医药与日化原料

苯并咪唑基取代的甾体衍生物合成及其抗肿瘤活性

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摘要：以孕烯醇酮为原料，通过对孕烯醇酮的 C-17′支链进行化学修饰，设计合成了 18 个甾核的 C-17′支链为苯并咪唑基取代的甾体化合物。通过 IR、^{1}HNMR、^{13}CNMR 和 HRMS 对化合物进行了结构表征，采用溴化噻唑蓝四氮唑（MTT）法测试了这些化合物对人口腔上皮癌细胞（KB）、宫颈癌细胞（HeLa）、人肝癌细胞（HepG）、人鼻咽癌细胞（CNE-2）、乳腺癌细胞（BT474）、卵巢癌细胞（SKOV3）的体外抑制活性。结果表明，部分化合物具有中等程度的活性，其中氟基取代的苯并咪唑甾体化合物 5c 和 6c 对人体卵巢癌细胞（SKOV3）具有良好的选择性抑制作用，IC_{50}（半抑制浓度）分别为（15.4±3.8）和（9.2±0.6）μmol/L。该类化合物可为设计开发新型抗肿瘤药物提供参考。

关键词：苯并咪唑；甾体化合物；孕烯醇酮；抗肿瘤活性；细胞毒性；医药与日化原料
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Synthesis and Antiproliferative Activity of Some Novel Steroidal Derivatives Containning Benzimidazole Groups

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Abstract: Eighteen steroidal derivatives containing benzimidazole heterocycle were designed and synthesized by suitable modification at C17' side chain of pregnenolone. Their structures were characterized by IR, ^{1}HNMR, ^{13}CNMR and HRMS. The antiproliferative activity of the target compounds *in vitro* was evaluated against human oral epithelial carcinoma cells (KB), cervical carcinoma (HeLa), liver cancer (HepG), human nasopharyngeal carcinoma (CNE-2), breast cancer (BT474) and ovarian cancer (SKOV3) cells by MTT method. The results showed that some compounds possessed distinct antiproliferative activity against the tested cells. Among these active compounds, compounds 5c and 6c exhibited better selective activity against SKOV3 cells with IC_{50} values of (15.4±3.8) μmol/L and (9.2±0.6) μmol/L, respectively. The information obtained from the studies may be useful for the design of novel chemotherapeutic drugs.

Key words: benzimidazole; steroids; pregnenolone; antiproliferative activity; cytotoxicity; drug and cosmetic materials

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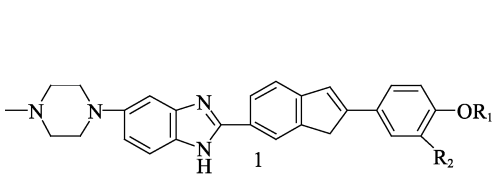
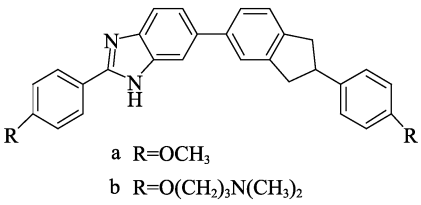
苯并咪唑杂环基团含有两个氮原子，可与生物体内的酶和受体形成氢键，是一类重要的药效基团[1]。以苯并咪唑环构筑的化合物具有广泛的生物活性，常作为有机合成反应中间体，在医药方面具有广泛用途，如作为组胺受体拮抗剂和质子泵抑制剂，具有抗高血压、抗病毒、抗菌、抗真菌、抗癌、镇痛等作用[2-8]，是目前医药研发中极为活跃的领域之一。

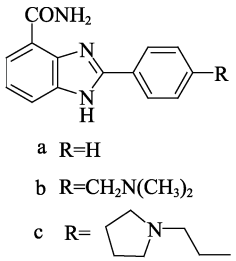
含有苯并咪唑结构片段的化合物具有较好的抗肿瘤活性，如，双苯并咪唑Ⅰa 和Ⅰb（结构如下所示）可作为 DNA 拓扑异构酶Ⅰ（Topo Ⅰ）抑制剂，对乳腺癌和前列腺癌具有强烈的抑制作用，其碘代物同样具有较好的抑制活性。化合物Ⅱ对乳腺癌和肺癌具有良好的抑制效果。化合物Ⅲa 是一种有效的 DNA 修复多聚酶抑制剂，可作为放疗和特定类型化疗的耐药修饰剂。其 2-芳基的对位被取代后得到的化合物Ⅲb 和Ⅲc 抑制活性相差不大，但代谢稳定性和水溶解性更好，对人体结肠、直肠癌细胞株具有良好的抑制活性，作为抗癌药物具有可开发的应用前景[9-10]。



双苯并咪唑化合物I

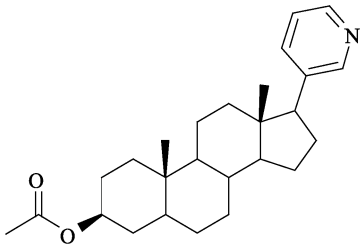


双苯并咪唑化合物Ⅱ

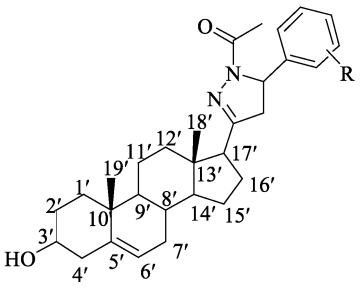


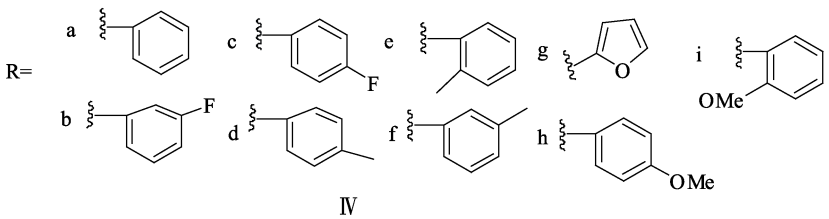
DNA修复多聚酶抑制剂Ⅲ

甾体类化合物对细胞及人体组织具有高度的渗透能力，对细胞核及细胞膜有一定的结合能力，对甾体化合物进行结构改造，有可能获得治疗人类疾病的药物。一些含有杂环结构的化合物具有良好的抗肿瘤活性[11-18]，Rafat 等以孕烯醇酮为原料，合成了 C-17′支链上含有吡唑苯环结构的孕烯醇酮衍生物，这些化合物对人体肿瘤细胞 MCF-7（乳腺癌）、NCI-H460（肺癌细胞）、SF-268（人神经癌细胞）均有极好的细胞毒活性[19]。Banday 等合成的系列支链杂环取代的甾体化合物Ⅳ（结构如下所示）对结肠癌、肺癌以及乳腺癌细胞具有较强的体外抑制作用[20]。文献中报道了一些具有 C-17′双键的 C-17′唑类甾体化合物及 C-17′苯并唑类甾体化合物的合成。其中，某些化合物对前列腺癌具有很好的体外抑制生长增殖作用，并且对雄性激素 17α-羟化酶也有很好的体外抑制作用[21-23]。此外，某些具有 C-17′杂环甾体结构的药物已用于临床治疗，如治疗前列腺癌（CRPC）患者的甾体药物醋酸阿比特龙（Abiraterone），结构如下所示。



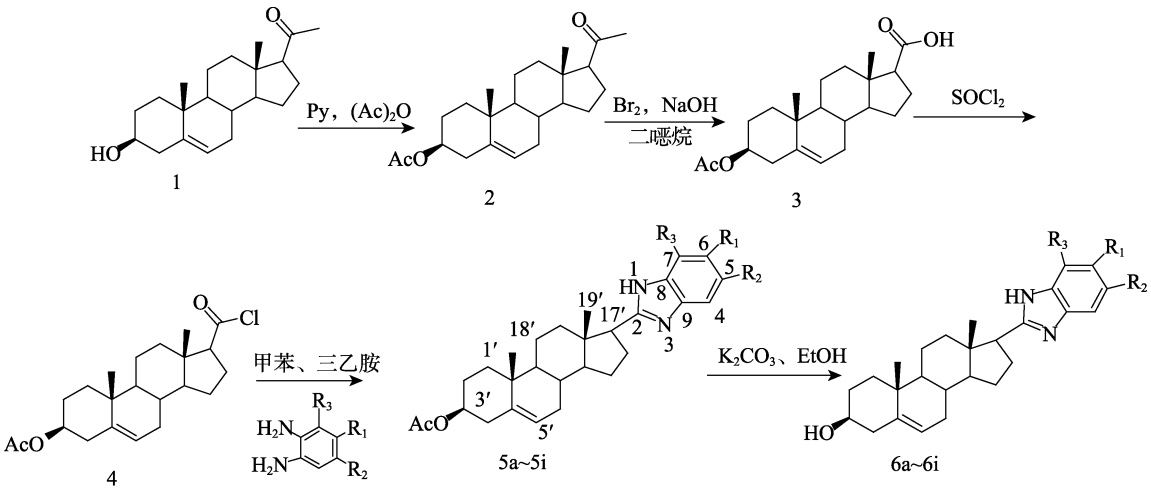
醋酸阿比特龙





Ⅳ

近年来，本课题组一直从事甾体杂环化合物的抗肿瘤活性研究。前期研究结果表明，当将苯并咪唑药效基团引入 B-降胆甾醇结构时，所得到的化合物具有很好的体外抑制肿瘤细胞生长增殖活性[24]。此外，将一些杂环基团引入甾核的 C-17′支链时，部分化合物也表现出良好的体外抑制肿瘤生长增殖活性[25]。作为工作的延伸，本文以孕烯醇酮为原料，经过卤仿反应，二氯亚砜酰化，然后在甾核的 C-17′位引入具有不同取代基结构的苯并咪唑基团，合成了 18 个新的含苯并咪唑杂环的孕烯醇酮衍生物，并采用 MTT 法对这些化合物进行了体外抑制肿瘤细胞生长增殖筛选，以期为抗肿瘤药物的研发提供理论参考。合成路线如下所示：



a: R_1=H, R_2=H, R_3=H; b: R_1=H, R_2=Cl, R_3=H; c: R_1=H, R_2=F, R_3=H; d: R_1=H, R_2=OCH_3, R_3=H;
e: R_1=H, R_2=CH_3, R_3=H; f: R_1=H, R_2=CF_3, R_3=H; g: R_1=CH_3, R_2=CH_3, R_3=H; h: R_1=Br, R_2=CH_3, R_3=H;
i: R_1=CH_3, R_2=H, R_3=CH_3

1　实验部分

1.1　试剂与仪器

孕烯醇酮、邻苯二胺、4-氯邻苯二胺、4-氟-1,2-苯二胺、4-甲氧基邻苯二胺、4-甲基邻苯二胺、4-三氟甲基-1,2-苯二胺、3,4-二甲基邻苯二胺、5-溴-3-甲基苯-1,2-二胺、4,5-二甲基-1,2-苯二胺均购于百灵威科技有限公司；所用试剂均为市售 AR，溶剂使用前经干燥处理。

RPMI 1640 培养基（美国 HyClone 公司）、MTT（溴化噻唑蓝四氮唑）（中国索莱宝公司）、胎牛血清（浙江天杭生物科技有限公司）、青霉素-链霉素溶液（100X）（中国 Beyotime 公司）、胰酶（美国 Sigma 公司）、DMSO（美国 Amresco 公司）。细胞增殖评估利用 MTT 方法采用美国 Thermo Scientific 公司 MLLTISKAN MK3 酶标仪测定。

肿瘤细胞株: KB、HeLa、HepG、CNE-2、BT474、SKOV3。

阳性对照: 顺铂（Cisplatin）。

熔点测定采用 X6 显微熔点测定仪，温度计未经校正；IR 光谱采用美国 Thermo Scientific 公司 IS-10 型傅里叶红外光谱仪测定；^{1}HNMR 和 ^{13}CNMR 谱采用德国 Bruker AV-300 型超导核磁共振仪测定；高分辨质谱采用美国 Agilent 公司 6210 型 TOFMS 质谱仪测定；细胞增殖活性评估采用 MTT 方法，使用美国 Thermo Scientific 公司的 MLLTISKAN MK3 酶标仪进行测定。

1.2　方法

1.2.1　化合物 2～3 的合成

化合物 2 和化合物 3 的合成参考文献[7,26-28]。其中，化合物 2 为白色固体，产率：90.2%，m.p. 146~148 ℃（文献值[26]: 149~151 ℃）；化合物 3 为白色固体，产率：64.4%，m.p. 256~258 ℃。

1.2.2　2-（3′-乙酰氧基-17′-孕甾）苯并咪唑类化合物（5a～5i）的合成

向干燥的 50 mL 茄形瓶中加入 65 mg（0.18 mmol）化合物 3 和 2.2 mL 的二氯亚砜，在 70 ℃的油浴中回流 2.5 h，当溶液由无色变为黄色时，停止反应，减压除去多余的二氯亚砜得到化合物 4，化合物 4 不经提纯直接进入下一步反应。

另取干燥的 100 mL 圆底烧瓶，加入 0.39 mmol 邻苯二胺或不同取代的邻苯二胺，再加入 1.0 mL 三乙胺，然后将化合物 4 溶解于 3.0 mL 无水甲苯中，室温下，利用恒压漏斗慢慢将化合物 4 的甲苯溶液滴加到邻苯二胺中进行搅拌反应，当溶液变成橘黄色时，控制滴速，滴完有大量固体生成。然后于 105 ℃的油浴中回流反应，TLC 跟踪至无原料点后终止反应〔流动相 V（乙酸乙酯）：V（石油醚）= 1：1〕。减压蒸馏除去溶剂，柱层析分离得到目标产物 5a~5i〔流动相 V（乙酸乙酯）：V（石油醚）= 1：1〕。

2-（3'-乙酰氧基-17'-孕甾）苯并咪唑（5a），白色固体，产率 33.4%，m.p. 106~108 ℃；^{1}HNMR ($CDCl_3$, 300 MHz), δ: 0.82 (s, 3H, 18′-CH_3), 1.05 (s, 3H, 19′-CH_3), 2.04 (s, 3H, 3′-CH_3CO), 4.58~4.68 (m, 1H, 3′-αH), 5.41 (d, J=4.8, 1H, 6′-H), 6.80 (d, J=7.5, 2H, Ph-H), 7.05 (t, J=7.5, 1H, Ph-H), 7.16~7.19 (m, 1H, Ph-H), 7.18 (s, 1H, -NH); ^{13}CNMR (75 MHz, DMSO-d_6), δ: 13.32 (18′-C), 19.30 (19′-C), 21.01 (11′-CH_3CO), 21.40 (3′-CH_3CO), 23.83 (15′-C), 24.58 (16′-C), 27.73 (2′-C), 31.80 (8′-C), 31.92 (7′-C), 36.67 (1′-C), 37.05 (10′-C), 38.07 (4′-C), 38.67 (12′-C), 44.21 (17′-C), 50.00 (9′-C), 56.46 (13′-C), 57.51 (14′-C), 73.84 (3′-C), 122.30 (6′-C), 118.20、119.40、

124.80、125.20、126.90、139.70 (苯并咪唑-C), 140.80 (5′-C), 170.60 (2-C), 171.60 (3′-$OCOCH_3$); IR (KBr), ν/cm^{-1}: 3345, 2938, 2847, 1730, 1649, 1627, 1375, 1251, 1030, 743; HREIMS, *m/Z*: 433.2843 $[M+H]^+$ (calcd for $C_{28}H_{37}N_2O_2$, 433.2855)。

2-(3′-乙酰氧基-17′-孕甾)-5-氯苯并咪唑 (5b)，淡黄色固体，产率 34.7%，m.p. 98~100 ℃; ^{1}HNMR ($CDCl_3$, 300 MHz), δ: 0.82 (s, 3H, 18′-CH_3), 1.05 (s, 3H, 19′-CH_3), 2.05 (s, 3H, 3′-$OCOCH_3$), 4.58~4.68 (m, 1H, 3′-H), 5.40 (d, *J* =4.50, 1H, 6′-H), 6.76 (dd, *J* = 8.4, 2.1, 1H, 6-H), 6.81 (d, *J* = 2.1, 1H, 4-H), 6.98 (s, 1H, N-H), 7.10 (d, *J* = 8.4, 1H, 7-H); ^{13}CNMR (75 MHz, DMSO-d_6), δ: 13.33 (18′-C), 19.30 (19′-C), 21.00 (11′-C), 21.40 (3′-CH_3CO), 23.82 (15′-C), 24.55 (16′-C), 27.73 (2′-C), 29.69 (8′-C), 31.93 (7′-C), 36.66 (1′-C), 37.04 (10′-C), 38.07 (4′-C), 38.67 (12′-C), 44.21 (17′-C), 50.00 (9′-C), 56.46 (13′-C), 57.51 (14′-C), 73.84 (3′-C), 117.76 (4-C), 119.21 (7-C), 122.27 (6′-C), 123.16 (6-C), 126.26 (5-C), 132.13 (9-C), 139.71 (8-C), 142.12 (5′-C), 170.59 (2-C), 171.66 (3′-$OCOCH_3$); IR(KBr), ν/cm^{-1}: 3343, 2933, 2849, 1730, 1663, 1373, 1246, 1035, 803; HREIMS, *m/Z*: 467.2460 $[M+H]^+$ (calcd for $C_{38}H_{36}ClN_2O_2$, 467.2456)。

2-(3′-乙酰氧基-17′-孕甾)-5-氟苯并咪唑(5c)，淡黄色固体，产率 53.6%，m.p.123~125 ℃; ^{1}HNMR ($CDCl_3$，300 MHz), δ: 0.80 (s, 3H，18′-CH_3), 1.05 (s, 3H, 19′-CH_3), 2.03 (s, 3H, 3′-$OCOCH_3$), 4.56~4.67 (m, 1H, 3′-αH), 5.40 (d, *J* = 4.50, 1H, 6′-H), 6.50~6.41 (m, 1H, 6-H), 7.05~ 7.01 (m, 1H, 7-H), 7.17 (d, J_{F-H} = 14.4, 1H, 4-H); ^{13}CNMR (75 MHz, DMSO-d_6), δ: 13.37 (18′-C), 19.29 (19′-C), 21.01 (11′-C), 21.39 (3′-CH_3CO), 23.85 (15′-C), 24.57 (16′-C), 27.72 (2′-C), 31.79 (8′-C), 31.92 (7′-C), 36.66 (1′-C), 37.07 (10′-C), 38.06 (4′-C), 38.60 (12′-C), 44.19 (17′-C), 50.02 (9′-C), 56.44 (13′-C), 57.23 (14′-C), 73.91 (3′-C), 104.20 ($^2J_{C-F}$ = 25.1, 4-C), 105.47 ($^2J_{C-F}$ = 22.7, 6-C), 122.30 (6′-C), 127.14 ($^3J_{C-F}$ = 9.9, 7-C), 129.02 (9-C), 139.70 (5′-C), 143.29 ($^3J_{C-F}$ = 43.8, 8-C), 161.77 (J_{C-F} = 242.0, 5-C), 170.69 (2-C), 171.90 (3′-$OCOCH_3$); IR (KBr), ν/cm^{-1}: 3350, 2938, 2844, 1718, 1375, 1658, 1605, 1251,1028, 841; HREIMS, *m/Z*: 451.2766 $[M+H]^+$ (calcd for $C_{28}H_{36}FN_2O_2$, 451.2761)。

2-(3′-乙酰氧基-17′-孕甾)-5-甲氧基苯并咪唑 (5d)，白色固体，产率 60.53%，m.p. 96~98 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 0.79 (s, 3H, 18′-CH_3), 1.03 (s, 3H, 19′-CH_3), 2.02 (s, 3H, 3′-$OCOCH_3$), 3.74 (s, 3H, 5′-OCH_3), 4.57~4.63 (m, 1H, 3′-αH), 5.38 (d, *J* =2.4, 1H, 6′-H), 6.32 (s, 1H, 4-H), 6.94 (d, 1H, 6-H), 6.96 (d, *J* = 4.5, 1H, 7-H); ^{13}CNMR (75MHz, DMSO-d_6), δ: 13.50 (18′-C), 19.45 (19′-C), 21.13 (11′-C), 21.57 (3′-CH_3CO), 23.96 (15′-C), 24.71 (16′-C), 27.85 (2′-C), 31.93 (8′-C), 32.05 (7′-C), 36.79 (1′-C), 37.16 (10′-C), 38.19 (4′-C), 38.75 (12′-C), 44.27 (17′-C), 50.12 (9′-C), 56.6 (13′-C), 57.42 (14′-C), 74.01 (3′-C), 103.15 (4-C), 104.83 (6-C), 117.65 (7-C), 122.47 (6′-C), 127.03 (9-C), 139.81 (5′-C), 142.97 (8-C), 159.10 (5-C), 170.80 (2-C), 171.94 (3′-$OCOCH_3$); IR (KBr), ν/cm^{-1}: 3413, 2930, 2857, 1716, 1621, 1516, 1030, 966, 843, 791; HREIMS, *m/Z*: 463.2954 $[M+H]^+$ (calcd for $C_{29}H_{39}N_2O_3$, 463.2961)。

2-(3′-乙酰氧基-17′-孕甾)-5-甲基苯并咪唑 (5e)，白色固体，产率 52.6%，m.p. 132~134 ℃; ^{1}HNMR ($CDCl_3$, 300 MHz), δ: 0.80 (s, 3H, 18′-CH_3), 1.04 (s, 3H, 19′-CH_3), 2.03 (s, 3H, 3′-$OCOCH_3$), 2.25 (s, 3H, 5-CH_3), 4.53~4.70 (m, 1H, 3′-αH), 5.40 (d, *J* =3.6, 1H, 6′-H), 6.58 (d, *J* =7.8, 1H, 6-H), 7.00 (d, *J* =7.8, 1H, 7-H), 7.20 (s, 1H, 4-H); ^{13}CNMR (75 MHz, DMSO-d_6), δ: 13.32 (18′-C), 19.30 (19′-C), 20.45 (5-CH_3), 21.00 (3′-CH_3CO), 21.40 (11′-C), 23.84 (15′-C), 24.59 (16′-C), 27.73 (2′-C), 31.82 (8′-C), 31.92 (7′-C), 36.66 (1′-C), 37.06 (10′-C), 38.07 (4′-C), 38.52 (12′-C), 44.15 (17′-C), 50.03 (9′-C), 56.42 (13′-C), 57.29 (14′-C), 73.91 (3′-C),118.63 (7-C), 120.05 (4-C), 122.06 (6′-C), 122.33 (6-C), 125.31 (5-C), 136.89 (8-C), 139.71 (9-C), 140.93 (5′-C), 170.65 (2-C), 171.61 (3′-$OCOCH_3$); IR (KBr), ν/cm^{-1}: 3339, 2925, 2850, 1733, 1654, 1369, 1260, 1030,794; HREIMS, *m/Z*: 447.3039 $[M+H]^+$ (calcd for $C_{29}H_{39}N_2O_2$, 447.3012)。

2-(3′-乙酰氧基-17′-孕甾)-5-三氟甲基苯并咪唑 (5f)，白色固体，产率 53.2%，m.p. 112~115 ℃; ^{1}HNMR ($CDCl_3$，300 MHz), δ: 0.82 (s, 3H, 18′-CH_3), 1.06 (s, 3H, 19′-CH_3), 2.03 (s, 3H, 3′-$OCOCH_3$), 4.58~4.68 (m, 1H, 3′-αH), 5.41 (d, *J* = 4.8, 1H, 6′-H), 6.83 (d, *J* = 8.4, 1H, 7-H), 7.33 (d, *J* = 8.4, 1H, 6-H), 7.40 (s, 1H, 4-H); ^{13}CNMR (75 MHz, DMSO-d_6), δ: 13.39 (18′-C), 19.28 (19′-C), 21.01 (11′-C), 21.36 (3′-CH_3CO), 23.87 (15′-C), 24.57 (16′-C), 27.70 (2′-C), 31.79 (8′-C), 31.94 (7′-C), 36.68 (1′-C), 37.08 (10′-C), 38.05 (4′-C), 38.62 (12′-C), 44.30 (17′-C), 50.00 (9′-C), 56.47 (13′-C), 57.35 (14′-C), 73.95 (3′-C), 117.51 (4-C), 120.73 (7-C), 122.28 (6′-C), 122.57 (5-CF_3), 122.88 (6-C), 123.88 (5-C), 126.16 (8-C), 139.73 (5′-C), 144.44 (9-C), 170.80 (2-C), 171.95 (C═O); IR (KBr), ν/cm^{-1}: 3351, 2937, 2903, 1716, 1324, 1110, 1075, 891, 624; HREIMS, *m/Z*: 501.2713 $[M+H]^+$ (calcd for $C_{29}H_{36}F_3N_2O_2$, 501.2729)。

2-(3′-乙酰氧基-17′-孕甾)-5,6-二甲基苯并咪唑 (5g)，白色固体，产率 68.1%，m.p. 120~123 ℃; ^{1}HNMR ($CDCl_3$，300 MHz), δ: 0.81 (s, 3H, 18′-CH_3), 1.05 (s, 3H, 19′-CH_3), 2.04 (s, 3H, 3′-CH_3CO), 2.11 (s, 3H, 4-CH_3), 2.26 (s, 3H, 5-CH_3), 4.57~4.68 (m, 1H, 3′-αH), 5.40 (d, *J* = 1.5, 1H, 6′-H), 6.62 (d, *J* = 8.1, 1H, 6-H), 6.93 (d, *J* = 8.1, 1H, 7-H), 7.09 (s, 1H, N-H); ^{13}CNMR (75 MHz, DMSO-d_6), δ: 13.34 (4-C), 13.38

(18′-CH_3), 19.31 (19′-C), 20.55 (5-CH_3), 21.01 (11′-C), 21.43 (3′-CH_3CO), 23.81 (15′-C), 24.59 (16′-C), 27.73 (2′-C), 31.81 (8′-C), 31.92 (7′-C), 36.66 (1′-C), 37.04 (10′-C), 38.07 (4′-C), 38.59 (12′-C), 44.14 (17′-C), 50.02 (9′-C), 56.43 (13′-C), 57.35 (14′-C), 73.89 (3′-C), 120.48 (7-C), 122.21 (6′-C), 122.34 (4-C), 122.70 (6-C), 122.80 (8-C), 135.09 (5-C), 139.49 (9-C), 139.69 (5′-C), 170.64 (2-C), 171.75 (C═O); IR (KBr), ν/cm^{-1}: 3361, 2935, 2900, 1733, 1509, 1369, 1242, 1035, 786, 729; HREIMS, *m/Z*: 461.3164 $[M+H]^+$ (calcd for $C_{30}H_{40}N_2O_2$, 461.3168)。

2-(3′-乙酰氧基-17′-孕甾)-5-甲基-6-溴苯并咪唑(5h)，淡黄色固体，产率 11.6%, m.p. 105~107 ℃; ^{1}HNMR ($CDCl_3$, 300 MHz), δ: 0.80 (s, 3H, 18′-CH_3), 1.05 (s, 3H, 19′-CH_3), 2.04 (s, 3H, 3′-$OCOCH_3$), 2.19 (s, 3H, 5-CH_3), 4.57~4.66 (m, 1H, 3′-αH), 5.40 (d, J = 4.5, 1H, 6′-H), 6.79 (s, 1H, N-H), 7.10 (s, 1H, 4′-H), 7.25 (s, 1H, 7′-H); ^{13}CNMR (75 MHz, DMSO-d_6), δ: 13.39 (18′-C), 17.64 (5-CH_3), 19.30 (19′-C), 21.00 (11′-C), 21.41 (3′-CH_3CO), 23.81 (15′-C), 24.58 (16′-C), 27.72 (2′-C), 29.69 (8′-C), 31.92 (7′-C), 36.66 (1′-C), 37.05 (10′-C), 38.06 (4′-C), 38.58 (12′-C), 44.25 (17′-C), 49.99 (9′-C), 56.43 (13′-C), 58.43 (14′-C), 73.902 (3′-C), 109.90 (7-C), 122.30 (6′-C), 125.40 (6-C), 125.89 (4-C), 126.52 (8-C), 130.66 (5-C), 138.47 (9-C), 139.70 (5′-C), 170.74 (2-C), 171.85 (3′-$OCOCH_3$); IR (KBr), ν/cm^{-1}: 2937, 2867,1786, 1726, 1589, 1372, 1115, 1030, 901, 794; HREIMS, *m/Z*: 525.2101 $[M+H]^+$ (calcd for $C_{29}H_{37}BrN_2O_2$, 525.2117)。

2-(3′-乙酰氧基-17′-孕甾)-6,7-二甲基苯并咪唑(5i)，淡黄色固体,产率 11.5%, m.p. 108~110 ℃; ^{1}HNMR ($CDCl_3$，300 MHz), δ: 0.81 (s, 3H, 18′-CH_3), 1.05 (s, 3H, 19′-CH_3), 2.05 (s, 3H, 3′-CH_3CO), 2.15 (s, 3H, 5′-CH_3), 2.17 (s, 3H, 6′-CH_3), 4.57~4.68 (m, 1H, 3′-αH), 5.40 (d, J = 4.8, 1H, 6′-H), 6.61 (s, 1H, 3-H), 6.94 (s, 1H, 6-H), 7.07 (s, 1H, N-H); ^{13}CNMR (75 MHz, DMSO-d_6), δ: 13.30 (18′-C), 18.74 (19′-CH_3), 19.31 (6-CH_3), 19.31 (5-CH_3), 21.02 (11′-C), 21.41 (3′-CH_3CO), 23.83 (15′-C), 24.58 (16′-C), 27.74 (2′-C), 31.81 (8′-C), 31.93 (7′-C), 36.66 (1′-C), 37.05 (10′-C), 38.08 (4′-C), 38.57 (12′-C), 44.12 (17′-C), 50.03 (9′-C), 56.43 (13′-C), 57.41 (14′-C), 73.88 (3′-C), 119.72 (7-C), 122.33 (4-C), 122.40 (6′-C), 126.03 (8-C), 127.56 (9-C), 135.22 (6-C), 138.30 (5-C), 139.71 (5'-C), 170.60 (2-C), 171.39 (3'-$OCOCH_3$); IR (KBr), ν/cm^{-1}: 3438, 2930, 2855, 1663, 1731, 1521, 1379, 1025, 871, 796; HREIMS, *m/Z*: 461.3170 $[M+H]^+$ (calcd for $C_{30}H_{40}N_2O_2$, 461.3168)。

1.2.3　2-(3′-羟基-17′-孕甾)苯并咪唑类化合物(6a~6i)的合成

称取 0.16mmol 化合物 5 放入圆底烧瓶中,加入 0.1 mol/L K_2CO_3 水溶液 1 mL 和甲醇 5 mL，常温搅拌反应 2.5 h, TLC 跟踪，至无原料点后终止反应〔流动相 V（乙酸乙酯）：V（石油醚）= 1：1〕。将反应物减压蒸去溶剂，然后加入 10 mL 蒸馏水，再用二氯甲烷分 3 次萃取，合并有机相，饱和食盐水洗涤，无水 Na_2SO_4 干燥，滤液减压除去溶剂得白色固体，进一步柱层析分离得到目标产物 6a~6i〔流动相 V（乙酸乙酯）：V（石油醚）= 1：1〕。

2-(3′-羟基-17′-孕甾)苯并咪唑(6a)，白色固体，产率 89.6%，m.p.124~126 ℃; ^{1}HNMR ($CDCl_3$, 300 MHz), δ: 0.83 (s, 3H, 18′-CH_3), 1.04 (s, 3H, 19′-CH_3), 3.50~3.60 (m, 1H, 3′-αH), 3.89 (br s, 1H, 3′-OH), 5.38 (d, J = 4.8, 1H, 6′-H), 6.82 (d, J = 7.5, 2H, 5-H 和 6-H), 7.03 (s, 1H, N-H), 7.06 (d, J = 7.5, 1H, 3-H), 7.19 (d, J = 7.5, 1H, 7-H); ^{13}CNMR (75 MHz, DMSO-d_6), δ: 13.31 (18′-C), 19.38 (19′-C), 21.07 (11′-C), 23.82 (15′-C), 24.57 (16′-C), 31.62 (8′-C), 31.81 (2′-C), 31.98 (7′-C), 36.58 (10′-C), 37.30 (1′-C), 38.70 (12′-C), 42.26 (4′-C), 44.19 (17′-C), 50.11 (9′-C), 56.54 (13′-C), 57.59 (14′-C), 71.71 (3′-C), 118.34 (4-C 和 7-C), 119.55 (5-C 和 6-C), 121.37 (6′-C), 125.06 (9-C), 126.96 (8-C), 140.81 (5′-C), 171.49 (2-C); IR (KBr), ν/cm^{-1}: 3357, 2926, 2851, 1653, 1589, 1380, 1457, 1045, 956, 745; HREIMS, *m/Z* : 391.2791 $[M+H]^+$ (calcd for $C_{26}H_{35}N_2O$, 391.2749)。

2-(3′-羟基-17′-孕甾)-5-氯苯并咪唑 (6b)，白色固体，产率：95.0%，m.p，93~95 ℃; ^{1}HNMR ($CDCl_3$, 300 MHz), δ: 0.80 (s, 3H, 18′-CH_3), 1.04 (s, 3H, 19′-CH_3), 3.49~3.59 (m, 1H, 3′-αH), 5.38 (d, J = 4.5, 1H, 6′-H), 6.97 (d, J = 8.1, 1H, 6-H), 7.24 (d, J = 8.1, 1H, 7-H), 7.75 (s, 1H, N-H), 7.90 (s, 1H, 4-H); ^{13}CNMR (75 MHz, DMSO-d_6), δ: 13.31 (18′-C), 19.41 (19′-C), 20.98 (11′-C), 23.79 (15′-C), 24.60 (16′-C), 31.60 (8′-C), 31.81 (2′-C), 31.95 (7′-C), 36.56 (10′-C), 37.30 (1′-C), 38.51 (12′-C), 42.23 (4′-C), 44.28 (17′-C), 50.10 (9′-C), 56.44 (13′-C), 57.64 (14′-C), 71.70 (3′-C), 121.35 (6′-C), 125.40 (4-C), 125.72 (7-C), 127.92 (6-C), 130.75 (5-C), 136.20 (9-C), 140.47 (8-C), 140.83 (5′-C), 172.20 (2-C); IR (KBr), ν/cm^{-1}: 3359, 2922, 2852, 1651, 1516, 1377, 1045, 789, 731; HREIMS, *m/Z*: 425.2359 $[M+H]^+$ (calcd for $C_{26}H_{33}ClN_2O$, 425.2360)。

2-(3′-羟基-17′-孕甾)-5-氟苯并咪唑 (6c)，白色固体,产率 95.1%,m.p. 225~227 ℃; ^{1}HNMR ($CDCl_3$，600 MHz), δ: 0.80 (s, 3H, 18′-CH_3), 1.02 (s, 3H, 19′-CH_3), 3.10~3.55 (m, 1H, 3′-αH), 5.36 (s, 1H, 6′-H), 6.47 (s, 1H, OH), 6.44~6.49 (m, 1H, 6-H), 6.89 (s, 1H, 4-H), 7.023 (t, J = 7.2, 1H, 7-H); ^{13}CNMR (150 MHz, DMSO-d_6), δ: 13.00 (18′-C), 19.02 (19′-C), 20.68 (11′-C), 23.44 (15′-C), 24.18 (16′-C), 31.21 (2′-C), 31.41 (7′-C), 31.58 (8′-C), 36.18 (12′-C), 36.90 (1′-C), 38.34 (10′-C), 41.85 (4′-C), 43.81 (13′-C), 49.68

(9′-C), 56.13 (14′-C), 57.00 (17′-C), 71.31 (3′-C), 103.99 ($^2J_{C-F}$ = 25.2, 4-C), 105.30 ($^2J_{C-F}$ = 22.8, 6-C), 119.73 (9-C), 120.98 (6′-C), 126.68 ($^3J_{C-F}$ = 10.1, 7-C), 140.40 (5′-C), 142.81 ($^3J_{C-F}$ = 11.0, 8-C), 161.40 ($^1J_{C-F}$ = 242.4, 5-C), 171.46 (2-C); IR (KBr), ν/cm^{-1}: 3411, 2923, 2850, 1673, 1636, 1511, 1383, 1462, 1053, 973, 791; HREIMS, *m/Z*: 409.2664 $[M+H]^+$ (calcd for $C_{26}H_{33}FN_2O$, 409.2655)。

2-(3′-羟基-17′-孕甾)-5-甲氧基苯并咪唑 (6d)，白色固体，产率 94.6%，m.p. 93~95 ℃; ^{1}HNMR ($CDCl_3$, 300 MHz), δ: 0.80 (s, 3H, 18′-CH_3), 1.02 (s, 3H, 19′-CH_3), 3.50~3.55 (m, 1H, 3′-αH), 3.75 (s, 1H, 5-OCH_3), 3.94 (br s, 1H, 3′-OH), 5.36 (d, J = 2.4, 1H, 6′-H), 6.32 (d, J = 4.5, 1H, 6-H), 6.33 (s, 1H, 3-H), 6.85 (s, 1H, N-H), 6.97 (d, J = 4.5, 1H, 7-H); ^{13}CNMR (75 MHz, DMSO-d_6), δ: 13.49 (18′-C), 19.54 (19′-C), 21.20 (11′-C), 23.95 (15′-C), 24.72 (16′-C), 29.84 (8′-C), 31.73(2′-C), 32.09 (7′-C), 36.70 (10′-C), 37.42 (1′-C), 38.83 (12′-C), 42.37 (4′-C), 44.28 (17′-C), 50.21 (9′-C), 55.50 (-OCH_3), 56.64 (13′-C), 57.49 (14′-C), 71.84 (3′-C), 103.19 (4-C), 104.87 (6-C), 117.63 (7-C), 121.52 (6′-C), 127.00 (9-C), 140.92 (5′-C), 142.95 (8-C), 159.11 (5-C), 171.90 (2-C)；IR (KBr), ν/cm^{-1}: 3369, 2922, 2853, 1656, 1629, 1514, 1382, 1040, 953, 731；HREIMS, *m/Z*: 421.2851 $[M+H]^+$ (calcd for $C_{27}H_{36}N_2O_2$, 421.2855)。

2-(3′-羟基-17′-孕甾)-5-甲基苯并咪唑(6e)，白色固体，产率 95.0%，m.p. 132~135 ℃；^{1}HNMR ($CDCl_3$，300 MHz), δ: 0.80 (s, 3H, 18′-CH_3), 1.04 (s, 3H, 19′-CH_3), 2.32 (s, 3H, Ph-CH_3), 3.49~3.59 (m, 1H, 3′-αH), 5.38 (d, J = 4.5, 1H, 6′-H), 6.98 (d, J = 8.1, 1H, 6-H), 7.24 (d, J = 8.1, 1H, 7-H), 7.27 (s, 1H, 4-H),7.90 (s, 1H, N-H); ^{13}CNMR (75 MHz, DMSO-d_6), δ: 13.32 (18′-C), 19.42 (19′-C), 20.92 (Ph-CH_3), 21.02 (11′-C), 23.83 (15′-C), 24.61 (16′-C), 29.70 (8′-C), 31.61 (2′-C), 31.96 (7′-C), 36.57 (10′-C), 37.30 (1′-C), 38.49 (12′-C), 42.23 (4′-C), 44.28 (17′-C), 50.10 (9′-C), 56.45 (13′-C), 57.53 (14′-C), 71.71 (3′-C), 121.35 (6′-C), 125.29 (7-C), 125.73 (4-C), 127.92 (6-C), 130.75 (5-C), 136.20 (8-C), 140.47 (9-C), 140.83 (5′-C), 172.21 (2-C); IR (KBr), ν/cm^{-1}: 3421, 2930, 2848, 1663, 1516, 1043, 811, 734, 594; HREIMS, *m/Z*: 405.2903 $[M+H]^+$ (calcd for $C_{27}H_{36}N_2O$, 405.2906)。

2-(3′-羟基-17′-孕甾)-5-三氟甲基苯并咪唑(6f)，白色固体，产率 89.6%，m.p. 140~143 ℃；^{1}HNMR ($CDCl_3$，300 MHz), δ: 0.81 (s, 3H, 18′-CH_3), 1.02 (s, 3H, 19′-CH_3), 3.48~3.61 (m, 1H, 3′-αH), 5.31 (s, 1H, -OH), 5.39 (d, J = 5.1, 1H, 6′-H), 7.54 (d, J = 8.4, 1H, 7-H), 8.18 (d, J = 8.4, 1H, 6-H), 8.83 (s, 1H, 4-H), 9.80 (s, 1H, N-H); ^{13}CNMR (75 MHz, DMSO-d_6), δ: 13.17 (18′-C), 19.41 (19′-C), 21.08 (11′-C), 23.44 (15′-C), 24.62 (16′-C), 29.69 (8′-C), 31.86 (2′-C), 32.02 (7′-C), 36.57 (10′-C), 37.29 (1′-C), 38.33 (12′-C), 42.27 (4′-C), 44.24 (17′-C), 50.17 (9′-C), 56.64 (13′-C), 58.60 (14′-C), 71.72 (3′-C), 116.26 (3-C), 121.26 (J_{C-F} = 27.0, 6-C), 121.49 (6′-C), 127.73 ($^2J_{C-F}$ = 35.6, 5-C), 134.76 (7-C), 136.30 (CF_3), 138.46 (8-C), 140.83 (5′-C), 148.16 (9-C), 171.75 (2-C); IR (KBr), ν/cm^{-1}: 3344, 2920, 2848, 1668, 1519, 1319, 1260, 1030, 786, 729; HREIMS, *m/Z*: 459.2615 $[M+H]^+$ (calcd for $C_{27}H_{33}F_3N_2O$, 459.2623)。

2-(3′-羟基-17′-孕甾)-5,6-二甲基苯并咪唑(6g)，白色固体，产率 92.5%，m.p. 138~141 ℃; ^{1}HNMR ($CDCl_3$，300 MHz), δ: 0.80 (s, 3H, 18′-CH_3), 1.02 (s, 3H, 19′-CH_3), 2.09 (s, 3H, 5-CH_3), 2.25 (s, 3H, 4-CH_3), 3.46~3.54 (m, 1H, 3′-αH), 5.35 (d, J = 4.5, 1H, 6′-H), 6.60 (d, J = 8.1, 1H, 6-H), 6.92 (d, J = 8.1, 1H, 7-H); ^{13}CNMR (75 MHz, DMSO-d_6), δ: 13.31 (4-CH_3), 13.37 (18′-C), 19.40 (19′-C), 20.52 (5-CH_3), 21.07 (11′-C), 23.83 (15′-C), 24.59 (16′-C), 31.57 (8′-C), 31.83 (2′-C), 31.97 (7′-C), 36.57 (10′-C), 37.33(1′-C), 38.63 (12′-C), 42.22 (4′-C), 44.15 (17′-C), 50.14 (9′-C), 56.50 (13′-C), 57.35 (14′-C), 71.61 (3′-C), 120.52 (7-C), 121.28 (6′-C), 122.21 (4-C), 122.70 (6-C), 122.88 (8-C), 135.10 (9-C), 139.45 (5-C), 140.89 (5′-C), 171.87 (2-C); IR (KBr), ν/cm^{-1}: 3357, 2926, 2851, 1653, 1589, 1380, 1457, 1045, 956, 745; HREIMS, *m/Z*: 419.3015 $[M+H]^+$ (calcd for $C_{28}H_{38}N_2O$, 419.3062)。

2-(3′-羟基-17′-孕甾)-5-甲基-6-溴苯并咪唑(6h)，白色固体，产率 94.0%，m.p. 92~95 ℃; ^{1}HNMR ($CDCl_3$, 300 MHz), δ: 0.78 (s, 3H, 18′-CH_3), 1.01 (s, 3H, 19′-CH_3), 2.16 (s, 3H, 5-CH_3), 3.50~3.54 (m, 1H, 3′-αH), 3.79 (br s, 1H, 3′-OH), 5.35 (d, J = 1.6, 1H, 6′-H), 7.07 (s, 1H, 4-H), 7.10 (s, 1H, N-H), 7.22 (s, 1H, 7-H); ^{13}CNMR (75 MHz, DMSO-d_6), δ: 13.52 (18′-C), 17.81 (5-CH_3), 19.55 (19′-C), 21.19 (11′-C), 23.91 (15′-C), 24.70 (16′-C), 29.84 (8′-C), 31.72 (2′-C), 32.08 (7′-C), 36.69 (10′-C), 37.42 (1′-C), 38.79 (12′-C), 42.35 (4′-C), 44.39 (17′-C), 50.18 (9′-C), 56.62 (13′-C), 57.57 (14′-C), 71.82 (3′-C), 110.03 (7-C), 121.49 (6′-C), 125.44 (6-C), 125.95 (4-C), 126.70 (8-C), 130.84 (5-C), 138.59 (9-C), 140.92 (5′-C), 171.96 (2-C); IR (KBr), ν/cm^{-1}: 3364, 2930, 2850, 1651, 1467, 1374, 1048, 901, 729; HREIMS, *m/Z*: 483.2005 $[M+H]^+$ (calcd for $C_{27}H_{35}BrN_2O$, 483.2011)。

2-(3′-羟基-17′-孕甾)-6,7-二甲基苯并咪唑(6i)，白色固体，产率 89.0%，m.p. 114~116 ℃; ^{1}HNMR ($CDCl_3$，300 MHz), δ: 0.82 (s, 3H, 18′-CH_3), 1.04 (s, 3H, 19′-CH_3), 2.15 (s, 3H, 5-CH_3), 2.18 (s, 3H, 6-CH_3), 3.49~3.60(m, 1H, 3′-αH), 5.38 (d, J = 4.5, 1H, 6′-H), 6.62 (s, 1H, 4-H), 6.95 (s, 1H, 7-H), 6.97 (s, 1H, N-H); ^{13}CNMR (75 MHz, DMSO-d_6), δ: 13.31 (18-C), 18.76 (6-CH_3), 19.33 (5-CH_3), 19.41 (19′-CH_3), 21.07 (11′-C), 23.81 (15′-C), 24.58 (16′-C), 31.61 (8′-C), 31.82 (2′-C), 31.97 (7′-C), 36.57 (10′-C), 37.30 (1′-C), 38.66 (12′-C), 42.25 (4′-C), 44.14 (13′-C), 50.11

(17′-C), 56.52 (9′-C), 57.50 (14′-C), 71.71 (3′-C), 119.77 (7-C), 121.39 (4-C), 122.35 (6-C), 126.01 (6-C), 127.68 (5-C), 135.30 (9-C), 138.25 (8-C), 140.80 (5′-C), 171.41 (2-C); IR (KBr), ν/cm^{-1}: 3346, 2932, 2852, 1658, 1511, 1280, 1040, 734, 608; HREIMS, *m/Z*: 419.3066 $[M+H]^+$ (calcd for $C_{28}H_{38}N_2O$,419.3062)。

1.3　MTT 实验方法

将细胞以约（3~4）×10^4 个/mL 的密度接种于 96 孔板，每孔接种 200 μL，置于 CO_2 培养箱中培养 24 h，按预设的浓度梯度（5, 10, 20, 40, 80, 160 μmol/L）加入待测样品，每一梯度设 3 个平行孔，同时设只含培养基的空白对照孔和只含培养基及细胞的阴性对照孔。在 CO_2 培养箱中于 37 ℃培养 72 h 后，每孔加入 20 μL 的 MTT（溴化噻唑蓝四氮唑）溶液（将 250 mg MTT 粉末溶于 50 mL PBS 溶液得到 5 g/L 的 MTT 染色液），然后在二氧化碳培养箱中继续温育 4 h。抽取上清液后加入 200 μL 的 DMSO，振荡 10 min 溶解沉淀，随后用酶标仪测定 OD（光密度）值。通过下式求出一定浓度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 IC_{50} 值。

2　结果与讨论

2.1　结构表征与讨论

含苯并咪唑杂环的孕烯醇酮衍生物中，以孕烯醇酮为原料，通过 3′β-羟基保护，得到化合物 2，化合物 2 与溴素、NaOH 反应，得到 C-17′-羧酸产物 3。化合物 3 与二氯亚砜反应得到 C-17′-酰氯化合物 4，4 没有分离直接与带有不同取代基的邻苯二胺反应，得到苯环上拥有不同取代基团的 C-17′-苯并咪唑基甾体化合物 5a~5i。在合成过程中，化合物 3 与二氯亚砜反应时在无水无氧条件下进行。由于化合物 4 是一个酰氯，化学性质比较活泼，因此化合物 4 生成后不经提纯直接与不同的邻苯二胺衍生物反应，得到产物 5a ~ 5i。5a ~ 5i 进一步在碱性条件下脱去 C-3′-位乙酰基得到目标产物 6a ~ 6i, 合成的这 18 个含有苯并咪唑杂环基取代的甾体化合物均为文献中未见报道的化合物。

在化合物 5a 的数据中，化学位移 6.79~7.18 处出现了 4 个苯环上的氢。相应的 ^{13}CNMR 中，在 170.6 处出现了苯并咪唑环中-C═N 的碳化学位移，由此说明甾核的 C-17′-位羧基已和邻苯二胺反应，生成了 C-17′-苯并咪唑甾体化合物。

与化合物 5a 相比，化合物 6a 在化学位移 4.58~4.68 低场处出现的 3′-αH 的多重峰移到了高场的 3.46~3.61，并且在化合物 5a 的 ^{13}CNMR 中，化学位移 171.6 处的-C═O 吸收峰已经消失，说明 3′β-乙酰氧基中的乙酰基已经脱掉，转换成为 6a 的 3′β-羟基。

类似地，其他化合物的结构得到了相应的结构确证。

2.2　体外抗增殖活性测试

分别采用 KB、HeLa、HepG、CNE-2、BT474 和 SKOV3 细胞株作为测试对象，对部分合成物进行体外抑制肿瘤细胞生长增殖活性筛选，结果见表 1。另外，5g、h 和 6g、h 在所有被测试肿瘤细胞中的 IC_{50} 均>80 μmol/L，故在表 1 中没有列出。

从表 1 结果分析，所测试的不同结构苯并咪唑甾体化合物对 SKOV3 细胞表现出明显的选择性抑

表 1　部分甾体苯并咪唑化合物的体外抗增殖活性（IC_{50}, μmol/L）

Table 1　Antiproliferative activity of steroidal benzimidazole compounds in *vitro* (IC_{50}, μmol/L)

Comp.	HeLa	HEPG2	CNE-2	KB	BT474	SKOV3
5a	>80	>80	65.1	ND	>80.0	75.8±9.1
5b	>80	>80	>80	>80	>80.0	76.3±5.7
5c	>80	>80	>80	ND	34.6±2.2	15.4±3.8
5e	>80	>80	>80	ND	>80.0	76.0±9.5
5f	>80	>80	ND	>80	>80.0	77.9±12.3
5i	67.8±5.2	76.4±6.5	>80	ND	>80.0	>80.0
6a	65.7±7.5	55.3±4.3	>80	>80	67.2±2.2	45.2±3.6
6b	>80	66.8±7.8	43.7±2.2	>80	58.8±2.2	57.4±1.2
6c	70.3±2.4	53.7±2.2	ND	ND	44.6±2.2	9.2±0.6
6e	>80	65.4±2.2	44.8±2.2	ND	67.5±2.2	54.4±2.7
6f	55.5±6.3	>80	ND	>80	78.6±2.2	37.6±1.6
6i	ND	>80	56±2.2	>80	72±2.2	>80.0
顺铂	12.9±2.2	9.4±0.6	12.7±1.5	8.2±0.6	14.3±0.7	12.5±1.7

注：各种肿瘤细胞是在 37 ℃，$\varphi(CO_2)$ = 5%恒温箱中培养，以 MTT 法进行体外抑制活性测试。

制作用。其中，以苯并咪唑基中苯环存在氟取代基时的化合物 5c 和 6c 表现较为显著，IC_{50}值分别为（15.4±3.8）和（9.2±0.6）μmol/L。这些化合物对其他测试细胞也表现出一定的体外选择性抑制作用，如 5a 对 CNE-2 细胞表现出一定的抑制，5i 和 6a 对 HeLa 和 HEPG2 细胞表现出选择性抑制，6b 对 CNE-2 和 BT474 表现明显的抑制作用。进一步将所合成化合物的抗肿瘤活性和其分子结构进行比较，初步发现如下规律：

（1）将孕甾醇酮 C-17′-位连接上苯并咪唑杂环药效基团后，化合物对与性激素相关的卵巢癌（SKOV3）及乳腺癌（BT474）细胞表现出较好的抑制生长增殖活性；

（2）从苯并咪唑的结构来看，当苯并咪唑的苯环上取代基为供电子基团（如 5e, 5i）时，化合物对肿瘤细胞基本无抑制作用。而当取代基为吸电子基团（如 6b, 6c）时，化合物均具有明显的抑制活性。

（3）从表 1 中数据可以看出，当化合物 5 中 3′β-乙酰氧基脱去乙酰基成为羟基后，所得到的具有 3′β-羟基结构的化合物 6 抑制活性得到不同程度的提升，这可能是提高了化合物的亲水性及氢键成键能力的缘故。

3　结论

以孕烯醇酮为原料，通过 5 步反应，设计合成了 18 个新的 C-17′苯并咪唑甾体化合物，并对合成物进行了结构表征。对部分目标化合物的体外抗肿瘤活性进行测试，结果表明，化合物对与性激素相关的卵巢癌（SKOV3）及乳腺癌（BT474）细胞表现出较好的抑制生长增殖活性。其中，苯并咪唑基苯环中存在氟取代基时，形成的化合物 5c 和 6c 对卵巢癌（SKOV3）细胞具有良好的选择性抑制活性，IC_{50}值分别为（15.4±3.8）和（9.2±0.6）μmol/L。另外，从苯并咪唑的结构来看，与苯环上取代基为供电子基团的产物相比，当苯环上存在吸电子取代基时，化合物对肿瘤细胞的抑制活性显著提高，甾核 C-3′位为羟基结构时可使化合物的抑制活性得到提升。此类化合物对性激素相关的其他肿瘤细胞的活性有待进一步深入研究，这些化合物的合成及抗肿瘤活性初筛结果为进一步设计和优化甾体抗肿瘤类药物提供了理论参考。

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（上接第 2051 页）

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