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Synthesis and cytotoxic activities of 2-substituted (25*R*)-spirostan-1,4,6-triene-3-ones via ring-opening/elimination and 'click' strategy

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ABSTRACT

To develop more effective antitumor steroidal drugs, we synthesized a library including twenty-two novel cytotoxic 2-alkyloxyl substituted (25*R*)-spirostan-1,4,6-triene-3-ones and corresponding 1,2,3-triazoles through an abnormal monoepoxide ring-opening/elimination and 'click' reactions. After the cytotoxic evaluations against HepG2, Caski and HeLa cell lines, three steroidal triazoles **5b**, **5f** and **5m** in this library were found to possess potent anti-proliferative effects against Caski cells with the half-inhibitory concentrations (IC_{50}) of 9.4–11.8 μM. The high-efficient and straightforward process was attractive feature for facile preparation of anti-tumor steroidal triazoles.

Currently, cancer has become a major cause of morbidity and mortality in all countries and regions. Development of novel anti-cancer drugs and therapies is always the basic mission for all medicinal chemists. Among the important natural organic compounds occurring in the animals, plants and fungi, steroids have been reported to possess great therapeutic value as antitumor,[1,2] anti-inflammatory,[3,4] and immunostimulant agents.[5] The natural steroidal skeleton has been therefore developed as original anti-cancer drug candidates, for example, the notable of Withaferin A[6,7] and oxysterols.[8] Modification of some these natural products have yielded lots of better cytotoxic steroidal compounds, for example OSW-1[9] and other semi-synthetic cytotoxic steroids with platinum complex or alkylating agent hybrids.[10,11]

Diosgenin is a C27 spiroacetal steroidal sapogenin abundantly available in *Dioscorea species*, *fenugreek*, and *Costus speciosus*,[12] which has been used as a healthy food, as well as the anti-hypercholesterolemia, antihypertriacyl glycerolemia, antidiabetes and antihyperglycemia ingredient in traditional Chinese medicine.[13] The anticancer effect of diosgenin has been also reported with the mechanism of transformation, induction of apoptosis, suppression of proliferation, and disruption of cell membrane integrity.[14] In order to design and develop safer cytotoxic agents, the synthesis of various diosgenin derivatives has been described in the recent literature.[15,16] Among the modified structures of diosgenin, the (25*R*)-spirostan-1,4,6-triene-3-one, an important building block for the synthesis of ruscogenin and 1β-hydroxy vitamin D analogues,[17] was found to possess a wide range of bioactivity including anti-malarial[18] and anti-tumor effects.[19]

Herein we report the library synthesis of 2-alkyloxyl substituted (25*R*)-spirostan-1,4,6-triene-3-ones in three steps including an abnormal monoepoxide ring-opening reaction and a Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction. These diosgenin derivatives have been examined the antiproliferative activities against three human cancer cell lines with the aim of finding more effective antitumor steroidal drugs.

Synthesis: *The abnormal monoepoxide ring-opening reaction:* Due to the widely pharmacological effects and synthetic usage for the (25*R*)-spirostan-1,4,6-triene-3-one analogues, the trienone **2**[18] was prepared by treating **1** with 9.1 equiv of DDQ dehydrogenation, which was further regioselectively converted into the monoepoxide **3**[19] by alkaline H_2O_2 in high yields according to modified Schmalz's method.[20] Flash column chromatography was used to remove most of dark hydroquinone, and trienone **2** was obtained in 83% yield after standard column chromatography. When exploring the ring-opening reaction of monoepoxide **3** with nucleophilic alcohols (R^1OH), we found that the reaction could not be catalyzed by strong acids in various solvents, such as 1 mol/L HCl in CH_3OH, 20% H_2SO_4 in MeOH, EtOH or acetone, 70% $HClO_4$ in acetone, BF_3-Et_2O in tetrahydrofuran (THF) or 1,4-dioxane.

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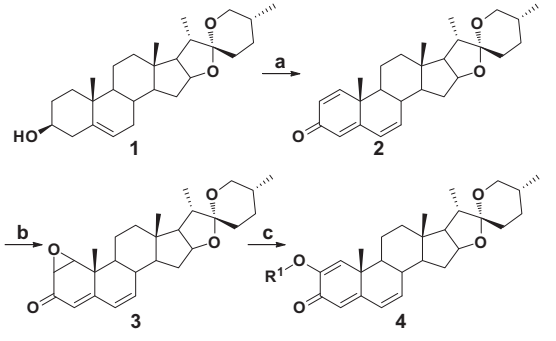
Furthermore, no catalyst or using strong base catalyst in aqueous solvent also resulted in the unreacted starting material. To our surprise, the monoepoxide **3** could be smoothly converted to 2-alkyloxyl substituted (25*R*)-spirostan-1,4,6-triene-3-ones **4** under catalytic amount of NaOH in anhydrous alcohol (Scheme 1).

During the synthesis and isolation of compound **4a**, no ring-opening intermediate of the monoepoxide **3a** was observed. When the reaction temperature was gradually lowered to −20 °C, only a small amount of **4a** was observed in the mixture. Elevated temperatures could shorten the reaction time, and the highest yield of **4a** reached 87% at 50 °C under 5% NaOH in anhydrous methanol. The higher temperatures over 50 °C or concentration of NaOH beyond 5% resulted in reduced yields. Finally, 5% NaOH in anhydrous alcohol was chosen as the best catalyst, and seven target (25*R*)-2-alkyloxyl-spirostan-1,4,6-triene-3-ones (**4a**–**4g**) were achieved in moderate to excellent yields (65–89%).

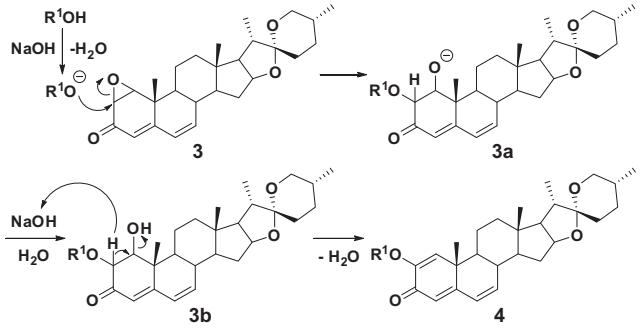
On the basis of the results obtained, the possible mechanism for the abnormal monoepoxide ring-opening reaction might be interpreted as consisting of the following steps (Scheme 2). Firstly, the alcohol R^1OH was deprotonated by NaOH to generate the alkoxy anion, which nucleophically attacked the monoepoxide **3** to give the ring-opening intermediate **3a**. After the protonating procedure, the most stable trienone system **4** was formed under the basic conditions through the elimination mechanism.

*Click synthesis of steroidal 1,2,3-triazoles (**5**):* Considering the attractive features of 1,2,3-triazole moieties in medicinal and biological chemistry,[21,22] a series of steroidal azoles were prepared through Huisgen's Cu(I)-catalyzed 1,3-dipolar cycloaddition of (25*R*)-2-propynyloxy-spirostan-1,4,6-triene-3-one **4e** with azides in this work (Scheme 3). According to the literature methods, the corresponding azides were synthesized by the substitution reaction of alkyl bromides with sodium azide.[23,24] We therefore synthesized fifteen (25*R*)-2-[(1H-1,2,3-triazol-4-yl)methoxy]-spirostan-1,4,6-triene-3-ones (**5a**–**5o**) via classical click chemistry conditions using copper(II) sulfate pentahydrate, sodium ascorbate, *tert*-butanol and H_2O at 80 °C in 20–93% yields.

Considering the unique characteristics and antitumor applications of the steroidal dimmers,[25,26] 1,4-xylyldiazide[27] was also used for the synthesis of bis-steroidal triazole derivatives. When equimolar of **4e** and 1,4-xylyldiazide were added, a steroidal triazole **5d** (yield 50%) and a bis-steroidal triazole **5e** (yield 20%) were spontaneously isolated in the reaction. Increasing the amount of **4e** and prolonged reaction time could result in the higher yield of the dimer **5e** (Scheme 4).



Scheme 1. Synthetic routes for the (25*R*)-2-alkyloxyl-spirostan-1,4,6-triene-3-one (**4**). Reagents and conditions: (a) DDQ, 1,4-dioxane, reflux, 15 h; (b) 30% H_2O_2, MeONa, MeOH, 20 °C, 12 h; (c) 5% NaOH, anhydrous R^1OH, 50 °C, 3 h.



Scheme 2. Possible reaction mechanism for (25*R*)-2-alkyloxyl-spirostan-1,4,6-triene-3-one (**4**).

Structure elucidation: All the structures of 2-alkyloxyl substituted (25*R*)-spirostan-1,4,6-triene-3-ones (**4a**–**4g**) and 1,2,3-triazoles derivatives (**5a**–**5o**) were confirmed by spectral analysis with satisfactory spectral data. In ^{1}H NMR spectrum of **4a**–**4g**, the olefinic proton of H-1 in the A-ring located at 5.92–6.04 ppm as singlet, and the single peak of H-4 located at relatively high field with chemical shift of 6.04–6.20 ppm. Both of H-6 and H7 in the B-ring showed two separated double of doublets at 6.22–6.24 and 6.02–6.07 ppm, respectively. The single peak of steroidal triazoles **5a**–**5o** could be clearly observed in the range of 7.55–8.15 ppm, and the proton in the methylene groups of N–CH_2 and O–CH_2 were assigned to 5.03–5.84 ppm as singlets or quartets. The strong infrared absorption peak at 2099 cm^{-1} was assigned to the azide group in **5d**, which disappeared when bis-steroidal triazole **5e** was formed. On the other side, only 33 carbon resonance peaks in the ^{13}C NMR spectra were found for the bis-steroidal triazole **5e**, which further indicated the symmetrical structure had been successfully synthesized. In the HR-ESI-MS spectrum, the ion adducts of $(M+Na)^+$ or $(M+K)^+$ were usually observed as the base peak ion for all the target compounds. The structure of compound **4b** was also confirmed by the X-ray crystallographic analysis (CCDC number: 1050838), and its ORTEP drawing with common atom numbering scheme was shown in Figure 1.

Biological evaluation: As our continuous interest in search of the biological steroidal antitumor agents,[28,29] all the target compound were also evaluated for in vitro anti-proliferative activities against three human cancer cell lines (Hep-G2, Caski and Hela) for 48 h at 37 °C by MTT assays. The growth inhibition (%) was determined in triplicate at concentrations 5, 10, 20, 40, 80 and 160 μM. All data were presented as mean ± standard deviation and analyzed by SPSS.[30] The half-inhibitory concentration (IC_{50} value) was calculated and listed in Table 1 based on the percentage inhibition of cell growth. Compared to the cytotoxicities against HeLa cells, most of (25*R*)-2-alkyloxyl-spirostan-1,4,6-triene-3-ones (**4**) and (25*R*)-2-[(1*H*-1,2,3-triazol-4-yl)methoxy]-spirostan-1,4,6-triene-3-one (**5**) displayed the better anti-proliferative activities against HepG2 and Caski cells.

Introduction of 1,2,3-triazoles to steroidal skeleton were found the promising cytotoxicities. Four steroidal 1,2,3-triazoles (**5c**, **5f**, **5g**, **5m**) showed moderate inhibitory effects on $HepG_2$ cells with IC_{50} values of 40.2–45.6 μM, and three triazoles of **5b**, **5f** and **5m** exhibited potent anti-proliferative effects against Caski cells with the experimental IC_{50} of 9.4–11.8 μM against Caski cells. Furthermore, compound **5f** were observed to possess the broadest spectrum of cytotoxic activities for all the tested tumor cells, especially against HeLa cells (IC_{50} 48.1 μM). These results indicated the introduction of an unsubstituted benzyl, 2-oxopropyl or 3-hydroxyphenylethyl group at 1-position of the triazole resulted in increasing cytostatic effect on Caski cells. The further structural exploration and anti-tumor mechanism study for these steroidal triazoles are under way in our research group.

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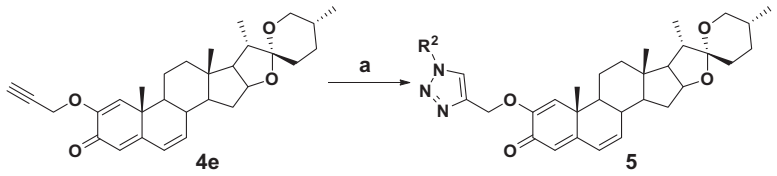
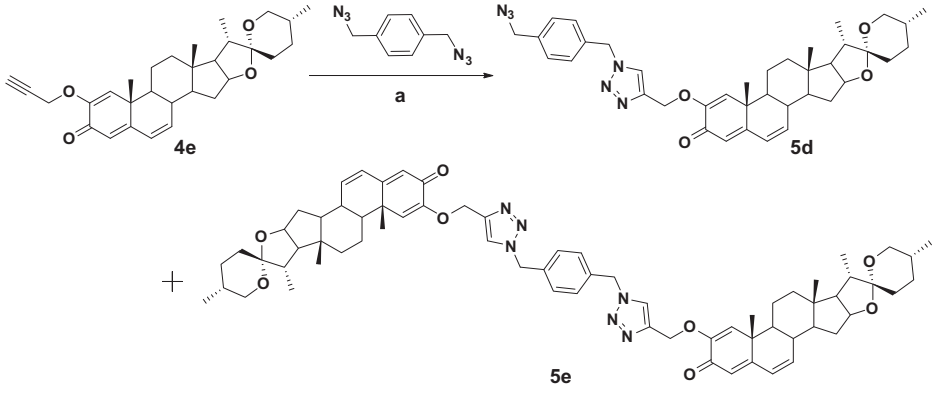


5a: R^2 = Ph
5b: R^2 = $PhCH_2$-
5c: R^2 = 4-MeO-$PhCH_2$-
5f: R^2 = CH_3COCH_2-
5g: R^2 = $CH_3CH_2OCOCH_2$-
5h: R^2 = $CNCH_2$-
5i: R^2 = $PhCOCH_2$-
5j: R^2 = 4-MeO-$PhCOCH_2$-
5k: R^2 = 4-Me-$PhCOCH_2$-
5l: R^2 = 4-HO-$PhCOCH_2$-
5m: R^2 = 3-HO-$PhCOCH_2$-
5n: R^2 = 4-Br-$PhCOCH_2$-
5o: R^2 = 3-Br-$PhCOCH_2$-

Scheme 3. Synthetic routes for the steroidal 1,2,3-triazoles. Reagents and conditions: (a) Vc-Na, $CuSO_4$–$5H_2O$, *t*-BuOH, H_2O, 80 °C.



Scheme 4. Synthetic routes for the steroidal dimer. Reagents and conditions: (a) Vc-Na, $CuSO_4$-$5H_2O$, *t*-BuOH, H_2O, 80 °C.

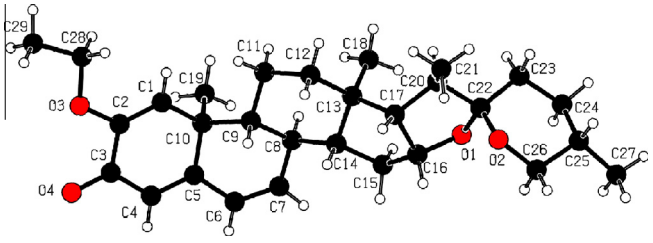


Figure 1. The molecular structure of **4b** with numbered atoms.

In summary, we have developed a facile method for the synthesis of a library consisting of twenty three 2-alkyloxyl and 1,2,3-triazol-4-methoxyl substituted (25*R*)-spirostan-1,4,6-triene-3-one derivatives through the monoepoxide ring-opening/elimination and 'click' chemistry. In this library, three cytotoxic steroidal triazoles (**5b**, **5f** and **5m**) with the IC_{50} values of 9.4–11.8 μM against Caski cells were found. The structure/cytotoxicity investigations implied that the benzyl, 2-oxopropyl or 3-hydroxyphenylethyl substituted steroidal triazoles exhibited the excellent anti-tumor activities. The present method provided a straightforward procedure for rapid modifying steroid skeleton with a wide range of biological substrates.

Table 1
Calculated antiproliferative IC_{50} values for all the synthesized compounds

Compounds	IC_{50} values (μM)		
	$HepG_2$	Caski	HeLa
4a	77.1	28.5	>100
4b	>100	>100	>100
4c	61.6	32.1	80.3
4d	61.3	29.3	>100
4e	90.7	23.2	57.4
4f	85.0	39.5	71.6
4g	53.4	33.5	>100
5a	>100	>100	>100
5b	53.4	10.9	>100
5c	41.8	67.0	>100
5d	>100	>100	>100
5e	>100	>100	>100
5f	40.2	11.8	48.1
5g	44.0	68.9	>100
5h	>100	>100	>100
5i	60.2	99.0	>100
5j	>100	>100	>100
5k	76.1	>100	>100
5l	52.8	98.7	>100
5m	45.6	9.4	>100
5n	53.7	>100	>100
5o	88.1	>100	>100

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.06.028. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Salvador, J. A.; Carvalho, J. F. S.; Neves, M. A. C.; Silvestre, S. M.; Leitão, A. J.; Silva, M. M. C.; Sá e Melo, M. L. *Nat. Prod. Rep.* **2013**, *30*, 324.
2. Challinor, V. L.; De Voss, J. J. *Nat. Prod. Rep.* **2013**, *30*, 429.
3. Khan, M. O.; Lee, H. J. *Chem. Rev.* **2008**, *108*, 5131.
4. Kessel, L.; Tendal, B.; Jørgensen, K. J.; Erngaard, D.; Flesner, P.; Andresen, J. L.; Hjortdal, J. *Ophthalmology* **2014**, *121*, 1915.
5. Cyster, J. G.; Dang, E. V.; Reboldi, A.; Yi, T. *Nat. Rev. Immunol.* **2014**, *14*, 731.
6. Vyas, A. R.; Singh, S. V. *AAPS J.* **2014**, *16*, 1.
7. Chen, L. X.; He, H.; Qiu, F. *Nat. Prod. Rep.* **2011**, *28*, 705.
8. Wijeratne, E. M.; Xu, Y. M.; Scherz-Shouval, R.; Marron, M. T.; Rocha, D. D.; Liu, M. X.; Costa-Lotufo, L. V.; Santagata, S.; Lindquist, S.; Whitesell, L.; Gunatilaka, A. A. *J. Med. Chem.* **2014**, *57*, 2851.
9. Tang, Y.; Li, N.; Duan, J. A.; Tao, W. *Chem. Rev.* **2013**, *113*, 5480.
10. Bansal, R.; Acharya, P. C. *Chem. Rev.* **2014**, *114*, 6986.
11. Zhang, P.; Yang, Y. W.; Shen, Z. R. *Mini Rev. Med. Chem.* **2013**, *13*, 265.
12. Rajput, S.; Mandal, M. *Eur. J. Cancer Prev.* **2012**, *21*, 205.
13. Raju, J.; Mehta, R. *Nutr. Cancer* **2009**, *61*, 27.
14. Chen, P. S.; Shih, Y. W.; Huang, H. C.; Cheng, H. W. *PLoS ONE* **2011**, *6*, e20164.
15. Fernández-Herrera, M. A.; López-Muñoz, H.; Hernández-Vázquez, J. M.; Sánchez-Sánchez, L.; Escobar-Sánchez, M. L.; Pinto, B. M.; Sandoval-Ramírez, J. *Eur. J. Med. Chem.* **2012**, *54*, 721.
16. Hamid, A. A.; Hasanain, M.; Singh, A.; Bhukya, B.; Omprakash, V. P. G.; Sarkar, J.; Chanda, D.; Khan, F.; Aiyelaagbe, O. O.; Negi, A. S. *Steroids* **2014**, *87*, 108.
17. Liu, M.; Yu, B.; Hui, Y. *Tetrahedron Lett.* **1998**, *39*, 415.
18. Ma, E.; Kim, J. *Molecules* **2003**, *8*, 886.
19. Quan, H. J.; Koyanagi, J.; Komada, F.; Saito, S. *Eur. J. Med. Chem.* **2005**, *40*, 662.
20. Sheikh, S. E.; Greffen, A. M.; Lex, J.; Neudörfl, J.-M.; Schmalz, H.-G. *Synlett* **2007**, 1881.
21. Agalave, S. G.; Maujan, S. R.; Pore, V. S. *Chem. Asian J.* **2011**, *6*, 2696.
22. Hou, J.; Liu, X.; Shen, J.; Zhao, G.; Wang, P. G. *Expert Opin. Drug Discov.* **2012**, *7*, 489.
23. Cuetos, A.; Bisogno, F. R.; Lavandera, I.; Gotor, V. *Chem. Commun.* **2013**, 2625.
24. Demko, Z. P.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2110.
25. Šegan, S.; Andrić, F.; Radoičić, A.; Opsenica, D.; Šolaja, B.; Zlatović, M.; Milojković-Opsenica, D. *J. Sep. Sci.* **2011**, *34*, 2659.
26. Shawakfeh, K. Q.; Al-Said, N. H. *Steroids* **2011**, *76*, 232.
27. Guo, Z. F.; Yan, H.; Li, Z. F.; Lu, Z. L. *Org. Biomol. Chem.* **2011**, *9*, 6788.
28. Liu, C. X.; Guo, Z. Y.; Xue, Y. H.; Cheng, J.; Huang, N. Y.; Zhou, Y.; Cheng, F.; Zou, K. *Fitoterapia* **2012**, *83*, 323.
29. Wang, G.; Huang, W.; He, H.; Fu, X.; Wang, J.; Zou, K.; Chen, J. *Int. J. Mol. Med.* **2013**, *31*, 219.
30. West, B. T. *Eval. Health Prof.* **2009**, *32*, 207.