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An intramolecular one-pot synthesis of steroidal triazoles via 1,3-dipolar cycloadditions of in situ generated diazo compounds

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ABSTRACT

A novel synthetic route is reported for the preparation of steroidal triazoles via intramolecular 1,3-dipolar cycloaddition of a steroidal 16,17-seco-17-diazo-16-nitrile system. The structures of the products are established by X-ray and NMR studies. The in vitro antiproliferative activity of the steroidal triazoles against three tumor cell lines was evaluated.

The 1,3-dipolar cycloaddition reaction is a powerful synthetic protocol for the synthesis of five-membered heterocycles via well-designed intramolecular sequences.[1] On the other hand, steroidal C-17 tethered heterocyclic compounds possess high antiproliferative activity toward breast and prostate cancer cell lines.[2] Steroidal[3,2-*c*]triazoles possess anti-androgenic activity.[3] Several nonsteroidal cytochrome P450 aromatase inhibitors containing a triazole ring (such as anastrozole and letrozole), exhibit potent antiproliferative activity against estrogen receptor positive breast adenocarcinoma MCF-7 and inhibit the growth of two different MCF-7 breast tumor xenografts in nude mice.[4,5] This inspired us to synthesize 1,2,3-triazole androgen and estrogen D-ring-fused derivatives via a 1,3-dipolar cycloaddition reaction, and to evaluate their antiproliferative activity. We used the 17-oxo-16,17-seco-16-nitriles **1** and **2**, which were synthesized earlier as substrates.[6,7] Compounds **1** and **2** were transformed into the corresponding tosylhydrazones **3** and **4** (Scheme 1). The reactions were carried out in refluxing ethanol over 2 h to afford hydrazones **3** and **4** in yields of 86% and 72%, respectively.

Aggarwal et al.[8] have reported previously that intermolecular 1,3-dipolar cycloaddition of diazo compounds onto alkenes and alkynes led to substituted pyrazoles. In this Letter, we report the synthesis of 1,2,3-triazoles via intramolecular 1,3-dipolar cycloadditions of diazo groups, generated in situ from hydrazones **3** and **4**, to a nitrile group. Thus, addition of NaOH in dioxane/H_2O, $NaBH_4$ in ethanol, or $LiAlH_4$ in dioxane, to tosylhydrazones **3** and **4** yielded the sodium salts **3a** and **4a**, further heating of which at reflux gave the 17-diazo compounds **5** and **6**. These in situ formed diazo compounds underwent intramolecular 1,3-dipolar cycloaddition to give the D-ring-fused triazole derivatives **7** and **8**. The structure of triazole **7**, which was previously prepared as a byproduct[9] under different reaction conditions, was established on the basis of spectroscopic data,[10] and X-ray analysis[11] (Fig. 1), whereas the structure of triazole **8** was established based on detailed spectroscopic data.[12]

Comprehensive analysis of the one- and two-dimensional NMR data of **8** including the results of ^{13}C DEPT, ^{1}H–^{1}H COSY, HSQC, and HMBC (500 MHz) experiments, allowed us to establish the structure of **8**. The strong HMBC correlations between the CH_3-18 methyl protons and C-17 (signal at 161.34 ppm) confirmed that the cycloaddition reaction took place at the C-17 position. The equally strong HMBC correlations between the NH proton and the C-16 and C-17 quaternary carbons suggested the symmetrical triazole structure of **8** in DMSO solution, while according to X-ray analysis this hydrogen is positioned on N-1.

In the case of compound **8** the benzyl protection was removed by catalytic hydrogenolysis in the presence of 10% Pd/C, which resulted in the steroidal triazole **9**[13] in a yield of 65%.

The steroidal triazoles **7** and **9** were preliminarily evaluated for their antiproliferative activity against three human tumor cell

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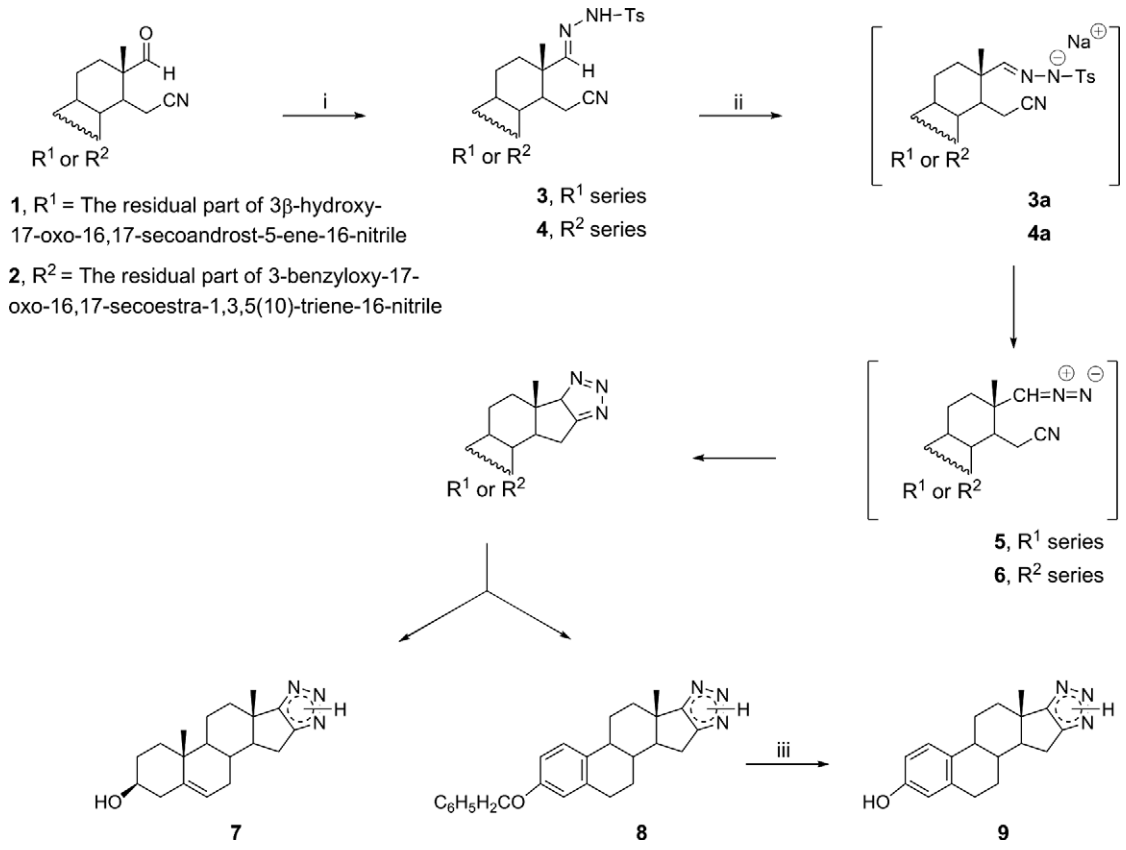

Scheme 1. Reagents and conditions: (i) $TsNHNH_2$, EtOH, reflux, 2 h, 86% of **3**, 72% of **4**; (ii) NaOH, dioxane/H_2O, reflux, 1 h for **7**, or 2 h for **8**, 55% of **7**, and 61% of **8**, $NaBH_4$, EtOH, reflux, 3 h, 65% of **7**, 76% of **8**, or $LiAlH_4$, dioxane, reflux, 6 h, 69% of **7**; (iii) H_2, 10% Pd/C, MeOH, CH_2Cl_2, rt, 45 h, 65%.

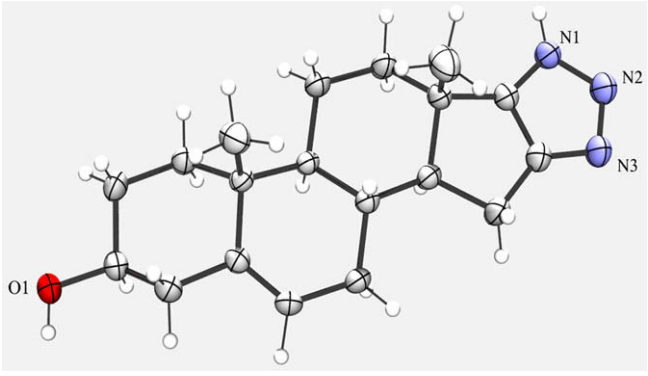

Figure 1. ORTEP representation of the X-ray structure of **7**.

lines. Table 1 shows 50% inhibitory concentrations (IC_{50}) of the tested compounds against human breast adenocarcinoma ER^-, MDA-MB-231, breast adenocarcinoma ER^+, MCF-7, prostate cancer PC-3, and normal fetal lung fibroblasts, MRC-5 cells. The results show that the triazole derivative **7** exhibited significant antiproliferative activity and selectivity against PC-3 cells, being almost eight times more potent than Doxorubicin. Compound **9** was active against MDA-MB-231, and was inactive against the MCF-7 and PC-3 cell lines. Both compounds were nontoxic against healthy MRC-5 cells, in contrast to Doxorubicin, which was extremely toxic against these cells.

Table 1
IC_{50} (μM)[a] values of the steroidal triazole derivatives **7** and **9** and Doxorubicin,[b] in vitro, against different cancer cells

Compound	MDA-MB-231	MCF-7	PC-3	MRC-5
7	>100	>100	12.27	>100
9	20.24	>100	108.64	>100
Doxorubicin	0.12	0.75	95.61	0.12

[a] Inhibitory concentrations (IC_{50}) were determined through the use of an established SRB method.[14]
[b] Doxorubicin (adriamycin) served as reference compound.

In conclusion, we have developed a very simple and convenient route for the synthesis of a steroidal 16,17-fused 1,2,3-triazole derivatives. This method involves the intramolecular 1,3-dipolar cycloaddition of a diazo group, generated in situ from C-17-hydrazones onto a C-16-nitrile group in 16,17-secosteroids. Triazole **7** showed potent antiproliferative activity against prostate cancer PC-3 cells, and can serve as the basis for obtaining more active agents against these cells. Compounds **7** and **9** did not exhibit any cytotoxicity toward normal fetal lung MRC-5 cells.

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10. *Selected data for* **7**: mp 299 °C (from MeOH); IR ν_{max} 3426 and 3241 (OH and NH); ^{1}H NMR (250 MHz, DMSO-d_6): δ 0.93 (s, 3H, H-18), 1.02 (s, 3H, H-19), 1.97 (m, 1H, H-14), 2.15 (m, 2H, H-4), 2.33 (dd, 1H, $J_{14,15\beta}$ = 11.2, $J_{15\alpha,15\beta}$ = 14.2 Hz, H-15β), 2.58 (dd, 1H, $J_{14,15\alpha}$ = 6.1, $J_{15\alpha,15\beta}$ = 14.2 Hz, H-15α), 3.27 (m, 1H, H-3), 4.64 (d, 1H, J = 4.5 Hz, OH), 5.31 (m, 1H, H-6), 14.00 (br s, 1H, NH); ^{13}C NMR (62.9 MHz, DMSO-d_6): δ 17.94 (CH_3), 19.09 (CH_3), 19.95 (CH_2), 23.27 (CH_2), 30.21 (CH), 30.73 (CH_2), 31.39 (CH_2), 33.68 (CH_2), 36.37 (qC), 36.73 (CH_2), 38.71 (qC), 42.22 (CH_2), 49.96 (CH), 61.49 (CH), 69.96 (CH), 120.00 (C-6), 141.57 (C-5), 152.20 (C-16), 161.42 (C-17); HR MS: *m/z* 312.2079 (M^+−H); calcd for $C_{19}H_{26}N_3O$: 312.2081.
11. X-ray crystallographic data for the structure in this Letter have been deposited with the Cambridge Crystallographic Data Centre as Supplementary Publication Number CCDC 713316. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 1223 336033 or email: deposit@ccdc.cam.ac.uk]. Selected crystallographic data: empirical formula $C_{19}H_{27}N_3O$, crystal system, space group: monoclinic, P212121; important bond lengths: C16-C17 1.357(3), N1-C17 1.340(3), N3-C16 1.355(3), N2-N3 1.324(2), N2-N1 1.361(2).
12. *Selected data for* **8**: mp 188 °C (from MeOH–H_2O); IR ν_{max} 3459 and 3150 (NH); ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.93 (s, 3H, H-18), 1.42 (H-7α), 1.57 (H-11β), 1.68 (H-8), 1.78 (H-12α), 1.91 (H-7β), 2.12 (H-14), 2.19 (H-12β), 2.30 (H-9), 2.39 (H-11α), 2.41 (H-15β), 2.69 (dd, 1H, $J_{14,15\alpha}$ = 6.4, $J_{15\alpha,15\beta}$ = 14.3 Hz, H-15α), 2.82 (m, 2H, H-6α, H-6β), 5.04 (s, 2H, $PhCH_2O$), 6.73 (d, 1H, $J_{2,4}$ = 2.5 Hz, H-4), 6.76 (dd, 1H, $J_{2,4}$ = 2.5, $J_{1,2}$ = 8.5 Hz, H-2), 7.18 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), 7.32–7.43 (5H, Ph), 14.03 (br s NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 18.29 (C-18), 23.12 (C-15), 25.66 (C-11), 26.92 (C-7), 29.06 (C-6), 33.78 (C-12), 36.97 (C-8), 39.10 (C-13), 43.74 (C-9), 60.62 (C-14), 68.98 (OCH_2, benzyl), 112.30 (C-2), 114.59 (C-4), 125.94 (C-1), 127.50 (2 × *m*-CH, Ph), 127.68 (*p*-CH, Ph), 128.39 (2 × *o*-CH, Ph), 132.16 (C-10), 137.32 (C-5), 137.38 (qC-benzyl), 151.18 (C-16), 156.23 (C-3), 161.34 (C-17); HR MS: *m/z* 386.2219 (M^++H); calcd for $C_{25}H_{28}N_3O$: 386.2227.
13. *Selected data for* **9**: mp 268 °C (from benzene–acetone); IR ν_{max} 3230 (OH and NH); ^{1}H NMR (250 MHz, acetone-d_6): δ 1.01 (s, 3H, H-18), 6.56 (d, 1H, $J_{2,4}$ = 2.0 Hz, H-4), 6.62 (dd, 1H, $J_{2,4}$ = 2.0, $J_{1,2}$ = 8.4 Hz, H-2), 7.12 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 8.03 (s, 1H, OH), 13.29 (br s, 1H, NH); ^{13}C NMR (62.9 MHz, acetone-d_6): δ 18.71 (C-18), 24.18 (C-15), 26.93 (C-11), 28.33 (C-7), 30.15 (C-6), 35.00 (C-12), 38.54 (C-8), 40.52 (C-13), 45.29 (C-9), 62.08 (C-14), 113.69 (C-2), 116.03 (C-4), 126.84 (C-1), 131.76 (C-10), 138.28 (C-5), 152.74 (C-16), 156.11 (C-3), 162.94 (C-17). HR MS: *m/z* 296.1765 (M^++H); calcd for $C_{18}H_{22}N_3O$: 296.1757.
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