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Synthesis and *In Vitro* Antiproliferative Evaluation of Some B-norcholesteryl thiazole Derivatives

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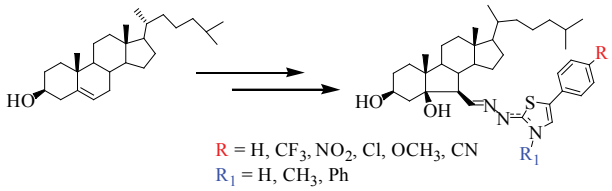
Abstract

Some novel B-norcholesteryl thiazole derivatives were synthesized and their structures were characterized by IR, NMR and HRMS. The antiproliferative activity of the compounds against human cervical carcinoma (HeLa), human lung carcinoma (A549), human liver carcinoma (HEPG2) and normal kidney epithelial (HEK293T) cells was assayed. The results revealed that compounds 14 and 17 with a structure of N-methylthiazole showed distinct antiproliferative activity against A549 and HEPG2 cells. Compounds 20-24 with a structure of N-phenylthiazole displayed a selective antiproliferative activity on HeLa cells and were almost inactive to normal kidney epithelial cells (HEK293T). The research provided a theoretical reference for the exploration of new anti-cancer agents and may be useful for the design of novel chemotherapeutic drugs.



R = H, CF_3, NO_2, Cl, OCH_3, CN
R_1 = H, CH_3, Ph

Keywords: Cholesterol; B-norcholesteryl thiazoles; B-norcholesteryl N-methylthiazoles; B-norcholesteryl N-phenylthiazoles; Antiproliferative activity

Introduction

Steroidal compounds show various biological activity and play a very important role in life. The steroidal drugs have been used widely in traditional medicines, such as anti-inflammatory, anti-microbial, anticarcinogen, hypotensive, hormone kind medication, hypocholesterolemic and diuretic activities, etc. [1-8].

Recently, a variety of steroidal compounds with unusual and interesting structures have been isolated from marine life or plants, and synthesized [9-12]. Among these steroidal compounds, steroids with the structure of B-nor-steroidal nucleus are rarely reported and are uncommon in nature. Wentworth et al. reported that 3β, 5β-dihydroxyl- B-norcholesteryl-6β-formaldehyde with a structure of [6,5,6,5]-fused ring might been generated by ozonolysis of cholesterol *in vivo* because of the cause of some human disease [13]. Moreover, several sterols possessing a skeleton of [6,5,6,5] fused ring were isolated by Wang et al. [14], Miyamoto et al. [15] and Xiaomei et al. [16] and synthesized by Xiaomei et al. [17].

In order to investigate the anti-tumor activity of new steroidal derivatives, we synthesized a series of steroidal derivatives possessing [6,5,6,5]-fused ring, various side chains and substituted groups on the steroidal nucleus, and determined their antiproliferative activities against different types of cancer cells [18-20]. The results showed that presence of a cholesterol-type side chain was very important in determining the cytotoxicity of these compounds, and presence of a thiosemicarbazone group on the C-6 position of steroidal nucleus could enhance the antiproliferative activity of the compound.

Heterosteroids have been obtained a great amount of attention over the years by medicinal chemists for drug discovery. Introducing a heterocycle or a heteroatom into steroids will greatly affect the chemical property of a steroid and often result in useful alterations in its biological activity [21,22]. So far, the steroids containing heterocycles or heteroactoms had been widely explored and reported [23]. Literatures suggested that such compounds displayed distinct cytotoxicity against cancer cell lines [24-28].

Recently, we synthesized some novel B-norcholesteryl benzimidazole and benzothiazole derivatives with [6,5,6,5]-fused ring and assayed their antiproliferative activity against some cancer cells [29]. The results showed that some B-norcholesteryl benzimidazole compounds exhibited an excellent antiproliferative activity and almost inactive to normal kidney epithelial cells (HEK293T). Thiazole ring is a pharmacophore with extensive bioactivities, such as antimicrobial, anti-inflammatory, anticancer, anti-HIV-1, anticonvulsant etc. [30-33]. In order to obtain biologically potent anticancer compounds with diverse structures, as an extension of our previous work, a series of B-norsteroidal derivatives possessing a [6,5,6,5]-fused ring and a structure of 6-thiazole had been prepared starting from cholesterol in the present study. Meanwhile the antiproliferative activity of compounds *in vitro* was evaluated further.

Results and Discussion

Chemistry

Scheme 1 outlines a synthetic procedure of B-norcholesteryl thiazole derivatives (5-10). Compound 4 was prepared according to Gan et al. [18]. The configurations of C-5 and C-7 in compound 3 had been described in references [13,34,35]. Compound 4 were obtained by the reaction of 3 with thiosemicarbazide. The reaction of compound 4 with ω-bromoacetophenone afforded a corresponding B-norcholesteryl

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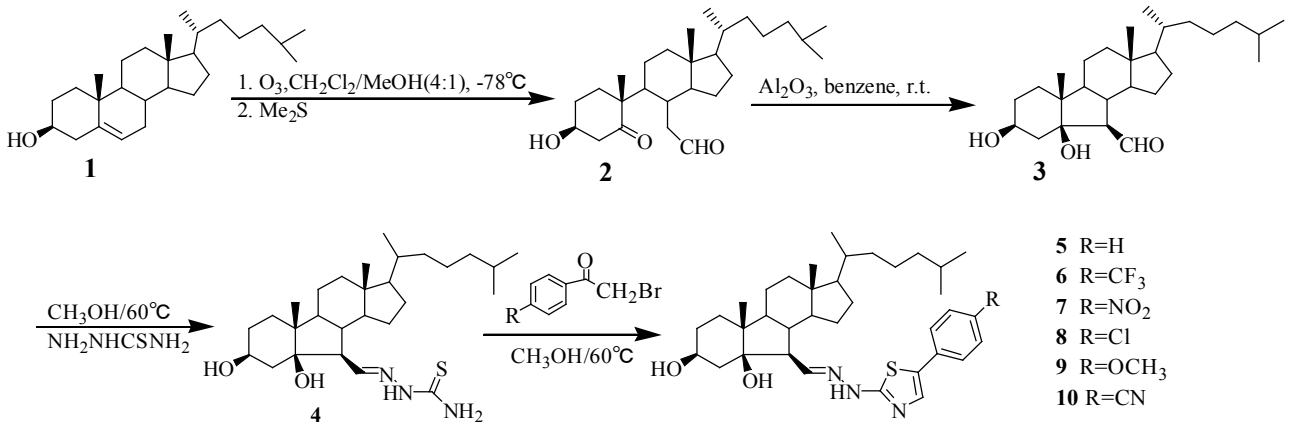


Scheme 1: Synthesis of compounds **5-10**.

thiazole derivative 5. The ^{1}H NMR and ^{13}C NMR spectra of 5 were the basis of its structure elucidation. In the NMR spectrum, resonances signals of Ph-H at δ 7.75, 7.38, 7.30 ppm and Ph-C at δ 128.7-102.8 ppm demonstrated the presence of a benzene ring in compound 5. The ^{1}H NMR spectrum showed a singlet at δ 7.14 indicating a thiazole proton. Moreover, the ^{13}C NMR spectrum showed three signals at δ 169.9, 147.7, 134.8 ppm indicating the formation of thiazole ring in 5 further. The HREI mass spectra of compound 5 exhibited molecular ion peak at m/z 592.3923. All spectrums confirmed the structure of compound 5.

To survey the effect of various substituent groups in benzene ring on the antiproliferative activity of compounds, compounds (6-10) were synthesized by the reaction of 4 with different 4-substituted-ω-bromoacetophenones. Their structures had been confirmed on analytical and spectral data.

In order to investigate the effect of substituted groups on thiazole ring against the antiproliferative activity, we prepared a series of B-norcholesteryl N-methyl- and N-phenylthiazole derivatives (12-24) by reacting compound 11 or 18 with various ω-bromoacetophenones (Scheme 2 and 3). The structures of all synthesized compounds had been confirmed by analysis of IR, NMR and HRMS.

Biological results and discussion

Lung cancer and liver cancer are a main cause of death in cancer patients. To investigate the antiproliferative activity of the new compounds, we determined their IC_{50} values on A549 (human lung carcinoma), HEPG2 (human liver carcinoma) and HeLa (human cervical carcinoma) using a MTT assay, and non-cancer cells HEK293T (Normal Kidney Epithelial Cells) were chosen as a control. The results were showed as IC_{50} values (concentration of a compound allowing survival of 50% of the cells in a population) in μM in Table 1.

As shown in Table 1, these B-norcholesteryl thiazole derivatives (5-19) didn't exhibited obvious antiproliferative activity against tested cancer cells except compound 14 which showed distinct cytotoxicity on all tested cancer cells and 17 possessed of a moderate cytotoxicity on A549 and HEPG2 cells. Compared with the result of our previous work [20,29], we think that a main cause of the less activity of these compounds may be a greater size of 5-phenyl group on thiazole ring resulted to the interaction of comopounds with the cells can't take place, which deserve further study.

However, it was interesting that compounds 20-24 with a N-phenylthiazole ring displayed distinct antiproliferative activity against HeLa cells and were almost inactive to other cancer cells and normal kidney epithelial cells. The results exhibited that compounds 20-24 were better selective inhibitors against HeLa cells, and are worth to study further.

Experimental Section

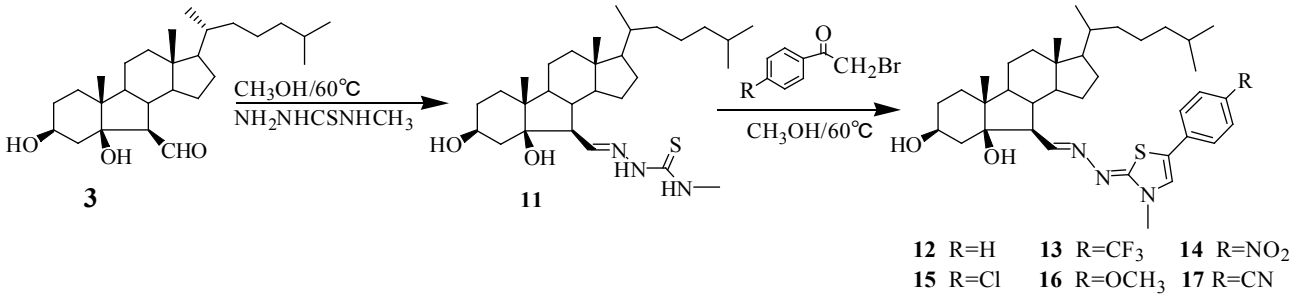
Chemistry

The sterols were purchased from Sinopharm Chemical Reagent Co., Ltd, Shanghai, China. All chemicals and solvents were analytical grade. Melting points were determined on an X_4 apparatus (Beijing Tech Instrument Co. Ltd., Beijing, China) and were uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AV-600 spectrometer at working frequencies 600 and 150 MHz, and a Bruker AV-300 spectrometer at working frequencies 300 and 75 MHz, respectively. Chemical shifts are expressed in parts per million (δ) values and coupling constants (J) in Hertz. Infrared spectra were measured with a Thermo Scientific Nicolet IS-10 Spectrophotometer (Thermo Scientific, America). HREIMS was measured on an Agilent 6210 TOFMS instrument (Agilent Technologies, America). The cell proliferation assay was undertaken by a MTT method using 96-well plates on a MLLTISKAN MK3 analysis spectrometer (Thermo Scientific, Shanghai, China).

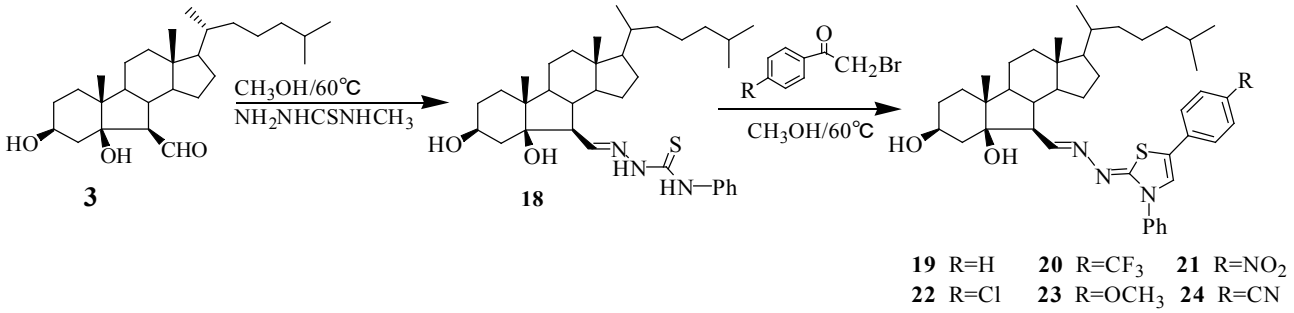
The compound **4** was prepared according to the method of Gan et al. [18].

General procedure for the synthesis of compounds 5-10: ω-Bromoacetophenone or its 4-substituted derivative (0.45 mmol) was added to a solution of compound 4 (0.3 mmol) in CH_3OH (50 mL). The solution was stirred for 2-3 h at 60°C until no starting material was observed (the progress of the reaction was monitored by TLC, petroleum ether/ethyl acetate=1:1). Then the reaction was stopped and the majority of solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (300-400 mesh) to afford the corresponding target products 5-10.

α) **1-(3β,5β-Dihydroxy-6'-B-norcholesteryl-6E-ylidene)-2-(5'-phenyl-2'-thiazol)hydrazone** (**5**): Yellow oil, yield: 71%. IR (KBr) ν/cm^{-1}: 3439, 2953, 2858, 1706, 1567, 1442, 1380, 1325, 1278, 1071, 1051, 1024; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.75 (1H, d, J=7.2, 2,6-PhH), 7.38 (2H, t, J=7.8, 3,5-PhH), 7.30 (1H, m, 4-PhH), 7.14 (1H, d, J=6.4, C6-H), 6.73 (1H, s, 4-ArH), 4.17 (1H, br s, -OH), 3.46 (1H, s, -OH), 2.18-2.12 (2H, m, C7-H and C8-H), 0.94 (3H, d, J=6.3, 21-CH_3), 0.90 (3H, s, 19-CH_3), 0.88 (6H, d, J=6.6, 26- and 27-CH_3), 0.72 (3H, s, 18-CH_3); ^{13}C



Scheme 2: Synthesis of compounds **12-17.**



Scheme 3: Synthesis of compounds **19-24**.

Compounds	Cells			
	HeLa	A549	HEPG2	HEK293T
5	>80	>80	>80	>80
6	>80	>80	>80	>80
7	>80	>80	>80	>80
8	>80	>80	>80	>80
9	>80	>80	>80	>80
10	>80	>80	>80	>80
12	53.5	>80	>80	>80
13	>80	>80	>80	>80
14	55.4	23.8	30.2	>80
15	>80	>80	>80	>80
16	>80	>80	>80	>80
17	>80	32.5	19.8	>80
19	>80	>80	>80	>80
20	50.9	>80	>80	>80
21	24.6	>80	>80	>80
22	17.8	>80	>80	>80
23	38.7	>80	>80	>80
24	21.7	>80	>80	>80
Cisplatin	10.1	2.3	67	10.3

Table 1: *In vitro* antiproliferative activities (IC_{50} in μM) of compounds **5-24**.

NMR (75 MHz, $CDCl_3$) *δ*: 169.9 (2-ArC), 150.7 (6-C), 147.7 (4-ArC), 134.8 (5-ArC), 128.6 (1'-PhC), 127.7 (2',6',-PhC), 126.0 (4'-PhC), 102.8 (3',5'-PhC), 83.7 (5-C), 67.0 (3-C), 56.4 (17-C), 56.05 (14-C), 55.7 (7-C), 52.0 (9-C), 45.4 (13-C), 44.7 (8-C), 43.5 (10-C), 42.5 (12-C), 39.8 (24-C), 39.5 (4-C), 36.3 (22-C), 35.6 (20-C), 28.7 (1-C), 28.6 (2-C), 28.0 (16-C), 27.7 (25-C), 24.7 (15-C), 23.9 (23-C), 22.8 (26-C), 22.6 (27-C), 21.6 (11-C), 18.8 (21-C), 18.2 (19-C), 12.6 (18-C); HREIMS *m/z*: 592.3923 $[M+H]^+$ (calcd. for $C_{36}H_{54}N_3O_2S$, 592.3937).

β) **1-(3β,5β-Dihydroxy-6'-B-norcholesteryl-6E-ylidene)-2-[5'-(p-trifluoromethylphenyl)-2'-thiazol]hydrazone** (**6**): Yellow oil, yield: 73%. IR (KBr) *ν*/cm^{-1}: 3416, 2945, 2863, 1709, 1614, 1564, 1524, 1448, 1409, 1377, 1320, 1170, 1125, 1066, 1013; ^1H NMR (300 MHz, CDCl3) *δ*: 8.11 (1H, d, *J*=8.1 Hz, Ar-H), 7.83 (2H, d, *J*=8.1, 2,6-PhH), 7.78 (1H, d, *J*=8.1, C6-H), 7.62 (2H, d, *J*=8.1, 3,5-PhH), 7.26 (1H, s, -NH), 6.82 (1H, s, 4-ArH), 4.46 (1H, s, -OH), 4.19 (1H, br s, C3-H), 4.13 (1H, dd, *J*=14.1, 6.9, C7-H), 3.45 (1H, s, -OH), 2.22-2.16 (2H, m, C4-H), 0.93 (3H, d, *J*=6.6, 21-CH_3), 0.92 (3H, s, 19-CH_3), 0.87 (6H, d, *J*=6.6, 26- and 27-CH_3), 0.72 (3H, s, 18-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 170.0 (2-ArC), 148.8 (6-C), 137.6 (5-ArC), 129.7 (1-PhC), 129.3 (4-ArC), 126.1 (2',6'-PhC), 125.9 (3',5'-PhC), 125.6 (4-PhC), 104.8 (CF_3), 83.7 (5-C), 67.1 (3-C), 56.4 (17-C), 56.1 (14-C), 55.6 (7-C),

51.9 (9-C), 45.4 (13-C), 44.7 (8-C), 43.6 (10-C), 42.3 (12-C), 39.8 (24-C), 39.5 (4-C), 36.2 (22-C), 35.6 (20-C), 30.3 (1-C), 28.5 (2-C), 28.0 (16-C), 27.6 (25-C), 24.7 (15-C), 23.8 (23-C), 22.8 (26-C), 22.5 (27-C), 21.6 (11-C), 18.8 (21-C), 18.3 (19-C), 12.5 (18-C); HREIMS *m/z*: 660.3806 $[M+H]^+$ (calcd. for $C_{37}H_{53}F_3N_3O_2S$, 660.3811).

χ) **1-(3β,5β-Dihydroxy-6'-B-norcholesteryl-6E-ylidene)-2-[5'-(p-nitrophenyl)-2'-thiazol]hydrazone** (**7**): Brown oil, yield: 73%. IR (KBr) ν/cm^{-1}: 3491, 2898, 1709, 1604, 1517, 1407, 1340, 1277, 1202, 1175, 1108, 1045, 1008; ^1H NMR (300 MHz, $CDCl_3$) δ: 8.21 (2H, d, *J*=8.8, 3',5'-PhH), 7.87 (2H, d, *J*=8.8, 2',6'-PhH), 7.23 (1H, d, *J*=6.6, C6-H), 6.94 (1H, s, 4-ArH), 4.20 (1H, br s, C3-H), 3.87 (1H, s, -OH), 3.44 (1H, s, -OH), 2.22-2.15 (2H, m, C7-H and C8-H), 0.93 (3H, d, *J*=6.6, 21-CH_3), 0.92 (3H, s, 19-CH_3), 0.86 (6H, d, *J*=6.6, 26- and 27-CH_3), 0.71 (3H, s, 18-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.0 (2-ArC), 148.7 (6-C), 148.4 (4-ArC), 146.8 (4'-PhC), 140.7 (1'-PhC), 126.4 (2',6'-PhC), 124.1 (3',5'-PhC), 106.9 (5-ArC), 83.7 (5-C), 67.1 (3-C), 56.4 (17-C), 56.1 (14-C), 55.6 (7-C), 52.0 (9-C), 45.4 (13-C), 44.7 (8-C), 43.6 (10-C), 42.4 (12-C), 39.8 (24-C), 39.5 (4-C), 36.2 (22-C), 35.6 (20-C), 28.6 (1-C), 28.5 (2-C), 28.0 (16-C), 27.7 (25-C), 24.7 (15-C), 23.8 (23-C), 22.8 (26-C), 22.5 (27-C), 21.6 (11-C), 18.8 (21-C), 18.3 (19-C), 12.5 (18-C); HREIMS *m/z*: 637.3767 $[M+H]^+$ (calcd. for $C_{36}H_{53}N_4O_4S$, 637.3788).

δ) **1-(3β,5β-Dihydroxy-6'-B-norcholesteryl-6E-ylidene)-2-[5'-(p-chlorophenyl)-2'-thiazol]hydrazone** (**8**): Yellow oil, yield: 84%. IR (KBr) ν/cm^{-1}: 3354, 2903, 1701, 1574, 1475, 1462, 1399, 1380, 1317, 1290, 1265, 1045, 1015; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.65 (2H, d, *J*=8.7, 3',5'-PhH), 7.32 (2H, d, *J*=8.7, 2',6'-PhH), 7.16 (1H, d, *J*=6.3, C6-H), 6.70 (1H, s, 4-ArH), 4.20-4.10 (1H, m, C3-H), 3.84 (1H, br s, -OH), 3.45 (1H, br s, -OH), 2.18-2.12 (2H, m, C7-H and C8-H), 0.93 (3H, d, *J*=6.3, 21-CH_3), 0.89 (3H, s, 19-CH_3), 0.88 (6H, *J*=6.6, 26- and 27-CH_3), 0.72 (3H, s, 18-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.1 (2-ArC), 149.5 (6-C), 147.9 (4-ArC), 133.4 (4'-PhC), 133.3 (1'-PhC), 128.8 (3',5'-PhC), 127.3 (2',6'-PhC), 103.2 (5-ArC), 83.7 (5-C), 67.0 (3-C), 56.4 (17-C), 56.1 (14-C), 55.7 (7-C), 52.0 (9-C), 45.4 (13-C), 44.7 (8-C), 43.6 (10-C), 42.4 (12-C), 39.8 (24-C), 39.5 (4-C), 36.2 (22-C), 35.6 (20-C), 28.6 (1-C), 28.6 (2-C), 28.0 (16-C), 27.6 (25-C), 24.7 (15-C), 23.9 (23-C), 22.8 (26-C), 22.6 (27-C), 21.0 (11-C), 18.8 (21-C), 18.2 (19-C), 12.6 (18-C); HREIMS *m/z*: 626.3552 $[M+H]^+$ (calcd. for $C_{36}H_{53}ClN_3O_2S$, 626.3547).

ε) **1-(3β,5β-Dihydroxy-6'-B-norcholesteryl-6E-ylidene)-2-[5'-(p-methoxyphenyl)-2'-thiazol]hydrazone** (**9**): Yellow oil, yield: 88%. IR (KBr) ν/cm^{-1}: 3426, 2925, 1711, 1607, 1566, 1511, 1499, 1459, 1374, 1165, 1108, 1026; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.65 (2H, d, *J*=8.7, 2',6'-PhH), 7.16 (1H, d, *J*=6.0, C6-H), 6.89 (2H, d, *J*=8.7, 3',5'-PhC), 6.57 (1H, s, 4-ArH), 4.15 (1H, br s, C3-H), 3.82 (3H, s, -OCH_3), 2.16-2.11 (2H, m, C7-H and C8-H), 0.93 (3H, d, *J*=6.6, 21-CH_3), 0.89 (3H, s, 19-CH_3), 0.88 (6H, d, *J*=6.6, 26- and 27-CH_3), 0.71 (3H, s, 18-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.0 (2-ArC), 159.3 (4'-PhC), 150.2 (6-C), 147.7 (5-ArC), 127.7 (4-ArC), 127.3 (2',6'-PhC), 114.0 (3',5'-PhC), 100.9 (1'-PhC), 83.6 (5-C), 67.0 (3-C), 56.4 (17-C), 56.0 (14-C), 55.7 (7-C), 55.3 (-OCH_3), 51.9 (9-C), 45.3 (13-C), 44.7 (8-C), 43.6 (10-C), 42.4 (12-C), 39.8 (24-C), 39.5 (4-C), 36.2 (22-C), 35.7 (20-C), 28.6 (1-C), 28.6 (2-C), 28.0 (16-C), 27.6 (25-C), 24.7 (15-C), 23.9 (23-C), 22.8 (26-C), 22.6 (27-C), 21.6 (11-C), 18.8 (21-C), 18.2 (19-C), 12.6 (18-C); HREIMS *m/z*: 622.4023 $[M+H]^+$ (calcd. for $C_{37}H_{56}N_3O_3S$, 622.4042).

ϕ) **1-(3β,5β-Dihydroxy-6'-B-norcholesteryl-6E-ylidene)-2-[5'-(p-cyanophenyl)-2'-thiazol] hydrazone** (**10**)L Brown oil, yield: 85%. IR (KBr) ν/cm^{-1}: 3384, 2928, 2223, 1706, 1602, 1556, 1456, 1407, 1325, 1263, 1169, 1043, 1009; ^1H NMR (600 MHz, $CDCl_3$) δ: 7.77 (2H, d, *J*=8.4, 2',6'-PhH), 7.58 (2H, d, *J*=8.4, 3',5'-PhH), 7.17 (1H, d, *J*=6.0, C6-H), 6.83 (1H, s, 4-ArH), 4.13 (1H, br s, C3-H), 3.95 (1H, s, -OH), 2.13-2.09 (2H, m, C8-H and C7-H), 0.89 (3H, d, *J*=6.0, 21-CH_3), 0.84 (3H, s, 19-CH_3), 0.84 (3H, d, *J*=6.6, 26- or 27-CH_3 s, 21-CH3), 0.83 (3H, d, *J*=6.6, 26- or 27-CH_3), 0.67 (3H, s, 18-CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 171.6 (2-ArC), 150.2 (6-C), 149.8 (5-ArC), 140.2 (1'-PhC), 133.9 (3',5'-PhC), 127.7 (2',6'-PhC), 120.4 (4-ArC), 112.0 (CN), 107.5 (4'-PhC), 85.1 (5-C), 68.4 (3-C), 57.7 (17-C), 57.4 (14-C), 57.0 (7-C), 53.0 (9-C), 46.8 (13-C), 46.1 (8-C), 45.0 (10-C), 43.7 (12-C), 41.1 (24-C), 40.9 (4-C), 37.6 (22-C), 37.0 (20-C), 30.0 (1-C), 29.7 (2-C), 29.4 (16-C), 29.0 (25-C), 26.1 (15-C), 25.2 (23-C), 24.3 (26-C), 24.0 (27-C), 23.0 (11-C), 20.2 (21-C), 19.8 (19-C), 14.0 (18-C); HREIMS *m/z*: 617.3898 $[M+H]^+$ (calcd. for $C_{37}H_{53}N_4O_2S$, 617.3889).

Compounds 12-17 were prepared similarly according to the procedure of 5-10, but using compound 11 as a reactive substrate. Compound 11 was prepared according to Gan et al. [20].

a)1-(3β,5β-Dihydroxy-6'-B-norcholesteryl-6E-ylidene)-2-(N-methyl-5'-phenylthiazol-2(3H)-ylidene)azine (**12**): Brown oil, yield: 76%. IR (KBr) ν/cm^{-1}: 3492, 2926, 1625, 1556, 1451, 1368, 1308, 1169, 1123; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.77 (1H, d, *J*=5.4, C6-H), 7.45-7.43 (3H, m, 3',4',5'-PhH), 7.36-7.34 (2H, m, 2',6'-PhH), 5.92 (1H, s, 4-ArH), 4.74 (1H, s, -OH), 4.01 (1H, br s, C3-H), 3.71 (1H, br s, -OH), 3.29 (3H, s, N-CH3), 2.28 (1H, dd, *J*=9.0, 5.1, C7-H), 2.11-2.04 (2H, m, C8-H and C4-H), 0.98 (3H, s, 19-CH_3), 0.93 (3H, d, *J*=6.6, 21-CH_3), 0.87 (6H, d, *J*=6.3, 26- and 27-CH3), 0.69 (3H, s, 18-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 169.8 (2-ArC), 157.7 (6-C), 140.9 (5-ArC), 131.0 (1'-PhC), 129.2 (4'-PhC), 128.8 (2',6'-PhC), 128.7 (3',5'-PhC), 98.8 (4-ArC), 83.2 (5-C), 67.2 (3-C), 56.6 (17-C), 55.7 (14-C), 54.3 (7-C), 50.0 (9-C), 45.2 (13-C), 44.9 (10-C), 44.8 (12-C), 44.6 (24-C), 39.9 (N-CH_3), 39.5 (4-C), 36.2 (8-C), 35.7 (22-C), 33.4 (20-C), 28.5 (1-C), 28.3 (2-C), 28.0 (16-C), 27.3 (25-C), 24.9 (15-C), 23.8 (23-C), 22.8 (26-C), 22.5 (27-C), 21.6 (11-C), 18.8 (21-C), 18.8 (19-C), 12.6 (18-C); HREIMS *m/z*: 606.4104 $[M+H]^+$ (calcd. for $C_{37}H_{56}N_3O_2S$, 606.4093).

b)1-(3β,5β-Dihydroxy-6'-B-norcholesteryl-6E-ylidene)-2-[N-methyl-5'-(p-trifluoromethyl phenyl)thiazol-2(3H)-ylidene]azine (**13**): Brownish oil, yield: 55%. IR (KBr) ν/cm^{-1}: 3449, 2922, 1721, 1622, 1544, 1508, 1320, 1251, 1173, 1120, 1015; ^1H NMR (600 MHz, $CDCl_3$) δ: 7.74 (1H, d, *J*=4.8, C6-H), 7.25 (2H, d, *J*=8.4, 3',5'-PhH), 6.94 (2H, d, *J*=8.4, 2',6'-PhH), 5.84 (1H, s, 4-ArH), 4.83 (1H, s, -OH), 3.98 (1H, br s, C3-H), 3.73 (1H, br s, -OH), 3.26 (3H, s, N-CH_3), 2.26 (1H, dd, *J*=9.0, 4.8, C7-H), 2.05 (2H, m, C8-H and C4-H), 0.96 (3H, s, 19-CH_3), 0.91 (3H, d, *J*=6.0, 21-CH_3), 0.85 (3H, d, *J*=6.6, 26- or 27-CH_3), 0.84 (3H, d, *J*=6.6, 26- or 27-CH_3), 0.66 (3H, s, 18-CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 171.3 (2-ArC), 161.7 (6-C), 158.9 (1'-PhC), 142.1 (5-ArC), 131.6 (2',6'-PhC), 124.6 (4'-PhC), 115.6 (3',5'-PhC), 99.4 (4-ArC), 84.6 (5-C), 68.6 (3-C), 58.1 (17-C), 57.1 (14-C), 56.8 (7-C), 55.5 (9-C), 51.2 (13-C), 46.5 (10-C), 46.4 (12-C), 46.2 (24-C), 46.0 (N-CH_3), 41.3 (4-C), 40.9 (8-C), 37.6 (22-C), 37.1 (20-C), 30.0 (1-C), 29.7 (2-C), 29.4 (16-C), 28.5 (25-C), 26.3 (15-C), 25.2 (23-C), 24.2 (26-C), 24.0 (27-C), 23.0 (11-C), 20.3 (21-C), 20.2 (19-C), 14.0 (18-C); HREIMS *m/z*: 674.3968 $[M+H]^+$ (calcd. for $C_{38}H_{55}F_3N_3O_2S$, 674.3967).

c)1-(3β,5β-Dihydroxy-6'-B-norcholesteryl-6E-ylidene)-2-[N-methyl-5'-(p-nitrophenyl) thiazol-2(3H)-ylidene]azine (**14**): Brown oil, yield: 64%. IR (KBr) ν/cm^{-1}: 3440, 2931, 1626, 1554, 1467, 1423, 1281, 1169, 1111, 1075; ^1H NMR (600 MHz, $CDCl_3$) δ: 8.28 (2H, d, *J*=8.4, 3',5'-PhH), 7.75 (1H, d, *J*=5.4, C6-H), 7.53 (2H, d, *J*=8.4, 2',6'-PhH), 6.08 (1H, s, 4-ArH), 4.48 (1H, s, -OH), 3.99 (1H, s, C3-H), 3.30 (3H, s, N-CH_3), 2.26 (1H, dd, *J*=9.0, 6.0, C7-H), 2.03-2.02 (2H, m, C8-H and C4-H), 0.93 (3H, s, 19-CH_3), 0.89 (3H, d, *J*=6.6, 21-CH_3), 0.84 (3H, d, *J*=6.6, 26- or 27-CH_3), 0.83 (3H, d, *J*=6.6, 26- or 27-CH_3), 0.65 (3H, s, 18-CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 170.6 (2-ArC), 160.2 (6-C), 149.3 (4'-PhC), 140.2 (1'-PhC), 138.5 (5-ArC), 130.6 (2',6'-PhC), 125.6

(3',5'-PhC), 103.5 (4-ArC), 84.6 (5-C), 68.6 (3-C), 58.1 (17-C), 57.1 (14-C), 55.9 (7-C), 51.4 (9-C), 46.6 (13-C), 46.2 (10-C), 46.0 (12-C), 45.8 (24-C), 41.2 (N-CH_3), 40.9 (4-C), 37.6 (8-C), 37.1 (22-C), 35.3 (20-C), 30.0 (1-C), 29.6 (2-C), 29.4 (16-C), 28.6 (25-C), 26.3 (15-C), 25.2 (23-C), 24.2 (26-C), 24.0 (27-C), 23.0 (11-C), 20.3 (21-C), 20.2 (19-C), 14.0 (18-C); HREIMS *m/z*: 651.3950 $[M+H]^+$ (calcd. For $C_{37}H_{55}N_4O_4S$, 651.3944).

d)1-(3β,5β-Dihydroxy-6'-B-norcholesteryl-6E-ylidene)-2-[N-methyl-5'-(p-chlorophenyl)thiazol-2(3H)-ylidene]azine (15): Yellow oil, yield: 69%. IR (KBr) v/cm-1: 3447, 2949, 2855, 1721, 1620, 1586, 1554, 1500, 1462, 1366, 1322, 1166, 1127, 1065, 1017; ^{1}H NMR (600 MHz, CDCl3) *δ*: 7.76 (1H, d, *J*=5.4, C6-H), 7.39 (2H, d, *J*=8.4, 3',5'-PhH), 7.26 (2H, d, *J*=8.4, 2',6'-PhH), 5.92 (1H, s, 4-ArH), 4.63 (1H, s, -OH), 3.99 (1H, br s, C3-H), 3.26 (3H, s, N-CH_3), 2.25 (1H, dd, *J*=9.0, 5.4, C7-H), 2.06-2.01 (2H, m, C8-H and C4-H), 0.94 (3H, s, 19-CH_3), 0.89 (3H, d, *J*=6.0, 21-CH_3), 0.84 (3H, d, *J*=6.6, 26- or 27-CH_3), 0.83 (3H, d, *J*=6.6, 26- or 27-CH_3), 0.66 (3H, s, 18-CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) *δ*: 170.9 (2-ArC), 159.6 (6-C), 141.2 (1'-PhC), 136.8 (4'-PhC), 131.4 (3',5'-PhC), 130.6 (3-ArC), 130.5 (2',6'-PhC), 101.1 (4-ArC), 84.6 (5-C), 68.6 (3-C), 58.1 (17-C), 57.1 (14-C), 55.8 (7-C), 51.4 (9-C), 46.6 (13-C), 46.2 (10-C), 46.1 (12-C), 45.8 (24-C), 41.3 (N-CH_3), 40.9 (4-C), 37.6 (8-C), 37.1 (22-C), 35.0 (20-C), 30.0 (1-C), 29.6 (2-C), 29.4 (16-C), 28.6 (25-C), 26.3 (15-C), 25.2 (23-C), 24.2 (26-C), 24.0 (27-C), 23.0 (11-C), 20.3 (21-C), 20.2 (19-C), 14.0 (18-C); HREIMS *m/z*: 640.3716 $[M+H]^+$ (calcd. For $C_{37}H_{55}ClN_3O_2S$, 640.3703).

e)1-(3β,5β-Dihydroxy-6'-B-norcholesteryl-6E-ylidene)-2-[N-methyl-5'-(p-methoxy phenyl)thiazol-2(3H)-ylidene]azine (16): Yellow oil, yield: 70%. IR (KBr) v/cm^{-1}: 3437, 2940, 2860, 1620, 1588, 1544, 1506, 1464, 1423, 1361, 1290, 1249, 1170, 1125, 1072, 1038; ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 7.76 (1H, d, *J*=5.1, C6-H), 7.27 (1H, d, *J*=8.7, 2',6'-PhH), 6.96 (2H, d, *J*=8.7, 3',5'-PhH), 5.85 (1H, s, 4-ArH), 4.79 (1H, s, -OH), 4.00 (1H, br s, C3-H), 3.85 (3H, s, -OCH_3), 3.73 (1H, s, -OH), 3.27 (3H, s, N-CH_3), 2.27 (1H, dd, *J*=9.0, 5.1, C7-H), 2.10-2.04 (2H, m, C8-H and C4-H), 0.97 (3H, s, 19-CH_3), 0.93 (3H, d, *J*=6.6, 21-CH_3), 0.87 (6H, d, *J*=6.6, 26- and 27-CH_3), 0.69 (3H, s, 18-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 169.8 (2-ArC), 160.3 (6-C), 157.5 (4'-PhC), 140.7 (5-ArC), 130.1 (2',6'-PhC), 123.3 (1'-PhC), 114.2 (3',5'-PhC), 98.0 (4-ArC), 83.2 (5-C), 67.2 (3-C), 56.8 (-OCH_3), 55.7 (17-C), 55.4 (14-C), 54.2 (7-C), 50.0 (9-C), 45.2 (13-C), 44.9 (10-C), 44.8 (12-C), 44.6 (24-C), 39.9 (N-CH_3), 39.5 (4-C), 36.2 (8-C), 35.7 (22-C), 33.3 (20-C), 28.5 (1-C), 28.3 (2-C), 28.0 (16-C), 27.2 (25-C), 24.9 (15-C), 23.8 (23-C), 22.8 (26-C), 22.5 (27-C), 21.6 (11-C), 18.8 (21-C), 18.8 (19-C), 12.6 (18-C); HREIMS *m/z*: 636.4200 $[M+H]^+$ (calcd. For $C_{38}H_{58}N_3O_3S$, 636.4199).

f)1-(3β,5β-Dihydroxy-6'-B-norcholesteryl-6E-ylidene)-2-[N-methyl-5'-(p-cyanophenyl) thiazol-2(3H)-ylidene]azine (17):Yellow oil, yield: 67%. IR (KBr) v/cm-1: 3467, 2858, 2225, 1634, 1583, 1551, 1499, 1464, 1425, 1306, 1269, 1173, 1072, 1017; ^{1}H NMR (600 MHz, $CDCl_3$) *δ*: 7.73-7.69 (4H, m, 2',3',5',6'-PhH), 7.47 - 7.45 (1H, d, *J*=7.8, C6-H), 6.01 (1H, s, 4-ArH), 4.47 (1H, s, -OH), 3.97 (1H, s, C3-H), 3.35 (1H, s, -OH), 3.26 (3H, s, N-CH_3), 2.24-2.22 (1H, m, C7-H), 2.02-1.99 (2H, m, C8-H and C4-H), 0.91 (3H, d, *J*=6.0, 21-CH_3), 0.87 (3H, s, 19-CH_3), 0.81 (6H, d, *J*=6.0, 26- and 27-CH_3), 0.64 (3H, s, 18-CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) *δ*: 170.7 (2-ArC), 160.0 (6-C), 140.5 (1'-PhC), 136.7 (5-ArC), 134.1 (3',5'-PhC), 130.5 (2',6'-PhC), 119.6 (4-ArC), 114.1 (CN), 102.9 (4'-PhC), 84.6 (5-C), 68.5 (3-C), 58.0 (17-C), 57.0 (14-C), 56.0 (7-C), 55.9 (9-C), 51.4 (13-C), 46.6 (10-C), 46.2 (12-C), 45.8 (24-C), 41.2 (N-CH3), 40.9 (4-C), 37.6 (8-C), 37.0 (22-C), 35.2 (20-C), 30.0 (1-C), 29.6 (2-C), 29.4 (16-C), 28.6 (25-C), 26.3 (15-C), 25.2 (23-C), 24.2 (26-C), 24.0 (27-C), 23.0 (11-C), 20.3 (21-C), 20.2 (19-C), 14.0 (18-C); HREIMS *m/z*: 631.4055 $[M+H]^+$ (calcd. For $C_{38}H_{55}N_4O_2S$, 631.4046).

Compounds 19-24 were prepared similarly according to the procedure of 5-10, but using compound 18 as a reactive substrate. Compound 18 was prepared according to Gan et al. [20].

a)1-(3β,5β-Dihydroxy-6'-B-norcholesteryl-6E-ylidene)-2-(3',5'-diphenylthiazol-2(3H)-ylidene)azine (19): Yellow oil, yield: 62%. IR (KBr) *ν*/cm^{-1}: 3439, 2933, 2855, 1624, 1569, 1544, 1492, 1455, 1295, 1165, 1123, 1011; ^{1}H NMR (600 MHz, $CDCl_3$) *δ*: 7.63 (1H, d, *J*=5.4, C6-H), 7.28-7.04 (10H, m, Ph-H), 6.08 (1H, s, 4-ArH), 4.75 (1H, s, -OH), 3.96 (1H, s, C3-H), 3.84 (1H, br s, -OH), 2.22 (1H, dd, *J*=9.6,6.0, C7-H), 2.05-2.02 (2H, m, C8-H and C4-H), 0.94 (3H, s, 19-CH_3), 0.91 (3H, d, *J*=6.6, 21-CH_3), 0.87 (3H, d, *J*=6.6, 26- or 27-CH_3), 0.86 (3H, d, *J*=6.6, 26- or 27-CH_3), 0.66 (3H, s, 18-CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) *δ*: 171.0 (2-ArC), 160.4 (6-C), 141.7 (5-ArC), 138.8 (1''-NPhC), 132.5 (1'-PhC), 130.3 (Ph-C), 130.0 (Ph-C), 129.9 (Ph-C), 129.8 (Ph-C), 129.7 (Ph-C), 129.6 (Ph-C), 129.1 (Ph-C), 102.0 (4-ArC), 84.7 (5-C), 68.5 (3-C), 58.2 (17-C), 57.1 (14-C), 55.8 (7-C), 51.3 (9-C), 46.6 (13-C), 46.2 (10-C), 45.9 (12-C), 45.8 (24-C), 41.3 (4-C), 40.9 (8-C), 37.6 (22-C), 37.1 (20-C), 30.0 (1-C), 29.6 (2-C), 29.4 (16-C), 28.5 (25-C), 26.2 (15-C), 25.3 (23-C), 24.3 (26-C), 24.0 (27-C), 23.1 (11-C), 20.4 (21-C), 20.2 (19-C), 14.1 (18-C); HREIMS *m/z*: 668.4248 $[M+H]^+$ (calcd. for $C_{42}H_{58}N_3O_2S$, 668.4250).

b)1-(3β,5β-Dihydroxy-6'-B-norcholesteryl-6E-ylidene)-2-[N-phenyl-5'-(p-trifluoromethylphenyl)thiazol-2(3H)-ylidene]azine (20): Yellow oil, yield: 63%. IR (KBr) *ν*/cm^{-1}: 3421, 2943, 2856, 1622, 1594, 1542, 1499, 1425, 1362, 1167, 1088; ^{1}H NMR (600 MHz, $CDCl_3$) *δ*: 7.63 (1H, d, *J*=5.4, C6-H), 7.42 (2H, d, *J*=8.4, 3',5'-PhH), 7.31 (2H, d, *J*=8.4, 2',6'-PhH), 7.26 (1H, t, *J*=7.2, N-PhH), 7.2-7.17 (4H, m, N-PhH), 6.20 (1H, s, 4-ArH), 4.65 (1H, br s, -OH), 3.98 (1H, br s, C3-H), 2.23 (1H, dd, *J*=9.6, 5.4, C7-H), 2.05-2.02 (2H, m, C8-H and C4-H), 0.94 (3H, s, 19-CH_3), 0.91 (3H, d, *J*=6.6, 21-CH_3), 0.86 (3H, d, *J*=6.6, 26- or 27-CH_3), 0.85 (3H, d, *J*=6.6, 26- or 27-CH_3), 0.65 (3H, s, 18-CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) *δ*: 170.6 (2-ArC), 161.0 (6-C), 140.3 (5-ArC), 138.5 (1''-NPhC), 136.0 (1'-PhC), 131.7 (-CF_3), 130.5 (3'',5''-NPhC), 129.8 (2',6'-PhC), 129.7 (2'',6''-NPhC), 126.7 (3',5'-PhC), 104.0 (4-ArC), 84.7 (5-C), 68.6 (3-C), 58.1 (17-C), 57.1 (14-C), 55.6 (9-C), 51.2 (13-C), 46.6 (10-C), 46.2 (12-C), 45.9 (24-C), 41.3 (4-C), 40.9 (8-C), 37.6 (22-C), 37.1 (20-C), 29.9 (1-C), 29.6 (2-C), 29.4 (16-C), 28.4 (25-C), 26.3 (15-C), 25.2 (23-C), 24.2 (26-C), 24.0 (27-C), 23.0 (11-C), 20.3 (21-C), 20.2 (19-C), 14.0 (18-C); HREIMS *m/z*: 736.4095 $[M+H]^+$ (calcd. for $C_{43}H_{57}F_3N_3O_2S$, 736.4124).

c)1-(3β,5β-Dihydroxy-6'-B-norcholesteryl-6E-ylidene)-2-[N-phenyl-5'-(p-nitrophenyl) thiazol-2(3H)-ylidene]azine (21): Brown oil, yield: 60%. IR(KBr)*ν*/cm^{-1}: 3451, 2935, 2855, 1629, 1591, 1546, 1492, 1452, 1410, 1198, 1167, 1105, 1078; ^{1}H NMR (600 MHz, $CDCl_3$) *δ*: 8.02 (2H, d, *J*=9.0, 3',5'-PhH), 7.63 (1H, d, *J*=5.4, C6-H), 7.32 (2H, t, *J*=7.2, N-PhH), 7.27 (1H, d, *J*=7.2, N-PhH), 7.22 (2H, d, *J*=9.0, 2',6'-PhH), 7.19 (2H, d, *J*=7.2, N-PhH), 6.31 (1H, s, 4-ArH), 4.52 (1H, s, -OH), 3.98 (1H, s, C3-H), 2.23 (1H, dd, *J*=9.6, 6.0, C7-H), 2.04-2.02 (2H, m, C8-H and C4-H), 0.93 (3H, s, 19-CH_3), 0.90 (3H, d, *J*=6.6, 21-CH_3), 0.85 (3H, d, *J*=6.6, 26- or 27-CH_3), 0.84 (3H, d, *J*=6.6, 26- or 27-CH_3), 0.65 (3H, s, 18-CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) *δ*: 170.2 (2-ArC), 161.4 (6-C), 148.5 (4'-PhC), 139.5 (5-ArC), 138.6 (1'-PhC), 138.5 (1''-NPhC), 130.7 (3'',5''-NPhC), 130.2 (4''-NPhC), 130.0 (2',6'-PhC), 129.6 (2'',6''-NPhC), 125.0 (3',5'-PhC), 105.8 (4-ArC), 84.7 (5-C), 68.6 (3-C), 58.1 (17-C), 57.1 (14-C), 55.8 (7-C), 51.3 (9-C), 46.6 (13-C), 46.2 (10-C), 45.9 (12-C), 45.8 (24-C), 41.3 (4-C), 40.9 (8-C), 37.6 (22-C), 37.1 (20-C), 29.9 (1-

C), 29.6 (2-C), 29.4 (16-C), 28.4 (25-C), 26.2 (15-C), 25.2 (23-C), 24.2 (26-C), 24.0 (27-C), 23.0 (11-C), 20.3 (21-C), 20.2 (19-C), 14.0 (18-C); HREIMS *m/z*: 713.4132 $[M+H]^+$ (calcd. For $C_{42}H_{57}N_4O_4S$, 713.4101).

d)1-(3β,5β-Dihydroxy-6'-B-norcholesteryl-6E-ylidene)-2-[N-phenyl-5'-(p-chlorophenyl) thiazol-2(3H)-ylidene]azine (**22**): Yellow oil, yield: 80%. IR(KBr)*v*/cm^{-1}: 3444, 2943, 2858, 1622, 1582, 1544, 1484, 1447, 1397, 1355, 1298, 1165, 1011; ^1H NMR (600 MHz, $CDCl_3$) *δ*: 7.61 (1H, d, *J*=5.4, C6-H), 7.29 (2H, t, *J*=7.2, 3",5"-NPhH), 7.24 (1H, d, *J*=7.2, 4",-NPhC), 7.16 (2H, d, *J*=7.2, 2",6"-NPhH), 7.13 (2H, d, *J*=8.4, 3',5'-PhH), 6.98 (2H, d, *J*=8.4, 2',6'-PhH), 6.09 (1H, s, 4-ArH), 4.67 (1H, s, -OH), 3.96 (1H, s, C3-H), 2.21 (1H, dd, *J*=9.5, 5.4, C7-H), 2.04-2.01 (2H, m, C8-H and C4-H), 0.93 (3H, s, 19-CH_3), 0.90 (3H, d, *J*=6.6, 21-CH_3), 0.86 (3H, d, *J*=6.6, 26- or 27-CH_3), 0.85 (3H, d, *J*=6.6, 26- or 27-CH_3), 0.65 (3H, s, 18-CH3); ^{13}C NMR (150 MHz, $CDCl_3$) *δ*: 170.7 (2-ArC), 160.7 (6-C), 140.5 (5-ArC), 138.7 (1"-NPhC), 135.8 (1'-PhC), 131.0 (4'-PhC), 130.8 (3",5"-NPhC), 130.4 (3',5'-PhC), 130.0 (2',6'-PhC), 129.9 (2",6"-NPhC), 129.3 (4"-NPhC), 102.6 (4-ArC), 84.6 (5-C), 68.5 (3-C), 58.2 (17-C), 57.1 (14-C), 55.7 (7-C), 51.3 (9-C), 46.6 (13-C), 46.2 (10-C), 45.9 (12-C), 45.8 (24-C), 41.3 (4-C), 40.9 (8-C), 37.6 (22-C), 37.1 (20-C), 30.0 (1-C), 29.6 (2-C), 29.4 (16-C), 28.5 (25-C), 26.2 (15-C), 25.2 (23-C), 24.3 (26-C), 24.0 (27-C), 23.0 (11-C), 20.3 (21-C), 20.2 (19-C), 14.0 (18-C); HREIMS *m/z*: 702.3851 $[M+H]^+$ (calcd. For $C_{42}H_{57}ClN_3O_2S$, 702.3860).

e)1-(3β,5β-Dihydroxy-6'-B-norcholesteryl-6E-ylidene)-2-[N-phenyl-5'-(p-methoxy-phenyl)thiazol-2(3H)-ylidene]azine (**23**): Yellow oil, yield: 75%. IR (KBr) *v*/cm^{-1}: 3471, 2945, 2855, 1592, 1544, 1502, 1455, 1357, 1295, 1248, 1175; ^1H NMR ($CDCl_3$, 600 MHz) *δ*: 7.61 (H, d, *J*=5.4, C6-H), 7.30-7.27 (2H, m, 3",5"-NPhH), 7.23 (1H, d, *J*=6.0, 4"-NPhH), 7.17 (2H, d, *J*=7.2, 2',6'-PhH), 6.96 (2H, d, *J*=8.4, 2",6"-NPhH), 6.67 (2H, d, *J*=7.2, 3',5'-PhH), 5.99 (1H, s, 4-ArH), 4.80 (1H, br s, -OH), 3.96 (1H, br s, C3-H), 3.82 (1H, br s, -OH), 3.71 (3H, s, -OCH_3), 2.21 (1H, dd, *J*=9.6, 5.4, C7-H), 2.04-2.02 (2H, m, C8-H and C4-H), 0.94 (3H, s, 19-CH_3), 0.91 (3H, d, *J*=6.6, 21-CH_3), 0.860 (3H, d, *J*=6.6, 26- or 27-CH_3), 0.855 (3H, d, *J*=6.6, 26- or 27-CH_3), 0.65 (3H, s, 18-CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) *δ*: 171.1 (2-ArC), 160.9 (6-C), 160.2 (4'-PhC), 141.5 (5-ArC), 138.9 (1"-NPhC), 131.0 (3",5"-NPhC), 130.3 (2',6'-PhC), 130.0 (2",6"-NPhC), 129.1 (1'-PhC), 125.0 (4"-NPhC), 115.0 (3',5'-PhC), 100.7 (4-ArC), 84.6 (5-C), 68.5 (3-C), 58.2 (-OCH_3), 57.1 (17-C), 56.6 (14-C), 55.6 (7-C), 51.2 (9-C), 46.6 (13-C), 46.2 (10-C), 45.9 (12-C), 45.8 (24-C), 41.3 (4-C), 40.9 (8-C), 37.6 (22-C), 37.1 (20-C), 30.0 (1-C), 29.6 (2-C), 29.4 (16-C), 28.4 (25-C), 26.2 (15-C), 25.2 (23-C), 24.3 (26-C), 24.0 (27-C), 23.1 (11-C), 20.4 (21-C), 20.2 (19-C), 14.0 (18-C); HREIMS *m/z*: 698.4365 $[M+H]^+$ (calcd. for $C_{43}H_{60}N_3O_3S$, 698.4355).

f)1-(3β,5β-Dihydroxy-6'-B-norcholesteryl-6E-ylidene)-2-[N-phenyl-5'-(p-cyanophenyl) thiazol-2(3H)-ylidene]azine (**24**): Yellow oil, yield: 49%. IR (KBr) *v*/cm^{-1}: 3454, 2940, 2227, 1624, 1587, 1554, 1494, 1454, 1362, 1295, 1083, 1013; ^1H NMR (600 MHz, $CDCl_3$) *δ*: 7.63 (1H, d, *J*=5.4, C6-H), 7.46 (2H, d, *J*=8.4, 2",6"-NPhH), 7.33 (2H, d, *J*=7.8, 2',6'-PhH), 7.28 (1H, *J*=8.4, 4"-NPhH), 7.18 (1H, d, *J*=7.8, 3',5'-PhH), 7.16 (2H, d, *J*=8.4 Hz, 3",5"-NPhH), 6.24 (1H, s, 4-ArH), 4.55 (1H, s, -OH), 3.98 (1H, m, C3-H), 2.22 (1H, dd, *J*=9.6, 5.4, C7-H), 2.04-2.02 (2H, m, C8-H and C4-H), 0.93 (3H, s, 19-CH_3), 0.90 (3H, d, *J*=6.6, 21-CH_3), 0.85 (3H, d, *J*=6.6, 26- or 27-CH_3), 0.84 (3H, d, *J*=6.6, 26- or 27-CH_3), 0.65 (3H, s, 18-CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) *δ*: 170.3 (2-ArC), 161.3 (6-C), 139.9 (5-ArC), 138.4 (1'-PhC), 136.8 (1"-NPhC), 133.5 (3',5'-PhC), 130.6 (3",5"-NPhC), 130.0 (4"-NPhC), 129.8 (2",6"-NPhC), 129.7 (2',6'-PhC), 129.6 (4-ArC), 119.7 (CN), 113.3 (4'-PhC), 84.7 (5-C), 68.6 (3-C), 58.1 (17-C), 57.1 (14-C), 55.7 (7-C), 51.2 (9-C), 46.6 (13-C), 46.2 (10-C), 45.9 (12-C), 41.3 (24-C), 40.9 (4-C), 37.6 (8-C), 37.0 (22-C), 31.1 (20-C), 30.0 (1-C), 29.6 (2-C), 29.4 (16-C), 28.4 (25-C), 26.2 (15-C), 25.2 (23-C), 24.2 (26-C), 24.0 (27-C), 23.0 (11-C), 20.3 (21-C), 20.2 (19-C), 14.0 (18-C); HREIMS *m/z*: 693.4203 $[M+H]^+$ (calcd. for $C_{43}H_{57}N_4O_2S$, 693.4202).

Biological assays

Materials: Stock solutions of the compounds were prepared in sterile dimethyl sulfoxide (DMSO) (Sigma) at a concentration of 10 mg/mL and afterward diluted with complete nutrient medium (RPMI-1640) supplemented with 10% heat inactivated fetal bovine serum and 0.1 g/L penicillin G+0.1 g/L streptomycin sulfate.

Cell culture: HeLa, A549, HEPG2 cancer cells and HEK293T cells were grown in the medium (RPMI-1640) supplemented with 10% heat inactivated fetal bovine serum and 0.1 g/L penicillin G+0.1 g/L streptomycin sulfate in a humidified atmosphere of 5% CO_2 at 37°C.

Assay for cell viability: The anticancer activity *in vitro* was measured using the MTT assay. Briefly, cells (1~2 × 10^4 cells per well) were seeded in 96-wells plates for 24 h. Different concentrations of the test compounds were added to the cells. An equal amount of DMSO was added to the cells used as negative controls. Triplicate wells were prepared for each individual dose. After reincubated for 72 h, the cells were washed with sterile phosphate buffer saline (PBS). 190 μL of RPMI-1640 and 10 μL of the tetrazolium dye (MTT) (5 mg/mL) solution were added to each well, and the cells were incubated for additional 4 h. After the supernatant was discarded, 200 μL of DMSO was added to dissolve the purple formazan crystals formed. The absorbance values (*A*) at 492 nm were determined using a MLLTISKAN MK3 analysis spectrometer. The IC_{50} values were calculated as the concentration of drug yielding 50% cell survival.

Conclusions

We synthesized some novel B-norcholesteryl thiazole derivatives. Their structures were characterized by IR, NMR and HRMS. The antiproliferative activity of the compounds against human cervical carcinoma (HeLa), human lung carcinoma (A549), human liver carcinoma cells (HEPG2) and normal kidney epithelial cells (HEK293T) was assayed. The results showed that some compounds with the structure of N-methylthiazole displayed distinct antiproliferative activity against A549 and HEPG2 cells. In addition, compounds with the structure of N-phenylthiazole exhibited a selective antiproliferative activity on HeLa cells and were almost inactive to normal kidney epithelial cells (HEK293T). The research provided a theoretical reference for the exploration of new anti-cancer agents and may be useful for the design of novel chemotherapeutic drugs.

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