Synthesis and Cytotoxicity of New Stigmasterol Derivatives

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Summary: This study identifies potential antitumor compounds from a series of new stigmasterol derivatives. Eleven stigmasterol derivatives were synthesized and their structures were confirmed by ^{1}H NMR, MS, and elemental analyses. Their cytotoxicity in vitro against three human cancer cell lines (MCF-7, A549 and HepG2) were evaluated by the MTT assay. Among these compounds, AB-5 and AB-11 shows much better cytotoxicity against MCF-7, A549, and HepG2 cells, and AB-10 exhibits selective cytotoxicity against MCF-7. Their structure–activity relationships were also investigated. In conclusion, AB-5, AB-10 and AB-11 serve as potential compounds for the new generation of anticancer drugs.

Keywords: Synthesis; Cytotoxicity; Stigmasterol derivatives; Phytosterol.

Introduction

Phytosterols are natural components of human diets and are largely derived from cereals, fruits, vegetables, and plant-derived oils[1]. Recently, there has been increased interest in the study of phytosterols for human health[2]. Consumption of phytosterols may assist in decreasing blood cholesterol levels[3]. Other important properties of phytosterols include antifungal, antibacterial, antiinflammatory, antitumor, antioxidant, and antiulcerative properties [4].

Phytosterols mainly include four structures: β-sitosterol, stigmasterol, campesterol and brassicasterol (Fig.1). The stigmasterol , which is also named as stigmasta-5,22-dien-3beta-ol ， exhibits strong activities in antitumor, reducing the blood fat concentration, preventing and treating cardiovascular diseases[5]. It also can be used to synthesize a variety of other drugs such as adrenal cortical hormone, steroid contraceptives, diuretic and sex hormone [6].

Despite its good medicinal properties, there are also limitations on its applications, such as low fat solubility and low activity[7]. Hence there is an urgent need for further search of more effective stigmasterol derivatives.

In the present study, a series of new stigmasterol derivatives AB-1-AB-11 (Fig. 2) are designed and synthesized. The aim of this study was to evaluate the cytotoxicity effect of these stigmasterol derivatives and to investigate their structure–activity relationships. The results of this study could be useful for the development of new agents for the control of tumor.

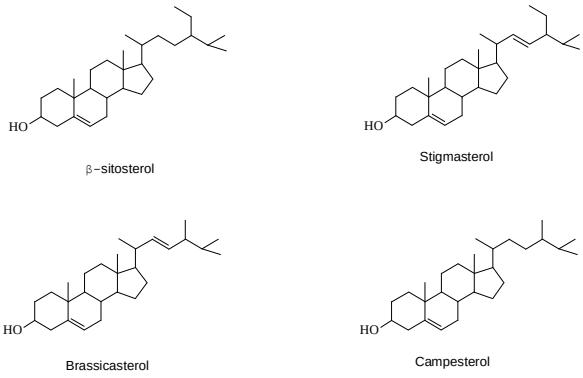

Fig. (1). The structure of four kinds of phytosterols

Experimental

Synthetic pathways of stigmasterol derivatives

The details for chemical structures and synthetic pathways of 11 stigmasterol derivatives are shown in Fig. 2.

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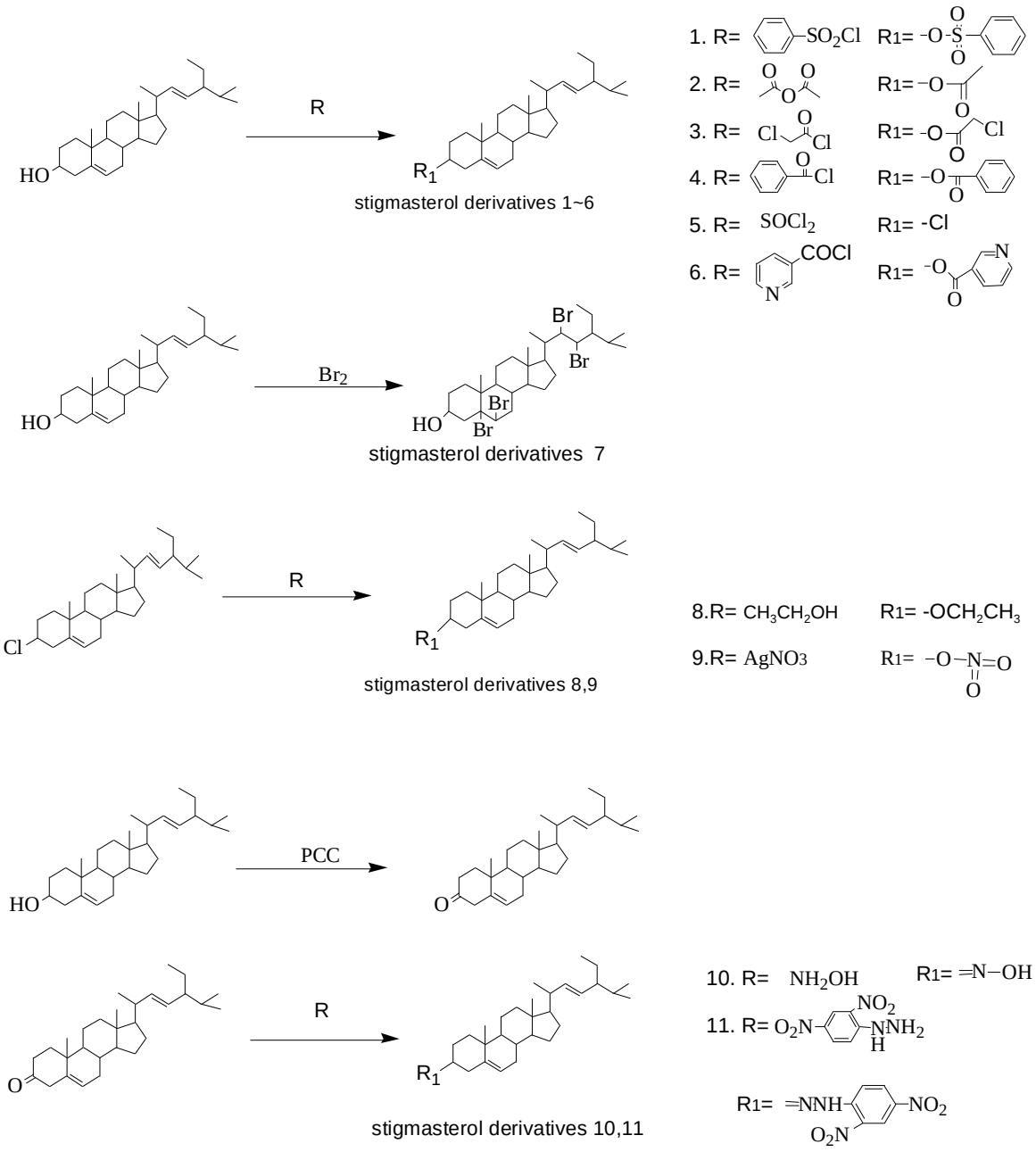

Fig.(2). Structures of Stigmasterol and its derivatives

Synthesis of 3-benzenesulfonyloxygen-5, 22-Diene-24-ethyl-cholesterol **(AB-1)**

Stigmasterol (0.5 g, 1.21 mmol) was dissolved in a 50 mL three-necked bottle which is protected by nitrogen, added in dry chloroform 20 mL, dry triethylamine 0.6 mL (about 4.3 mmol), controlling the temperature at 60 ℃, 1 mL of benzene sulfonyl chloride (6.0 mmol) and 3 mL of chloroform was slowly added dropwise, and completed all the addition within half an hour, then continue stirring with the process of followed reaction of thin layer chromatography, terminated after 7 hours. Filtered and the filtrate washed with saturated sodium chloride solution three times, dry the organic with over anhydrous sodium sulfate overnight, filtered again, evaporated to dryness the filtrate and silica gel column chromatography (silica gel, 200–300 mesh;

eluent, petroleum ether/acetic acid acetate = 20 : 1) to get a white solid powder 0.523 g, yield 78.2%.

3-acetoxy-5, 22-diene-24-ethyl-cholesterol (AB-2), 3-chloroacetoxy-5, 22-diene-24-ethyl-cholesterol（AB-3）and 3-benzoyl -5, 22-diene-24-ethyl-cholesterol (AB-4) were obtained by the same method.

Synthesis of 3-chlorine -5, 22-diene-24-ethyl-cholesterol **(AB-5)**

Stigmasterol (0.5 g, 1.21 mmol) was dissolved in a 50 mL three-necked bottles which is protected by nitrogen, added in dry petroleum ether 20 mL, controlling the temperature at 60 ℃, 0.9 mL of thionyl chloride (6.0 mmol) and 3 mL of petroleum ether was slowly added dropwise, and completed all the addition within 15 minute, then continue stirring with the process of followed reaction of thin layer chromatography, terminated after half an hour. Dry the organic with over anhydrous sodium sulfate overnight, filtered again, evaporated to dryness the filtrate and silica gel column chromatography (silica gel, 200–300 mesh; eluent, petroleum ether) to get a white solid powder 0.421 g, yield 80.9%.

Synthesis of 3-(3-carbonyl -pyridine) - 5, 22-diene-24-ethyl-cholesterol **(AB-6)**

Nicotinoyl chloride hydrochloride (1.0 g, 6 mmol）was dissolved in a 50 mL three-necked bottle which is protected by nitrogen, added in dry chloroform 30 mL with ice bath, then added in dry pyridine 0.6 mL and stirred for half an hour adding chlorinated stigmasterol 0.5 g (1.21 mmol), uped to 70 ℃, within the process of followed reaction of thin layer chromatography, terminated after 10 hours. Evaporated to dryness of the chloroform, washed the solid precipitate with water several times to get a white solid powder 0.557 g, yield 88.9%.

Synthesis of 3-hydroxyl -5, 6, 22, 23-tetrabromo-24-ethyl-cholesterol **(AB-7)**

Stigmasterol (0.5 g, 1.21 mmol) was dissolved in a 50 mL three-necked bottle which is protected by nitrogen, added in dry chloroform 20 mL, room temperature, 2 ml of benzoyl chloride (12.1 mmol) and 3 ml of choroform was slowly added dropwise, and completed all the addition within half an hour, then continue stirring with the process of followed reaction of thin layer chromatography, terminated after 3.5 hours. Filtered and the filtrate washed with saturated dicarbonate solution three times, dry the organic with over anhydrous sodium sulfate overnight, filtered again, evaporated to dryness the filtrate and silica gel column chromatography (silica gel, 200–300 mesh; eluent, petroleum ether/ acetic acid acetate/methyl aclohol = 20 : 1 : 0.05) to get a reddish brown solid powder 0.377 g, yield 42.6%.

Synthesis of 3-ethoxy -5, 22-diene-24-ethyl-cholesterol **(AB-8)**

Chlorinated stigmasterol (0.5 g, 1.21 mmol) was dissolved in a 50 mL three-necked bottle which is protected by nitrogen, added in dry chloroform 20 mL, controlling the temperature at 60 ℃, then continue stirring with the process of followed reaction of thin layer chromatography, terminated after 5 hours. Filtered, evaporated to dryness the filtrate and silica gel column chromatography (silica gel, 200–300 mesh; eluent, petroleum ether/ethyl acetate = 20 : 1) to get a white solid powder 0.174 g, yield 32.7%.

Synthesis of 3-nitro -5, 22-diene-24-ethyl-cholesterol **(AB-9)**

Stigmasterol (0.5 g, 1.21 mmol) was dissolved in a 50 mL three-necked bottle which is protected by nitrogen, added in dry chloroform 20 mL. At room temperature, 1.0 ml ethanol solution (6.0 mmol) of silver nitrate was slowly added dropwise, and completed all the addition within half an hour, then continues stirring with the process of followed reaction of thin layer chromatography, terminated after 2 hours. Filtered, evaporated to dryness the filtrate and silica gel column chromatography (silica gel, 200–300 mesh; eluent, petroleum ether/ethyl acetate = 3 : 1) to get a white solid powder 0.266 g, yield, 48.1%.

Synthesis of 3-oximido -5, 22-diene-24-ethyl-cholesterol **(AB-10)**

Stigmasterol (1.0 g, 2.42 mmol) was dissolved in a 50 mL three-necked bottle which is protected by nitrogen, added in dry chloroform 30 mL with ice bath, then added in PPC 1.0 g (4.84 mmol) stirring within the process of followed reaction of thin layer chromatography, terminated after 2.5 hours. Evaporated to dryness of the chloroform, and silica gel column chromatography (silica gel, 200–300 mesh; eluent, petroleum

ether/ethyl acetate = 20 : 1) to purifed to get a white solid powder (bean steroidal ketone) 0.61 g[15], yield 61.8%. Got bean stigmastone 0.5 g (1.21 mmol) dissolved in a bottel three mouths 50ML which is protected by nigtrogen, added in dry chloroform 30 mL, dry pyridine 0.6 mL and hydroxylamine 0.3 g (2.42 mmol) at room temperature, stirred, terminated after 10 hours, filtered, evaporated to dryness and silica gel column chromatography (silica gel, 200–300 mesh; eluent, petroleum ether/ethyl acetate = 10: 1), purified to get a white solid powder 0.385 g, yield 74.7%.

3-(2, 4-dinitrophenyl) - 5, 22-diene-24-ethyl-cholesterol (AB-11) was obtained by the same method.

Structural and elemental analyses

^{1}HNMR spectra was obtained on a Bruker Avance 600 FT-NMR spectrometer (Bruker, Switzerland) operating at 600 MHz and ^{1}H NMR chemical shifts (δ) being relative to tetramethylsilane. A mass of spectra were obtained from ESI-MS (Waters2695-ZQ4000 spectrometer, Waters Corporation, Milford, America). All melting points (mp) were measured by Electrothermal engineering 9200 apparatus (Syngene Corporation, Cambridge shire, England). Element analyses were conducted using TQ-3A element analysis instrument (Henan MingShengKeji Company, China).

Cytotoxicity assay

The cytotoxic effect of stigmasterol and stigmasterol derivatives were assayed with 3-(4,5-dimethyl-thiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) method in three human cancer cell lines (MCF-7, A549 and HepG2) with some modification

25% trypsin would be digested during the phase of cells logarithmic growth and then made into cell suspension, the cell density was determined as: 1×10^4/ml，were cultured in 96-well plates with under 5% CO_2 at 37 ℃. After 24 h, the appropriate test compound was added with different indicated concentrations of 3.125, 6.25, 12.5, 25, 50 and 100 μmol/L, respectively, for another 72 h incubation. Then, 100 μL of 0.5 g/L MTT was added to each well after discarding the culture medium, and an additional 4 h incubation was allowed. The resulting formazan was dissolved in 200 μL dimethyl sulfoxide (DMSO) after aspiration of the culture medium. After shaking for 30 min at a plate shaker, the plate was read immediately at 570 nm using a microplate reader (Bio-Rad Model 550). The percent specific cytotoxicity of each compound was determined based on (1-OD experiments / OD positive controls) ×100%.

Statistical analysis

All of the data shown represents the mean ± SD of triplicate experiments using three independent determinations. All of the statistical analyses were performed using Excel for Windows, and p values equal to or less than 0.05 were considered statistically significant by Student's t test.

Result and Discussion

Structural and chemical parameter of 11 stigmasterol derivatives

3-benzenesulfonyloxygen-5, 22-Diene-24-ethyl-cholesterol **(AB-1)**

mp 103.8–105.2 ℃. ^{1}H NMR($CDCl_3$) δ: 0.686 (s, 3H), 0.788–0.799 (m,6H), 0.845 (d, J = 6.6 Hz, 3H), 1.010–1.135 (m, 7H), 1.151–1.162 (m, 1H), 1.170–1.253 (m, 3H), 1.429–1.441 (m, 2H), 1.450–1.469 (m, 4H), 1.510–1.519 (m, 2H), 1.528–1.543 (m, 2H), 1.555–1.603 (m, 2H), 1.8931–.910 (m, 1H), 1.967–2.094 (m, 3H), 2.495 (d, J = 7.8 Hz, 1H), 4.196–4.229 (m, 1H), 5.013–5.041 (m, 1H), 5.125–5.165 (m, 1H), 5.399–5.414 (m, 1H), 7.527–7.545 (m, 3H), 7.718–7.734 (m, 2H). ESI-MS m/z 544.38 ([M + H]+, 80%). Anal. Calcd for $C_{35}H_{52}O_3S$: C, 76.04; H, 9.48. Found: C, 75.99; H, 9.50.

3-acetoxy-5, 22-diene-24-ethyl-cholesterol **(AB-2)**

mp 140.9–142.4 ℃. ^{1}H NMR (CDCl3) δ: 0.696 (s, 3H), 0.790–0.800 (m, 6H), 0.846 (d, J = 6.0 Hz, 3H), 0.911–0.974 (m, 1H), 1.016–1.127 (m, 7H), 1.129–1.137 (m, 1H), 1.142–1.191 (m, 4H), 1.225–1.248 (m, 1H), 1.469–1.510 (m, 9H), 1.520–1.529 (m, 1H), 1.853–1.873 (m, 2H), 1.978–2.035 (m, 6H), 2.300–2.347 (m, 2H), 4.603–4.612 (m, 1H), 4.992–5.032 (m, 1H), 5.130–5.169 (m, 1H), 5.374 (t, J = 2.4 Hz, 1H). ESI-MS m/z 456 ([M + H]+, 20%). Anal. Calcd for $C_{31}H_{50}O_2$: C, 81.88; H, 11.08. Found: C, 81.90; H, 11.07.

3-chloroacetoxy-5, 22-diene-24-ethyl-cholesterol （**AB-3**）

mp 175.1–176.9 ℃. ^{1}H NMR (CDCl3) δ: 0.696 (s, 3H), 0.789–0.816 (m, 6H), 0.846 (d, J = 6.6 Hz, 3H), 0.912–0.977 (m, 1H), 1.016–1.094 (m, 7H), 1.127–1.136 (m, 1H), 1.144–1.296 (m, 6H), 1.375–1.455 (m, 2H), 1.494–1.571 (m, 5H), 1.611–1.624 (m, 1H), 1.693–1.721 (m, 1H), 1.821–1.891 (m, 2H), 1.932–2.002 (m, 3H), 2.353–2.365 (m, 2H), 4.040 (s, 2H), 4.684–4.723 (m, 1H), 4.964–5.033 (m, 1H), 5.129–5.169 (m, 1H), 5.389 (t, J = 1.8 Hz, 1H). ESI-MS m/z 490 ([M + H]+, 10%). Anal. Calcd for $C_{31}H_{49}O_2Cl$: C, 76.11; H, 10.10. Found: C, 76.10; H, 10.09.

3-benzoyl -5, 22-diene-24-ethyl-cholesterol **(AB-4)**

mp 156.4–157.7 ℃. ^{1}H NMR (CDCl3) δ: 0.712 (s, 3H), 0.797–0.811 (m, 6H), 0.852 (d, J = 6.0 Hz, 3H), 0.954–1.117 (m, 9H), 1.145–1.348 (m, 5H), 1.395–1.677 (m, 8H), 1.690–1.726 (m, 2H), 1.909–1.931 (m, 1H), 1.989–2.099 (m, 4H), 2.461–2.474 (m, 2H), 4.821–4.899 (m, 1H), 5.031–5.045 (m, 1H), 5.144–5.169 (m, 1H), 5.420 (t, J = 2.4 Hz, 1H), 7.429 (t, J = 7.8 Hz, 2H), 7.540 (t, J = 7.8 Hz, 1H), 8.043 (d, J = 7.8 Hz, 2H). ESI-MS m/z 518 ([M + H]+, 10%). Anal. Calcd for $C_{36}H_{52}O_2$: C, 83.67; H, 10.14. Found: C, 83.65; H, 10.13.

3-chlorine -5, 22-diene-24-ethyl-cholesterol **(AB-5)**

mp 154.4–155.8 ℃. ^{1}H NMR (CDCl3) δ: 0.694 (s, 3H), 0.789–0.816 (m, 6H), 0.845 (d, J = 6.0 Hz, 3H), 0.918–0.940 (m, 1H), 0.999–1.014 (m, 2H), 1.018–1.047 (m, 6H), 1.121–1.184 (m, 3H), 1.250–1.256 (m, 1H), 1.409–1.561 (m, 9H), 1.881–1.904 (m, 1H), 1.958–1.997 (m, 2H), 2.001–2.108 (m, 1H), 2.494–2.501 (m, 1H), 2.524–2.577 (m, 1H), 3.746–3.811 (m, 1H), 4.997–5.033 (m, 1H), 5.126–5.165 (m, 1H), 5.365–5.374 (m, 1H). ESI-MS m/z 431.30 ([M + H]+, 80%). Anal. Calcd for $C_{29}H_{47}Cl$: C, 80.79; H, 10.99. Found: C, 80.79; H, 10.97.

3-(3-carbonyl -pyridine) - 5, 22-diene-24-ethyl-cholesterol **(AB-6)**

mp 125.4–127.3 ℃. ^{1}H NMR (CDCl3) δ: 0.712 (s, 3H), 0.795–0.809 (m, 6H), 0.845 (d, J = 6.6 Hz, 3H), 0.997–1.078 (m, 4H), 1.157–1.173 (m, 4H), 1.183–1.345 (m, 6H), 1.421–1.609 (m, 8H), 1.712–1.799 (m, 2H), 1.924–2.025 (m, 5H), 2.408–2.497 (m, 2H), 4.802–4.875 (m, 1H), 5.001–5.234 (m, 1H), 5.138–5.152 (m, 1H), 5.444 (t, J = 3 Hz, 1H), 7.390–7.412 (m, 1H), 8.303–8.323 (m, 1H), 8.767–8.778 (m, 1H), 9.230 (d, J = 1.8 Hz, 1H). ESI-MS m/z 519 ([M + H]+, 20%). Anal. Calcd for $C_{35}H_{51}NO_2$: C, 81.19; H, 9.93; N, 2.71; Found: C, 81.16; H, 9.94, N, 2.73.

3-hydroxyl -5, 6, 22, 23-tetrabromo-24-ethyl-cholesterol **(AB-7)**

mp 101.9–103.8 ℃. ^{1}H NMR (CDCl3) δ: 0.713 (s, 1H), 0.732 (s, 2H), 0.896–1.191 (m, 7H), 1.199–1.397 (m, 8H), 1.401–1.810 (m, 18H), 1.839–1.924 (m, 2H), 1.940–2.012 (m, 2H), 2.197–2.225 (m, 1H), 2.289–2.345 (m, 1H), 2.486–2.511 (m, 1H), 2.813–2.841 (m, 1H), 4.190–4.198 (m, 1H), 4.394–4.416 (m, 1H), 4.480–4.534 (m, 1H), 4.842–4.871 (m, 1H). ESI-MS m/z 733 ([M + H]+, 60%). Anal. Calcd for $C_{29}H_{48}BrO$: C, 47.56; H, 6.61. Found: C, 47.60; H, 6.60.

3-ethoxy -5, 22-diene-24-ethyl-cholesterol **(AB-8)**

mp 132.5–133.9 ℃. ^{1}H NMR (CDCl3) δ: 0.696 (s, 3H), 0.790–0.824 (m, 6H), 0.845 (d, J = 6.6 Hz, 3H), 0.897–0.945 (m, 1H), 0.986–1.094 (m, 9H), 1.187–1.213 (m, 6H), 1.292–1.342 (m, 1H), 1.345–1.601 (m, 8H), 1.645–1.723 (m, 1H), 1.847–1.979 (m, 2H), 1.998–2.104 (m, 3H), 2.178–2.224 (m, 1H), 2.347–2.392 (m, 1H), 3.152–3.199 (m, 1H), 3.516–3.534 (m, 2H), 5.023–5.038 (m, 1H), 5.133–5.172 (m, 2H), 5.342 (t, J = 3 Hz, 1H); Cl, 8.22. ESI-MS m/z 442 ([M + H]+, 30%). Anal. Calcd for $C_{31}H_{52}O$: C, 84.48; H, 11.89. Found: C, 84.49; H, 11.87.

3-nitro -5, 22-diene-24-ethyl-cholesterol **(AB-9)**

mp 90.1–92.0 ℃. ^{1}H NMR (CDCl3) δ: 0.702 (s, 3H), 0.792–0.807 (m, 6H), 0.848 (d, J = 6.0 Hz, 3H), 0.901–1.103 (m, 9H), 1.145–1.211 (m, 3H), 1.265–1.293 (m, 1H), 1.402–1.599 (m, 10H), 1.623–1.745 (m, 2H), 2.004–2.099 (m, 4H), 2.435–2.448 (m, 2H), 4.746–4.801 (m, 1H), 5.028–5.043 (m, 1H), 5.135–5.174 (m, 1H), 5.440 (t, J = 3 Hz, 1H). ESI-MS m/z 458 ([M + H]+, 10%). Anal. Calcd for $C_{29}H_{47}NO_3$: C, 76.10; H, 10.35; N, 3.06. Found: C, 76.07; H, 10.36, N, 3.07.

3-oximido -5, 22-diene-24-ethyl-cholesterol **(AB-10)**

mp 125.4–127.1 ℃. ^{1}H NMR (CDCl3) δ: 0.696(s, 3H), 0.792–0.820 (m, 6H), 0.845 (d, J = 6.0

Hz, 3H), 0.923–0.945 (m, 1H), 1.001–1.013 (m, 2H), 1.019–1.052 (m, 6H), 1.118–1.194 (m, 3H), 1.251 (s, 1H), 1.408–1.559 (m, 8H), 1.842–1.899 (m, 2H), 1.965–2.004 (m, 2H), 2.050–2.069 (m, 4H), 2.486–2.507 (m, 1H), 2.560–2.578 (m, 1H), 4.098–4.131 (m, 1H), 5.020–5.039 (m, 1H), 5.131–5.168 (m, 1H), 6.619 (s, 1H). ESI-MS m/z 426.31 ([M + H]+, 100%). Anal. Calcd for $C_{35}H_{47}NO$: C, 81.82; H, 11.13; N, 3.29. Found: C, 81.80; H, 11.14, N, 3.30.

3-(2, 4-dinitrophenyl) - 5, 22-diene-24-ethyl-cholesterol **(AB-11)**

mp 146.7–148.1 ℃. ^{1}H NMR (CDCl3) δ: 0.702(s, 3H), 0.793–0.820 (m, 6H), 0.845 (d, J = 6.0 Hz, 3H), 0.921–0.940 (m, 1H), 1.009–1.017 (m, 2H), 1.021–1.049 (m, 6H), 1.124–1.187 (m, 3H), 1.254 (s, 1H), 1.409–1.563 (m, 8H), 1.844–1.902 (m, 2H), 1.965–2.001 (m, 2H), 2.058–2.067 (m, 1H), 4.310 (s, 1H), 5.019–5.033 (m, 1H), 5.134–5.170 (m, 1H), 6.024 (s, 1H), 7.956 (d, J = 9.6 Hz, 1H), 8.293 (t, J = 4.8 Hz, 1H), 9.138 (s, 1H). ESI-MS m/z 592.05 ([M + H]+, 70%). Anal. Calcd for $C_{35}H_{50}N_4O_4$: C, 71.15; H, 8.53; N, 9.48. Found: C, 71.13; H, 8.56, N, 9.49.

Cytotoxicity of compounds

Using doxorubicin as a positive control, the antitumor activity of stigmasterol and its synthetic derivatives AB-1–AB-11 in MCF-7, A549 and HEPG2 cell lines was initially evaluated by MTT method, and the results were reported in Table 1.

Table-1: Cytotoxicity of stigmasterol and synthesized derivatives against MCF-7, A549 and HepG2 cells.

Compound	Cytotoxicity (IC_{50}, μmol·L^{-1})		
	MCF-7	A549	HepG2
AB-1	>50	>50	>50
AB-2	>50	>50	>50
AB-3	>50	>50	>50
AB-4	>50	>50	>50
AB-5	3.69±0.12	5.98±0.09	6.50±0.21
AB-6	>50	>50	>50
AB-7	>50	>50	>50
AB-8	>50	>50	>50
AB-9	>50	>50	>50
AB-10	4.37±0.26	23.12±0.38	18.95±0.45
AB-11	1.98±0.06	2.39±0.11	2.35±0.07
Stigmasterol	5.80±0.15	8.95±0.23	12.50±0.12
Doxorubicin	3.39±0.06	3.84±0.10	8.99±0.05

As shown in Table 1, in general the derivatives AB-5, AB-10 and AB-11 show better cytotoxicities than natural stigmasterol and similar cytotoxicities as doxorubicin, while the other derivatives show poor cytotoxic activities against MCF-7, A549 and HepG2 cell lines.

AB-10 shows an IC_{50} value of 4.37 μmol·L^{-1} in the MCF-7 cell line which is more active than stigmasterol (IC_{50} 5.8 μmol·L^{-1}). AB-5 shows higher anti-tumor activity (IC_{50} 6.5 μmol·L^{-1}) than doxorubicin (IC_{50} 8.99 μmol·L^{-1}) in the HepG2 cell line and almost the same activity (IC_{50} 3.69 μmol·L^{-1}) as doxorubicin (IC_{50} 3.39 μmol·L−1) in MCF-7 cell line. Great antitumor activity in the three tumor cell lines is shown by AB-11 with an IC_{50} value of 2.35 μmol·L^{-1} in the HepG2 cell line, which is three times more active than doxorubicin (IC_{50} 8.99 μmol·L^{-1}). AB-11 also has the most significant antitumor activity for the MCF-7 (IC_{50} 1.98 μmol·L^{-1}) and A549 (IC_{50} 2.39 μmol·L^{-1}) cell lines in all synthetic derivatives and the positive control.

Cancer and cardiovascular disease are the most two causes of mortality worldwide. Natural products from plant source , which used in traditional medicines, have been accepted currently as one of the main source of cancer and cardiovascular disease chemo-preventive drug[8].

Natural plant sterols, namely phytosterols have been received special attention for their capacity to reduce the concentration of blood cholesterol and prevent the onset of cardiovascular disorders, as well as their anti-inflammatory, anticancer and antioxidative effects[9].

Stigmasterol is one of the phytosterols. It was reported that the hepatoma cell SMMC-7721, BEL-7402 were inhibited in vitro and the hepatoma cell H22 was inhibited in vivo by stigmasterol[10]. The proliferation cycle the hepatoma cell was inhibited and the apoptosis of the hepatoma cell was promoted by stigmasterol[11]. Although stigmasterol has a very wide range of applications of biotechnology and pharmaceutical sectors[12], there are also some limitations. Natural stigmasterol have poor solubility in fat. And its stability and activity in vivo need to be further improved.

For that reason, evaluating the biological activity of synthetic stigmasterol derivatives was deemed important. There are several reports on the antiviral activity against human herpes virus 1 of synthetic stigmasterol derivatives with oxygenated side chains[13]. By contrast, little research has been conducted on the antitumor activity of synthetic stigmasterol derivatives, including their structure–activity relationships .

In our study, 11 new stigmasterol derivatives were synthesized and their antitumor activities were investigated on cancer cells MCF-7, A549 and HepG2. The stigmasterol derivatives which containing ester and bromine addition structure display weaker antitumor effect than natural stigmasterol. The derivatives whose hydroxy is substituted by heteroatoms, especially by imide, show better antitumor effect than natural stigmasterol. AB-11 displays the strongest inhibition to the three cancer cells among all derivatives, stigmasterol and doxorubicin. After comparing the structure of AB-5, AB-10 and AB-11, we discovered that the compound whose hydroxy was substituted by heteroatoms with aromatic nuclecs will show stronger antitumor activity than compounds with other groups.

Our results implying that the aromatic nucleus may help to increase the rigidity and these compounds in the spatial structure can combine with the target spot very well. With an unique oximido structure, AB-11 displays stronger inhibition than masculine Doxorubicin and Stigmasterol in MCF-7, A549 and HepG2. We speculate that the derivative containing oximido induces cell apoptosis by inhibiting the formation of membranes [14-15]. AB-5 displays better inhibition to MCF-7, A549, and HepG2 than stigmasterol, inferring that the chlorine atom with oxidability in the derivative plays an vital role in the antitumor activity.

Conclusion

In this study, eleven new stigmasterol derivatives were synthesized and their antitumor activities were investigated on cancer cells MCF-7, A549 and HepG2. The cytotoxicities of derivatives AB-5, AB-10 and AB-11 are better than that of natural stigmasterol and are similar as that of doxorubicin. Moreover, these derivatives present good solubility in fat, which show the potential in the developing of new anticancer drugs.

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