33** (14 mg, 0.03 mmol, 31%). HR-ESI-MS calculated for $C_{29}H_{49}O_3^+$: 445.3682; found 445.36678. ^{1}H NMR: 0.68 (3H, s, H-18); 0.92 (3H, t, J = 7.5 Hz, H-29); 0.94 and 0.95 (each: 3H, d, J = 6.8 Hz, H-26 and H-27), 1.01 (3H, d, J = 6.8 Hz, H-21); 1.04 (3H, s, H-19); 2.51 (2H, m, H-22 and H-23); 3.58 (1H, m, H-3); 3.85 (1H, dt, J = 1.3 and 7.6 Hz, H-7); 5.29 (1H, t, J = 1.3 Hz, H-6); ^{13}C NMR: 11.90, 12.51, 16.38, 18.36, 19.55, 20.37, 20.85, 21.12, 24.66, 27.26, 29.48, 29.95, 31.53, 37.17, 37.69, 38.96, 39.24, 42.17, 42.47, 42.68, 49.04, 55.68, 55.95, 63.07, 63.08, 65.44, 71.48, 124.02, 146.34; EIMS (TMS-derivative, *m/z*, I, %): 588 [M]+ (46), 573(11), 571(4), 570(4), 498(100), 408(74), 155(6), 127(13).

4.2. Cell cultures

Human hepatoma Hep G2 cells (purchased from ECACC) and human breast carcinoma MCF-7 cells (purchased from ATCC) were cultured in 96-well plates at 37 °C in an atmosphere containing 5% CO_2 in RPMI-1640 medium supplemented with 10% FCS. Before the experiments, cells were incubated for 24 h in a serum-free medium. The tested compounds at concentrations of 1.0, 3.0, 10, 15, and 30 μM were added to the culture medium in ethanolic solutions, the EtOH concentration in all experiments (including corresponding controls) was 0.4% by vol.

4.3. Cytotoxicity evaluation

Toxicity of synthesized compounds in Hep G2 cells and MCF-7 cells was determined by MTT assay based on mitochondrial reduction of the yellow MTT tetrazolium dye to a highly colored blue formazan product.[14] Cells in 96-well plates were incubated with compounds tested for 48 h at 37 °C in serum-free medium. Then the medium was aspirated, 125 μl of MTT solution in PBS (1 mg/ml) was added to each well, and plates were incubated at 37 °C for 4 h more, thereafter 125 μl of stop solution (0.1 M HCl in iPrOH containing 10% Triton X-100) was added to each well, and the plates were stored overnight at room temperature. The absorbance at 630 nm in each well was measured on a LKB microplate reader. The values for each point were calculated from six wells; all experiments were carried out in triplicate; toxicity of compounds tested was calculated from plot: cell viability (% from control) versus concentration of compounds tested in medium.

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