某些 B-降胆甾噻唑化合物的合成及抗肿瘤活性研究

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摘要 从胆甾醇出发, 合成了系列 B-降胆甾噻唑化合物, 通过 IR、NMR 及 HRMS 等现代分析方法对合成物进行了结构表征。同时, 分别采用人肺癌细胞(A549)、宫颈癌细胞(HeLa)、肝癌细胞(HEPG2)和人正常肾上皮细胞(HEK293T)对合成产物进行体外抑制肿瘤细胞增殖活性研究, 结果表明具有 *N*-甲基噻唑结构的化合物 **14** 和 **17** 对 A549 和 HEPG2 细胞的生长增殖表现出较好的抑制活性; 具有 *N*-苯基噻唑结构的化合物 **20**~**24** 对 HeLa 细胞表现出选择性的增殖抑制作用, 但对人正常肾上皮细胞却几乎没有作用。

关键词 胆甾醇; 甾体杂环化合物; B-降甾体噻唑化合物; 抗肿瘤活性

Synthesis and Evaluation of Some New B-Norcholesteryl Thiazole Compounds as Anticancer Agents

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Abstract Some B-norcholesteryl thiazole compounds were synthesized and their structures were determined by IR, NMR and HRMS. The antiproliferative activity of the compounds against human lung carcinoma (A549), cervical carcinoma (HeLa), liver carcinoma (HEPG2) and normal kidney epithelial (HEK293T) cells was assayed. The results showed that some compounds with a structure of *N*-methylthiazole showed distinct antiproliferative activity against A549 and HEPG2 cells. The compounds with a structure of *N*-phenylthiazole displayed a selective antiproliferative activity against HeLa cells and were almost inactive to HEK293T cells. The research provided a theoretical reference for the exploration of new anti-cancer agents.

Keywords cholesterol; heterosteroids; B-norcholesteryl thiazoles; antiproliferative activity

甾体化合物表现出不同的生物活性, 它们在生命体中起着非常重要的作用。甾体药物在传统药物中已经得到广泛应用, 如抗炎、抗菌、抗肿瘤、降压、激素类药物、降胆固醇及利尿药等^[1-8]。

最近, 从海洋生物中分离得到许多具有特殊结构的甾体化合物^[9-12]。在这些甾体化合物中, 具有 B-降结构的甾体由于在自然界中存在稀缺, 其很少被报道。Wentworth 等^[13]报道了一种具有[6, 5, 6, 5]稠环结构的

3 β ,5 β -二羟基-B-降胆甾-6 β -甲醛, 它是由于人类某些疾病的原因, 在生命体中通过胆甾醇的臭氧化所产生。另外, Wang^[14]、Miyamoto^[15]和 Scott^[16]等也分别从植物及海洋生物中分离得到某些具有[6, 5, 6, 5]稠环结构甾核的甾体, 而 Wei 等^[17]对此类化合物进行了合成研究。

为了研究新甾体化合物的抗肿瘤活性, 探索开发新的抗肿瘤药物, 我们曾合成了系列具有[6, 5, 6, 5]稠环甾核、不同支链结构和不同药效团取代的新型甾体衍生

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物,同时调查了它们对不同肿瘤细胞的抑制细胞增殖活性^[18~20]。结果表明,在此类化合物中,一条胆甾烷结构的支链对化合物的抗肿瘤活性非常重要。同时,在甾核的 C-6 位置一个极性药效团的存在(如羟基、氰基、胍基、缩氨基脲基等)可以大大增强化合物的抗肿瘤活性。

多年来,在药物探索研究领域,杂环甾体一直受到药物化学家的很大关注。在甾体中引入一个杂原子或杂环将会影响到甾体的化学性质,同时将导致其生物活性的改变^[21,22]。至今,含有杂环或杂原子的甾体已经得到广泛报道^[23]。文献中认为,这样一些杂环甾体往往表现出显著的抑制肿瘤细胞增殖或抗菌活性^[24~28]。

最近,我们合成了某些奇特的具有[6, 5, 6, 5]稠环结构的 B-降胆甾苯并咪唑和苯并噻唑衍生物,并评估了它们对某些肿瘤细胞的体外抗增殖活性^[29]。结果表明,某些 B-降胆甾苯并咪唑衍生物表现出优越的抑制肿瘤细胞增殖活性,而对人体正常肾上皮细胞(HEK-293T)几乎没有作用。噻唑环是一种具有广泛生物活性的药效团,如抗菌、抗炎、抗肿瘤、抗 HIV-1 及抗疟等^[30~34]。为了获得具有不同结构的生物有效的抗肿瘤化合物,作为我们前面工作的继续,我们从胆甾醇出发,合成了系列具有 6-噻唑环结构的 B-降甾体衍生物,并进一步评估了合成物的抑制肿瘤细胞生长增殖活性。

1 结果与讨论

1.1 化学部分

图 1 列出了 B-降胆甾噻唑衍生物 5~10 的合成路线

图式 1 化合物 5~10 的合成路线
Scheme 1 Synthetic route of compounds 5~10

线。化合物 4 根据文献[18]中的制备程序制备。化合物 3 中 C-5 和 C-7 的立体构型已在参考文献[13, 36, 37]中描述。通过化合物 3 和氨基硫脲的反应,得到化合物 4。化合物 4 和 ω -溴代苯乙酮反应得到相应的 B-降胆甾噻唑衍生物 5, 化合物 5 的结构通过 IR、NMR 及 HRMS 的表征。在 NMR 谱中,在 δ 7.75, 7.38, 7.30 处出现了 Ph-H 的化学位移,在 δ 128.7~102.8 处出现了 Ph-C 的化学位移,说明化合物 5 存在一个苯环结构。而在 ^1H NMR 中, δ 7.14 表明一个噻唑环质子的存在, δ 169.9, 147.7, 134.8 说明了噻唑环的形成。由此可见,化合物 5 的结构得到了相应 NMR 数据的表征。为了调查在化合物 5 中苯环上不同取代基对化合物抗肿瘤活性的影响,通过化合物 4 和不同的 4-取代- ω -溴代苯乙酮反应,得到了化合物 6~10, 其结构同样通过了其波谱及光谱的表征。

为了调查噻唑环中取代基对化合物抗肿瘤活性的影响,通过化合物 11 或 18 和不同的 ω -溴代苯乙酮反应,我们制备得到了 B-降胆甾噻唑的 N-甲基和 N-苯基噻唑衍生物 12~17 和 19~24, 所有合成物的结构均得到了 IR, NMR 和 HRMS 的结构证实(Scheme 2)。

1.2 生物部分

肺癌和肝癌是癌症病人死亡的主要原因。为了调查新化合物的抗肿瘤活性,我们采用 MTT 评估方法,确定了化合物对人卵巢癌细胞(HeLa)、肺癌细胞(A549)、肝癌细胞(HEPG2)及人正常肾上皮细胞(HEK293T)的抑制生长增殖活性,测试结果见表 1 所示。

图式 3 化合物 12~17 和 19~24 的合成路线

Scheme 3 Synthetic route of compounds 12~17 and 19~24

表 1 化合物 5~24 体外抑制肿瘤细胞生长增殖的 IC_{50} 值^aTable 1 *In vitro* antiproliferative activities (IC_{50}) of compounds 5~24

化合物	$\text{IC}_{50}/(\mu\text{mol}\cdot\text{L}^{-1})$			
	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
11	5.4	ND	ND	ND
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	5.2	22.2	10.3

^a ND: 没有测定.

正如表 1 所示, 对于所有的 N 上没有取代基的 B-降胆甾烷噻唑衍生物 5~10, 它们对所有被测试的肿瘤细胞株都没有表现出明显的抑制活性. 而对于 N 上具有取代基的 B-降胆甾烷噻唑衍生物来说, 化合物 14 对所有被测试的肿瘤细胞株都表现出中等程度的抑制活性, 但是对人体正常肾上皮细胞却几乎是是非活性. 而化合物 17 却对 A549 和 HEPG2 细胞表现出明显生长增殖抑制作用, 同样地对人体正常肾上皮细胞也是非活性. 可以看到, 化合物 14 和 17 都属于 N-甲基噻唑衍生物, 同时两者在苯环上都存在一个吸电子基团. 有趣的是对于具有 N-苯基噻唑环结构的化合物 20~24, 它们对 HeLa 肿瘤细胞株表现出明显的生长增殖抑制作用, 但对其它细

胞株却没有表现活性, 结果表明化合物 20~24 对卵巢癌 HeLa 细胞是较好的选择性生长增殖抑制剂, 值得进一步的深入研究.

但是, 所有合成的 B-降胆甾烷噻唑衍生物对所测试的肿瘤细胞株的抑制活性都低于阳性对照物顺铂. 同时, 也低于其前体化合物 11 (HeLa 细胞) 及我们先前报道的 B-降胆甾烷苯并咪唑和苯并噻唑衍生物^[29], 主要原因可能是在噻唑环上连接一个苯环后, 大大增加了 6-位取代基的链长及体积, 影响了其与肿瘤细胞中蛋白质或 DNA 的亲合作用的缘故.

2 实验部分

2.1 仪器与试剂

胆甾醇及其它试剂均购于百灵威科技有限公司; 所用试剂如无特别说明均为分析纯, 溶剂按常规方法进行处理干燥. 熔点测定采用 X₆ 显微熔点测定仪, 温度计未经校正; IR 光谱采用 Thermo Scientific Nicolet IS-10 傅立叶红外光谱仪测定 (Thermo Scientific, America); ¹H NMR 和 ¹³C NMR 谱采用德国 Bruker AV-300 型或 Bruker AV-600 型超导核磁共振仪测定; 高分辨质谱采用美国 Agilent 6210 TOFMS 质谱仪测定 (Agilent Technologies, America); 细胞增殖活性评估采用 MTT 方法, 使用 MLLTISKAN MK3 分光仪测定 (Thermo Scientific, Shanghai, China).

2.2 实验方法

2.1.2 化合物 5~10 的一般合成步骤

0.3 mmol 化合物 4 溶解于 50 mL CH₃OH 中, 然后加入 α -溴代苯乙酮或 4-取代- α -溴代苯乙酮 0.45 mmol, 加热至 60 °C, 在此条件下继续反应 2~3 h. TLC 跟踪检测无原料点后停止反应 [展开剂: $V(\text{石油醚}): V(\text{乙酸乙酯}) = 1:1$]. 减压蒸出所有溶剂, 得到的固体通过快速硅胶柱层析分离得到目标产物 5~10.

5-苯基噻唑-2-(3',5'-二羟基-6E-B-降)胆甾-6'-甲醛

脒(5): 黄色油状液体, 产率 71%. ^1H NMR (300 MHz, CDCl_3) δ : 7.75 (d, $J=7.2$ Hz, 1H, 2,6-PhH), 7.38 (t, $J=7.8$ Hz, 2H, 3,5-PhH), 7.32~7.28 (m, 1H, 4-PhH), 7.14 (d, $J=6.4$ Hz, 1H, C6-H), 6.73 (s, 1H, 4-ArH), 4.17 (br s, 1H, OH), 3.46 (s, 1H, OH), 2.18~2.12 (m, 2H, C7-H and C8-H), 0.94 (d, $J=6.3$ Hz, 3H, 21- CH_3), 0.90 (s, 3H, 19- CH_3), 0.88 (d, $J=6.6$ Hz, 6H, 26- and 27- CH_3), 0.72 (s, 3H, 18- CH_3); ^{13}C 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); IR (KBr) ν : 3439, 2953, 2858, 1706, 1567, 1442, 1380, 1325, 1278, 1071, 1051, 1024 cm^{-1} ; HREIMS calcd for $\text{C}_{36}\text{H}_{54}\text{N}_3\text{O}_2\text{S}$ $[\text{M}+\text{H}]^+$ 592.3937, found 592.3923.

5-(4'-三氟甲基)苯基噻唑-2-(3 β' ,5 β' -二羟基-6*E*-*B*-降)胆甾-6'-甲醛脒(6): 黄色油状液体, 产率 73%. ^1H NMR (300 MHz, CDCl_3) δ : 8.11 (d, $J=8.1$ Hz, 1H, Ar-H), 7.83 (d, $J=8.1$ Hz, 2H, 2,6-PhH), 7.78 (d, $J=8.1$ Hz, 1H, C6-H), 7.62 (d, $J=8.1$ Hz, 2H, 3,5-PhH), 7.26 (s, 1H, NH), 6.82 (s, 1H, 4-ArH), 4.46 (s, 1H, OH), 4.19 (br s, 1H, C3-H), 4.13 (dd, $J=14.1, 6.9$ Hz, 1H, C7-H), 3.45 (s, 1H, OH), 2.22~2.16 (m, 2H, C4-H), 0.93 (d, $J=6.6$ Hz, 3H, 21- CH_3), 0.92 (s, 3H, 19- CH_3), 0.87 (d, $J=6.6$ Hz, 6H, 26- and 27- CH_3), 0.72 (s, 3H, 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); IR (KBr) ν : 3416, 2945, 2863, 1709, 1614, 1564, 1524, 1448, 1409, 1377, 1320, 1170, 1125, 1066, 1013 cm^{-1} ; HREIMS calcd for $\text{C}_{37}\text{H}_{53}\text{F}_3\text{N}_3\text{O}_2\text{S}$ $[\text{M}+\text{H}]^+$ 660.3811, found 660.3806.

5-(4'-硝基)苯基噻唑-2-(3 β' ,5 β' -二羟基-6*E*-*B*-降)胆甾-6'-甲醛脒(7): 黄色油状液体, 产率 73%. ^1H NMR (300 MHz, CDCl_3) δ : 8.21 (d, $J=8.8$ Hz, 2H, 3',5'-PhH), 7.87 (d, $J=8.8$ Hz, 2H, 2',6'-PhH), 7.23 (d, $J=6.6$ Hz, 1H,

C6-H), 6.94 (s, 1H, 4-ArH), 4.20 (br s, 1H, C3-H), 3.87 (s, 1H, OH), 3.44 (s, 1H, OH), 2.22~2.15 (m, 2H, C7-H and C8-H), 0.93 (d, $J=6.6$ Hz, 3H, 21- CH_3), 0.92 (s, 3H, 19- CH_3), 0.86 (d, $J=6.6$ Hz, 6H, 26- and 27- CH_3), 0.71 (s, 3H, 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); IR (KBr) ν : 3491, 2898, 1709, 1604, 1517, 1407, 1340, 1277, 1202, 1175, 1108, 1045, 1008 cm^{-1} ; HREIMS calcd for $\text{C}_{36}\text{H}_{53}\text{N}_4\text{O}_4\text{S}$ $[\text{M}+\text{H}]^+$ 637.3788, found 637.3767.

5-(4'-氯)苯基噻唑-2-(3 β' ,5 β' -二羟基-6*E*-*B*-降)胆甾-6'-甲醛脒(8): 黄色油状液体, 产率 84%. ^1H NMR (300 MHz, CDCl_3) δ : 7.65 (d, $J=8.7$ Hz, 2H, 3',5'-PhH), 7.32 (d, $J=8.7$ Hz, 2H, 2',6'-PhH), 7.16 (d, $J=6.3$ Hz, 1H, C6-H), 6.70 (s, 1H, 4-ArH), 4.20~4.10 (m, 1H, C3-H), 3.84 (br s, 1H, OH), 3.45 (br s, 1H, OH), 2.18~2.12 (m, 2H, C7-H and C8-H), 0.93 (d, $J=6.3$ Hz, 3H, 21- CH_3), 0.89 (s, 3H, 19- CH_3), 0.88 ($J=6.6$ Hz, 6H, 26- and 27- CH_3), 0.72 (s, 3H, 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); IR (KBr) ν : 3354, 2903, 1701, 1574, 1475, 1462, 1399, 1380, 1317, 1290, 1265, 1045, 1015 cm^{-1} ; HREIMS calcd for $\text{C}_{36}\text{H}_{53}\text{ClN}_3\text{O}_2\text{S}$ $[\text{M}+\text{H}]^+$ 626.3547, found 626.3552.

5-(4'-甲氧基)苯基噻唑-2-(3 β' ,5 β' -二羟基-6*E*-*B*-降)胆甾-6'-甲醛脒(9): 黄色油状液体, 产率 88%. ^1H NMR (300 MHz, CDCl_3) δ : 7.65 (d, $J=8.7$ Hz, 2H, 2',6'-PhH), 7.16 (d, $J=6.0$ Hz, 1H, C6-H), 6.89 (d, $J=8.7$ Hz, 2H, 3',5'-PhC), 6.57 (s, 1H, 4-ArH), 4.15 (br s, 1H, C3-H), 3.82 (s, 3H, OCH_3), 2.16~2.11 (m, 2H, C7-H and C8-H), 0.93 (d, $J=6.6$ Hz, 3H, 21- CH_3), 0.89 (s, 3H, 19- CH_3), 0.88 (d, $J=6.6$ Hz, 6H, 26- and 27- CH_3), 0.71 (s, 3H, 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); IR (KBr) ν : 3426, 2925, 1711, 1607, 1566, 1511, 1499, 1459, 1374, 1165, 1108, 1026 cm^{-1} ; HREIMS calcd for $\text{C}_{37}\text{H}_{56}\text{N}_3\text{O}_3\text{S}$ $[\text{M}+\text{H}]^+$ 622.4042, found 622.4023.

5-(4'-氰基)苯基噻唑-2-(3 β' ,5 β' -二羟基-6*E*-*B*-降)胆甾-6'-甲醛(10): 黄棕色油状液体, 产率 85%. ^1H NMR (600 MHz, CDCl_3) δ : 7.77 (d, $J=8.4$ Hz, 2H, 2',6'-PhH), 7.58 (d, $J=8.4$ Hz, 2H, 3',5'-PhH), 7.17 (d, $J=6.0$ Hz, 1H, C6-H), 6.83 (s, 1H, 4-ArH), 4.13 (br s, 1H, C3-H), 3.95 (s, 1H, OH), 2.13~2.09 (m, 2H, C8-H and C7-H), 0.89 (d, $J=6.0$ Hz, 3H, 21- CH_3), 0.84 (s, 3H, 19- CH_3), 0.84 (d, $J=6.6$ Hz, 3H, 26- or 27- CH_3), 0.83 (d, $J=6.6$ Hz, 3H, 26- or 27- CH_3), 0.67 (s, 3H, 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); IR (KBr) ν : 3384, 2928, 2223, 1706, 1602, 1556, 1456, 1407, 1325, 1263, 1169, 1043, 1009 cm^{-1} ; HREIMS calcd for $\text{C}_{37}\text{H}_{53}\text{N}_4\text{O}_2\text{S}$ $[\text{M}+\text{H}]^+$ 617.3889, found 617.3898.

3.1.3 化合物 12~17 的合成

化合物 12~17 按照化合物 5~10 的合成程序进行制备, 但是使用化合物 11 作为反应底物, 化合物 11 根据参考文献[20]中的方法进行制备.

(3 β' ,5 β' -二羟基-6*E*-*B*-降)胆甾-6'-甲醛 *N*-甲基-5-(4'-硝基)苯基噻唑-2(3*H*)-吡嗪(12): 黄棕色油状液体, 产率 76%. ^1H NMR (300 MHz, CDCl_3) δ : 7.77 (d, 1H, $J=5.4$, C6-H), 7.45~7.43 (m, 3H, 3',4',5'-PhH), 7.36~7.34 (m, 2H, 2',6'-PhH), 5.92 (s, 1H, 4-ArH), 4.74 (s, 1H, OH), 4.01 (br s, 1H, C3-H), 3.71 (br s, 1H, OH), 3.29 (s, 3H, *N*- CH_3), 2.28 (dd, 1H, $J=9.0$, 5.1 Hz, C7-H), 2.11~2.04 (m, 2H, C8-H and C4-H), 0.98 (s, 3H, 19- CH_3), 0.93 (d, 3H, $J=6.6$

Hz, 21- CH_3), 0.87 (d, 6H, $J=6.3$ Hz, 26- and 27- CH_3), 0.69 (s, 3H, 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); IR (KBr) ν : 3492, 2926, 1625, 1556, 1451, 1368, 1308, 1169, 1123 cm^{-1} ; HREIMS calcd for $\text{C}_{37}\text{H}_{56}\text{N}_3\text{O}_2\text{S}$ $[\text{M}+\text{H}]^+$ 606.4093, found 606.4104.

(3 β' ,5 β' -二羟基-6*E*-*B*-降)胆甾-6'-甲醛 *N*-甲基-5-(4'-三氟甲基)苯基噻唑-2(3*H*)-吡嗪(13): 黄棕色油状液体, 产率 55%. ^1H NMR (600 MHz, CDCl_3) δ : 7.74 (d, $J=4.8$ Hz, 1H, C6-H), 7.25 (d, $J=8.4$ Hz, 2H, 3',5'-PhH), 6.94 (d, $J=8.4$ Hz, 2H, 2',6'-PhH), 5.84 (s, 1H, 4-ArH), 4.83 (s, 1H, OH), 3.98 (br s, 1H, C3-H), 3.73 (br s, 1H, OH), 3.26 (s, 3H, *N*- CH_3), 2.26 (dd, $J=9.0$, 4.8 Hz, 1H, C7-H), 2.05 (m, 2H, C8-H and C4-H), 0.96 (s, 3H, 19- CH_3), 0.91 (d, $J=6.0$ Hz, 3H, 21- CH_3), 0.85 (d, $J=6.6$ Hz, 3H, 26- or 27- CH_3), 0.84 (d, $J=6.6$ Hz, 3H, 26- or 27- CH_3), 0.66 (s, 3H, 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); IR (KBr) ν : 3449, 2922, 1721, 1622, 1544, 1508, 1320, 1251, 1173, 1120, 1015 cm^{-1} ; HREIMS calcd for $\text{C}_{38}\text{H}_{55}\text{F}_3\text{N}_3\text{O}_2\text{S}$ $[\text{M}+\text{H}]^+$ 674.3967, found 674.3968.

(3 β' ,5 β' -二羟基-6*E*-*B*-降)胆甾-6'-甲醛 *N*-甲基-5-(4'-硝基)苯基噻唑-2(3*H*)-吡嗪(14): 黄棕色油状液体, 产率 64%. ^1H NMR (600 MHz, CDCl_3) δ : 8.28 (d, $J=8.4$ Hz, 2H, 3',5'-PhH), 7.75 (d, $J=5.4$ Hz, 1H, C6-H), 7.53 (d, $J=8.4$ Hz, 2H, 2',6'-PhH), 6.08 (s, 1H, 4-ArH), 4.48 (s, 1H, OH), 3.99 (s, 1H, C3-H), 3.30 (s, 3H, *N*- CH_3), 2.26 (dd, $J=9.0$, 6.0 Hz, 1H, C7-H), 2.03~2.02 (m, 2H, C8-H and C4-H), 0.93 (s, 3H, 19- CH_3), 0.89 (d, $J=6.6$ Hz, 3H, 21- CH_3), 0.84 (d, $J=6.6$ Hz, 3H, 26- or 27- CH_3), 0.83 (d, $J=6.6$ Hz, 3H, 26- or 27- CH_3), 0.65 (s, 3H, 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*-CH₃), 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); IR (KBr) ν : 3440, 2931, 1626, 1554, 1467, 1423, 1281, 1169, 1111, 1075 cm^{-1} ; HREIMS calcd for $\text{C}_{37}\text{H}_{55}\text{N}_4\text{O}_4\text{S}$ $[\text{M}+\text{H}]^+$ 651.3944, found 651.3950.

(3 β ',5 β '-二羟基-6*E*-*B*-降)胆甾-6'-甲醛 *N*-甲基-5-(4'-氯)苯基噻唑-2(3*H*)-吡嗪(**15**): 黄色油状液体, 产率 69%. ¹H NMR (600 MHz, CDCl_3) δ : 7.76 (d, $J=5.4$ Hz, 1H, C6-H), 7.39 (d, $J=8.4$ Hz, 2H, 3',5'-PhH), 7.26 (d, $J=8.4$ Hz, 2H, 2',6'-PhH), 5.92 (s, 1H, 4-ArH), 4.63 (s, 1H, OH), 3.99 (br s, 1H, C3-H), 3.26 (s, 3H, *N*-CH₃), 2.25 (dd, $J=9.0, 5.4$ Hz, 1H, C7-H), 2.06~2.01 (m, 2H, C8-H and C4-H), 0.94 (s, 3H, 19-CH₃), 0.89 (d, $J=6.0$ Hz, 3H, 21-CH₃), 0.84 (d, $J=6.6$ Hz, 3H, 26- or 27-CH₃), 0.83 (d, $J=6.6$ Hz, 3H, 26- or 27-CH₃), 0.66 (s, 3H, 18-CH₃); ¹³C 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*-CH₃), 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); IR (KBr) ν : 3447, 2949, 2855, 1721, 1620, 1586, 1554, 1500, 1462, 1366, 1322, 1166, 1127, 1065, 1017 cm^{-1} ; HREIMS calcd for $\text{C}_{37}\text{H}_{55}\text{ClN}_3\text{O}_2\text{S}$ $[\text{M}+\text{H}]^+$ 640.3703, found 640.3716.

(3 β ',5 β '-二羟基-6*E*-*B*-降)胆甾-6'-甲醛 *N*-甲基-5-(4'-甲氧基)苯基噻唑-2(3*H*)-吡嗪(**16**): 黄色油状液体, 产率 70%. ¹H NMR (300 MHz, CDCl_3) δ : 7.76 (d, $J=5.1$ Hz, 1H, C6-H), 7.27 (d, $J=8.7$ Hz, 1H, 2',6'-PhH), 6.96 (d, $J=8.7$ Hz, 2H, 3',5'-PhH), 5.85 (s, 1H, 4-ArH), 4.79 (s, 1H, OH), 4.00 (br s, 1H, C3-H), 3.85 (s, 3H, OCH₃), 3.73 (s, 1H, OH), 3.27 (s, 3H, *N*-CH₃), 2.27 (dd, $J=9.0, 5.1$ Hz, 1H, C7-H), 2.10~2.04 (m, 2H, C8-H and C4-H), 0.97 (s, 3H, 19-CH₃), 0.93 (d, $J=6.6$ Hz, 3H, 21-CH₃), 0.87 (d, $J=6.6$ Hz, 6H, 26- and 27-CH₃), 0.69 (s, 3H, 18-CH₃); ¹³C 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 (-OCH₃), 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*-CH₃), 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); IR (KBr) ν : 3437, 2940, 2860, 1620, 1588, 1544, 1506, 1464, 1423, 1361, 1290, 1249, 1170, 1125, 1072, 1038 cm^{-1} ; HREIMS calcd for $\text{C}_{38}\text{H}_{58}\text{N}_3\text{O}_3\text{S}$ $[\text{M}+\text{H}]^+$ 636.4199, found 636.4200.

(3 β ',5 β '-二羟基-6*E*-*B*-降)胆甾-6'-甲醛 *N*-甲基-5-(4'-氰基)苯基噻唑-2(3*H*)-吡嗪(**17**): 黄色油状液体, 产率 67%. ¹H NMR (600 MHz, CDCl_3) δ : 7.73~7.69 (m, 4H, 2',3',5',6'-PhH), 7.47~7.45 (d, $J=7.8$ Hz, 1H, C6-H), 6.01 (s, 1H, 4-ArH), 4.47 (s, 1H, OH), 3.97 (s, 1H, C3-H), 3.35 (s, 1H, OH), 3.26 (s, 3H, *N*-CH₃), 2.24~2.22 (m, 1H, C7-H), 2.02~1.99 (m, 2H, C8-H and C4-H), 0.91 (d, $J=6.0$ Hz, 3H, 21-CH₃), 0.87 (s, 3H, 19-CH₃), 0.81 (d, $J=6.0$ Hz, 6H, 26- and 27-CH₃), 0.64 (s, 3H, 18-CH₃); ¹³C 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); IR (KBr) ν : 3467, 2858, 2225, 1634, 1583, 1551, 1499, 1464, 1425, 1306, 1269, 1173, 1072, 1017 cm^{-1} ; HREIMS calcd for $\text{C}_{38}\text{H}_{55}\text{N}_4\text{O}_2\text{S}$ $[\text{M}+\text{H}]^+$ 631.4046, found 631.4055.

3.1.4 化合物 19~24 的合成

化合物 19~24 按照化合物 5~10 的合成程序进行制备, 但是使用化合物 18 作为反应底物, 化合物 18 根据参考文献[20]中的方法进行制备.

(3 β ',5 β '-二羟基-6*E*-*B*-降)胆甾-6'-甲醛 *N*-苯基-5-苯基噻唑-2(3*H*)-吡嗪(**19**): 黄色油状液体, 产率 62%. ¹H NMR (600 MHz, CDCl_3) δ : 7.63 (d, $J=5.4$ Hz, 1H, C6-H), 7.28~7.04 (m, 10H, Ph-H), 6.08 (s, 1H, 4-ArH), 4.75 (s, 1H, OH), 3.96 (s, 1H, C3-H), 3.84 (br s, 1H, OH), 2.22 (dd, $J=9.6, 6.0$ Hz, 1H, C7-H), 2.05~2.02 (m, 2H, C8-H and C4-H), 0.94 (s, 3H, 19-CH₃), 0.91 (d, $J=6.6$ Hz, 3H, 21-CH₃), 0.87 (d, $J=6.6$ Hz, 3H, 26- or 27-CH₃), 0.86

(d, $J=6.6$ Hz, 3H, 26- or 27-CH₃), 0.66 (s, 3H, 18-CH₃); ¹³C NMR (150 MHz, CDCl₃) 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); IR (KBr) ν : 3439, 2933, 2855, 1624, 1569, 1544, 1492, 1455, 1295, 1165, 1123, 1011 cm⁻¹; HREIMS calcd for C₄₂H₅₈N₃O₂S [M+H]⁺ 668.4250, found 668.4248.

(3 β' ,5 β' -二羟基-6*E*-*B*-降)胆甾-6'-甲醛 *N*-苯基-5-(4'-三氟甲基)苯基噻唑-2(3*H*)-吡嗪(**20**): 黄色油状液体, 产率 63%. ¹H NMR (600 MHz, CDCl₃) δ : 7.63 (d, $J=5.4$ Hz, 1H, C6-H), 7.42 (d, $J=8.4$ Hz, 2H, 3',5'-PhH), 7.31 (d, $J=8.4$ Hz, 2H, 2',6'-PhH), 7.26 (t, $J=7.2$ Hz, 1H, *N*-PhH), 7.2~7.17 (m, 4H, *N*-PhH), 6.20 (s, 1H, 4-ArH), 4.65 (br s, 1H, OH), 3.98 (br s, 1H, C3-H), 2.23 (dd, $J=9.6, 5.4$ Hz, 1H, C7-H), 2.05~2.02 (m, 2H, C8-H and C4-H), 0.94 (s, 3H, 19-CH₃), 0.91 (d, $J=6.6$ Hz, 3H, 21-CH₃), 0.86 (d, $J=6.6$ Hz, 3H, 26- or 27-CH₃), 0.85 (d, $J=6.6$ Hz, 3H, 26- or 27-CH₃), 0.65 (s, 3H, 18-CH₃); ¹³C NMR (CDCl₃, 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 (CF₃), 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); IR (KBr) ν : 3421, 2943, 2856, 1622, 1594, 1542, 1499, 1425, 1362, 1167, 1088 cm⁻¹; HREIMS calcd for C₄₃H₅₇F₃N₃O₂S [M+H]⁺ 736.4124, found 736.4095.

(3 β' ,5 β' -二羟基-6*E*-*B*-降)胆甾-6'-甲醛 *N*-苯基-5-(4'-硝基)苯基噻唑-2(3*H*)-吡嗪(**21**): 黄棕色油状液体, 产率 60%. ¹H NMR (600 MHz, CDCl₃) δ : 8.02 (d, $J=9.0$ Hz, 2H, 3',5'-PhH), 7.63 (d, $J=5.4$ Hz, 1H, C6-H), 7.32 (t, $J=7.2$ Hz, 2H, *N*-PhH), 7.27 (d, $J=7.2$ Hz, 1H, *N*-PhH), 7.22 (d, $J=9.0$ Hz, 2H, 2',6'-PhH), 7.19 (d, $J=7.2$ Hz, 2H, *N*-PhH), 6.31 (s, 1H, 4-ArH), 4.52 (s, 1H, OH), 3.98 (s, 1H, C3-H), 2.23 (dd, $J=9.6, 6.0$ Hz, 1H, C7-H), 2.04~

2.02 (m, 2H, C8-H and C4-H), 0.93 (s, 3H, 19-CH₃), 0.90 (d, $J=6.6$ Hz, 3H, 21-CH₃), 0.85 (d, $J=6.6$ Hz, 3H, 26- or 27-CH₃), 0.84 (d, $J=6.6$ Hz, 3H, 26- or 27-CH₃), 0.65 (s, 3H, 18-CH₃); ¹³C NMR (150 MHz, CDCl₃) 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); IR (KBr) ν : 3451, 2935, 2855, 1629, 1591, 1546, 1492, 1452, 1410, 1198, 1167, 1105, 1078 cm⁻¹; HREIMS calcd for C₄₂H₅₇N₄O₄S [M+H]⁺ 713.4101, found 713.4132.

(3 β' ,5 β' -二羟基-6*E*-*B*-降)胆甾-6'-甲醛 *N*-苯基-5-(4'-氯)苯基噻唑-2(3*H*)-吡嗪(**22**): 黄色油状液体, 产率 80%. ¹H NMR (600 MHz, CDCl₃) δ : 7.61 (d, $J=5.4$ Hz, 1H, C6-H), 7.29 (t, $J=7.2$ Hz, 2H, 3'',5''-NPhH), 7.24 (d, $J=7.2$ Hz, 1H, 4''-NPhC), 7.16 (d, $J=7.2$ Hz, 2H, 2'',6''-NPhH), 7.13 (d, $J=8.4$ Hz, 2H, 3',5'-PhH), 6.98 (d, $J=8.4$ Hz, 2H, 2',6'-PhH), 6.09 (s, 1H, 4-ArH), 4.67 (s, 1H, OH), 3.96 (s, 1H, C3-H), 2.21 (dd, $J=9.5, 5.4$ Hz, 1H, C7-H), 2.04~2.01 (m, 2H, C8-H and C4-H), 0.93 (s, 3H, 19-CH₃), 0.90 (d, $J=6.6$ Hz, 3H, 21-CH₃), 0.86 (d, $J=6.6$ Hz, 3H, 26- or 27-CH₃), 0.85 (d, $J=6.6$ Hz, 3H, 26- or 27-CH₃), 0.65 (s, 3H, 18-CH₃); ¹³C NMR (150 MHz, CDCl₃) 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); IR (KBr) ν : 3444, 2943, 2858, 1622, 1582, 1544, 1484, 1447, 1397, 1355, 1298, 1165, 1011 cm⁻¹; HREIMS calcd for C₄₂H₅₇Cl-N₃O₂S [M+H]⁺ 702.3860, found 702.3851.

(3 β' ,5 β' -二羟基-6*E*-*B*-降)胆甾-6'-甲醛 *N*-苯基-5-(4'-甲氧基)苯基噻唑-2(3*H*)-吡嗪(**23**): 黄色油状液体, 产率 75%. ¹H NMR (CDCl₃, 600 MHz) δ : 7.61 (d, $J=5.4$ Hz,

1H, C6-H), 7.30~7.27 (m, 2H, 3'',5''-NPhH), 7.23 (d, $J=6.0$ Hz, 1H, 4''-NPhH), 7.17 (d, $J=7.2$ Hz, 2H, 2',6'-PhH), 6.96 (d, $J=8.4$ Hz, 2H, 2'',6''-NPhH), 6.67 (d, $J=7.2$ Hz, 2H, 3',5'-PhH), 5.99 (s, 1H, 4-ArH), 4.80 (br s, 1H, OH), 3.96 (br s, 1H, C3-H), 3.82 (br s, 1H, OH), 3.71 (s, 3H, OCH₃), 2.21 (dd, $J=9.6, 5.4$ Hz, 1H, C7-H), 2.04~2.02 (m, 2H, C8-H and C4-H), 0.94 (s, 3H, 19-CH₃), 0.91 (d, $J=6.6$ Hz, 3H, 21-CH₃), 0.860 (d, $J=6.6$ Hz, 3H, 26- or 27-CH₃), 0.855 (d, $J=6.6$ Hz, 3H, 26- or 27-CH₃), 0.65 (s, 3H, 18-CH₃); ¹³C NMR (CDCl₃, 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 (OCH₃), 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); IR (KBr) ν : 3471, 2945, 2855, 1592, 1544, 1502, 1455, 1357, 1295, 1248, 1175 cm⁻¹; HREIMS calcd for C₄₃H₆₀N₃O₃S [M+H]⁺ 698.4355, found 698.4365.

(3 β ',5 β '-二羟基-6 E -B-降)胆甾-6'-甲醛 *N*-苯基-5-(4'-氰基)苯基噻唑-2(3 H)-吡嗪(**24**): 黄色油状液体, 产率 49%. ¹H NMR (600 MHz, CDCl₃) δ : 7.63 (d, $J=5.4$ Hz, 1H, C6-H), 7.46 (d, $J=8.4$ Hz, 2H, 2'',6''-NPhH), 7.33 (d, $J=7.8$ Hz, 2H, 2',6'-PhH), 7.28 ($J=8.4$ Hz, 1H, 4''-NPhH), 7.18 (d, $J=7.8$ Hz, 1H, 3',5'-PhH), 7.16 (d, $J=8.4$ Hz, 2H, 3'',5''-NPhH), 6.24 (s, 1H, 4-ArH), 4.55 (s, 1H, OH), 3.98 (m, 1H, C3-H), 2.22 (dd, $J=9.6, 5.4$ Hz, 1H, C7-H), 2.04~2.02 (m, 2H, C8-H and C4-H), 0.93 (s, 3H, 19-CH₃), 0.90 (d, $J=6.6$ Hz, 3H, 21-CH₃), 0.85 (d, $J=6.6$ Hz, 3H, 26- or 27-CH₃), 0.84 (d, $J=6.6$ Hz, 3H, 26- or 27-CH₃), 0.65 (s, 3H, 18-CH₃); ¹³C NMR (150 MHz, CDCl₃) 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); IR

(KBr) ν : 3454, 2940, 2227, 1624, 1587, 1554, 1494, 1454, 1362, 1295, 1083, 1013 cm⁻¹; HREIMS calcd for C₄₃H₅₇N₄O₂S [M+H]⁺ 693.4202, found 693.4203.

2.2 生物活性评估

2.2.1 材料

所有测试物溶解于无菌 DMSO (Sigma)中, 配制成为 10 mg/mL 浓度的溶液备用, 使用时采用 RPMI-1640 培养液(加入 10%牛血清和 0.1 g/L 青霉素 G+0.1 g/L 硫酸链霉素)进行稀释使用.

2.2.2 细胞培养

HeLa、A549、HEPG2 癌细胞和 HEK293T 细胞采用 RPMI-1640 培养液(加入 10%牛血清和 0.1 g/L 青霉素 G+0.1 g/L 硫酸链霉素)在 37 °C 于 5% CO₂ 的潮湿气氛中培养.

2.2.3 细胞活力评估

体外抗肿瘤活性评估使用 MTT 法进行. 简单地说, 细胞被接种到 96 孔培养板中(每孔 1~2×10⁴ 个细胞), 在 CO₂ 培养箱中培育 24 h 待细胞贴壁, 然后在孔中加入不同浓度的被测试化合物. 等量的 DMSO 加入到细胞中作为阴性对照, 对于每一独立剂量采用一式三份进行平行试验. 在培育 72 h 后, 细胞采用无菌的磷酸盐缓冲液(PBS)进行洗涤. 接着每孔加入 190 μ L 的 RPMI-1640 和 10 μ L MTT 溶液(5 mg/mL), 继续培育 4 h. 弃去上清液, 每孔加入 200 μ L DMSO, 在震荡器上震荡 10 min 溶解形成的紫色甲瓚晶体沉淀, 随后用酶标仪在 492 nm 波长测定 OD 值, 然后通过下式求出样品一定浓度下样品对细胞的抑制率:

$$\text{抑制率(\%)} = \frac{[(\text{对照 OD} - \text{空白 OD}) - (\text{给药 OD} - \text{空白 OD})]}{(\text{对照 OD} - \text{空白 OD})} \times 100\%$$

然后以抑制率对药物浓度作图, 求出每个样品的 IC₅₀ 值(化合物导致 50%细胞生存浓度).

3 结论

合成了某些新的 B-降胆甾烷噻唑衍生物, 通过 IR, NMR 和 HRMS 证实了它们的结构. 使用 MTT 方法, 对化合物抑制人宫颈癌细胞(HeLa)、肺癌细胞(A549)、肝癌细胞(HEPG2)和人正常肾上皮细胞(HEK293T)的生长增殖活性进行了评估. 结果表明, 噻唑环 N 上不存在取代基时, 化合物对所有被测试细胞都没有表现出明显的抑制活性. 当噻唑环 N 上存在取代基时, 某些具有 *N*-甲基噻唑结构的化合物对 A549 和 HEPG2 细胞表现出明显的抑制增殖活性. 此外, 具有 *N*-苯基噻唑结构的化合物对 HeLa 细胞表现出选择性的抑制生长增殖作用. 所有这些化合物对人正常肾上皮细胞(HEK293T)都没有

表现出活性。但是,合成的 B-降胆甾烷噻唑衍生物对所测试的肿瘤细胞株的抑制活性都低于阳性对照物顺铂,也低于其前体化合物。研究结果对新的抗肿瘤甾体药物的设计合成提供了某些有用的理论参考。

辅助材料(Supporting Information) 所合成化合物的 NMR 谱图。这些材料可以免费从本刊网站(<http://sioc-journal.cn/>)上下载。

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