Structural Modification of Antitumor Nitrogenous Steroids (Postprint)

Authors: XIE Tian-Peng, YANG Kang, ZHANG Li-Zhi, CHEN De-Sheng, Kun Zou, HUANG Nian-Yu

Date: 2017-11-05T00:00:00+00:00

Abstract

Natural steroids have demonstrated notable cytotoxic activities and represent promising lead compounds for the development of anticancer drugs, including estramustine and prednimustine. Given that these semi-synthetic molecules are nitrogen mustard-functionalized steroidal derivatives, this review focuses on both the methodologies for introducing nitrogen atoms or nitrogen-containing heterocycles onto the A–D rings or side chains of steroids, and the analysis of the structure-activity relationship (SAR) for these synthetic cytotoxic steroids.

Full Text

Structural Modification for Antitumor Nitrogenous Steroids

Tian-Peng Xie, Kang Yang, Li-Zhi Zhang, De-Sheng Chen, Kun Zou, Nian-Yu Huang
Hubei Key Laboratory of Natural Products Research and Development, College of Biological and Pharmaceutical Sciences, China Three Gorges University, Yichang 443002, China

ABSTRACT
Natural steroids have demonstrated notable cytotoxic activities, representing promising lead compounds for anticancer drug development, including agents such as estramustine and prednimustine. Considering that these semi-synthetic molecules are nitrogen mustard-functionalized steroidal derivatives, this review focuses on methodologies for introducing nitrogen atoms or nitrogen-containing heterocycles onto the A–D rings or side chains of steroids, and analyzes the structure-activity relationships (SAR) for these synthetic cytotoxic steroids.

Keywords: steroids; structural modification; N-heterocycles; antitumor; structure-activity relationship

1. Introduction

Cancer is one of the leading causes of morbidity and mortality worldwide according to the WHO, and the incidence of most cancers—including gastric cancer, liver cancer, and esophageal cancer—has gradually increased over the past 20 years from 1989 to 2008 [1]. Despite the availability of numerous chemotherapeutic drugs, significant medical needs remain unmet due to drawbacks such as drug resistance, lack of selectivity, and cancer metastasis [2]. Natural products represent an important source for many widely used anticancer drugs [3]. As a widespread class of natural organic compounds found in plants, animals, and fungi, the steroid family has exhibited potent antitumor effects through direct suppression of tumor growth, including reduced cell cycle progression and apoptosis induction, as well as inhibition of tumor metastasis [4,5]. Numerous steroidal derivatives have been discovered with potent antineoplastic activity and high tissue selectivity [6]. Cytotoxic steroids such as estramustine phosphate, a hybrid structure combining estradiol and nitrogen mustard through a carbamate linkage with alkylating capability and estrogen-induced specificity, are marketed for prostate cancer treatment [7,8] and can be used as single agents or in combination with other drugs such as 5 -reductase inhibitors [9]. Given the pivotal role of antineoplastic nitrogenous steroids, this review discusses recent cases of structural modification on the cyclic backbone (Fig. 1 [Figure 1: see original paper]) and corresponding SAR for these steroidal derivatives as anticancer agents.

Fig. 1. Cyclic backbone of steroids

2. Modification of A–D Rings of Steroid Skeleton

2.1 Modification on the A-ring

Poirier' s group reported the preparation of five libraries of 2 -piperazino-5 -androstane-3 ,17 -diol derivatives via parallel solid-phase synthesis in 2011 [10]. Biological evaluation against HL-60 leukemia cells revealed interesting SAR for these aminosteroids: amide coupling functionalities showed stronger cytotoxic activity compared to corresponding sulfonamides or benzylamines, with six of the most active amide derivatives (Fig. 2 [Figure 2: see original paper], compounds 1–6) exhibiting low half-maximal inhibitory concentration (IC50) values of 1.7–3.1 M and high selectivity indices of 5–55. In 2012, amino-pregnane derivatives (7–9) were further prepared using a sequence of liquid- and solid-phase reactions to extend SAR data on antileukemic activities [11]; derivative 9, substituted with a 3-acetylbenzoyl group, showed strong anti-proliferative effects against HL-60 leukemia cells (IC50: 1.9 M) and low toxicity toward normal peripheral blood lymphocytes (IC50: 31 M). Subsequently, a 5 -androstane-3 ,17 -diol derivative bearing a quinoline nucleus at the end of a piperazine-proline side chain at position 2 and an ethinyl group at position 17 (10) [12,13]

demonstrated the most potent cytotoxic activity against five cancer cell lines (IC50: 0.1–1.1 M for HL-60, MCF-7, T-47D, LNCaP, and WEHI-3), while selectivity experiments toward liver enzymes CYP3A4 and CYP2D6 indicated this aminosteroid possessed very low risk of drug-drug interactions. To increase in vivo drug potency, an optimized 3-dimethylcarbamate aminosteroid derivative (11) was finally synthesized in 2016 using a prodrug strategy targeting the 3 -hydroxyl group [14]; this lead compound showed greater selectivity for cancer cells over normal cells and much higher stability in liver microsomes.

Fig. 2. Chemical structures of compounds 1–11

To develop more effective steroidal drugs, a library of (25R)-2-[(1H-1,2,3-triazol-4-yl)methoxy]-spirostan-1,4,6-triene-3-ones was synthesized from diosgenin for antitumor screening, and three steroidal triazoles (Fig. 3 [Figure 3: see original paper], compounds 12–14) exhibited potent anti-proliferative effects against Caski cells with IC50 values of 9.4–11.8 M [15]. Structure-cytotoxicity investigations suggested that benzyl, 2-oxopropyl, or 3-hydroxyphenylethyl substituted steroidal triazoles displayed excellent antitumor activities.

Fig. 3. Synthesis of compounds 12–14

Krstić reported the synthesis and anticancer activity of mono-/bis-thiosemicarbazones and mono-/bis-thiadiazolines substituted at the C-3 position of steroids [16]. The most cytotoxic products were 3-thiosemicarbazones (Fig. 4 [Figure 4: see original paper], compounds 15–17) and 3,17-bis(thiadiazolines) (18, 19) against six cancer cell lines (HeLa, K562, MDA-MB-361, MDA-MB-453, LS174, and A549), with activity comparable to cisplatin. The spiro heterocyclic substituent at the C-17 position, along with the presence of an , -unsaturated thiosemicarbazone moiety at C-3, enhanced the activity of the tested compounds.

Fig. 4. Chemical structures of compounds 15–19

Bufadienolides are natural cardiac steroids with potent antitumor activities derived from the Traditional Chinese medicine Chan' Su. To improve their biological activity and water solubility, Hu' s group designed and synthesized bufalin 3-nitrogen-containing-ester derivatives in 2013 [17]. SAR studies revealed that the C3 moiety of the A-ring significantly influenced cytotoxic activity. Bufalin-3-piperidinyl-4-carboxylate (Fig. 5 [Figure 5: see original paper], compound 21) displayed significant cytotoxic potency compared to the parent compound bufalin, with IC50 values of 0.76 and 0.34 nM against HeLa and A549 cell lines, respectively. Several years later, they found that bufalin-3-yl nitrogen-containing-carbamate hydrochloride (22, IC50: 0.30 to 1.09 nM against ten tumor cell lines) and C4' -substituted oleandrin analogues (23 and 24, IC50: 10.9 to 21.7 nM against human cervical carcinoma cell lines) exhibited significant in vitro anti-proliferative activities [18,19].

Fig. 5. Chemical structures of compounds 20–24

To clarify the SAR of antitumor activity for diosgenin derivatives in vitro,

Fan' s group designed and synthesized 3-azole bromide-substituted diosgenin derivatives (Fig. 6 [Figure 6: see original paper], compounds 25–30) based on three-dimensional pharmacophore docking simulation for Bcl-2 inhibitors [20,21]. SAR results indicated that derivatives with larger hydrophobic heterocyclic groups showed better activity through hydrogen bonding and dipole-dipole interactions. Due to molecular length restrictions, the side chain of compound 30 could not occupy the P2 and D3 sites compared to other derivatives, resulting in poor antitumor activity.

Fig. 6. Chemical structures of compounds 25–30

Duan' s group first prepared E-salignone (Fig. 7 [Figure 7: see original paper], compound 31) [22] through eight reaction steps in 16.3% total yield, finding it could inhibit invasion of human breast cancer MDA-MB-231 cells and non-small cell lung cancer cells (A549) induced by the chemokine epidermal growth factor (EGF) with IC50 values of 0.36 and 5.77 M, respectively. After modification, a series of antimetastatic E-salignone amide derivatives (32–34) were synthesized [23], with compound 32 showing anti-migration effects in wound-healing assays. More recently, they prepared pregn-17(20)-en-3-amine derivatives (35–42) as anti-breast cancer agents [24]. Compared with the positive control LY294002 (IC50: 0.38 M), the most potent anti-metastatic compound 40 exhibited an IC50 value of 30 nM through inhibition of phosphorylations of integrin 1, PI3K, Akt, and PKC . Preliminary SAR indicated that 3 -substituted steroid derivatives showed better anti-invasion activities than 3 -substituted ones, and the , -unsaturated fragment in ring D might be critical for anti-metastatic activity.

Fig. 7. Chemical structures of compounds 31–42

Beyond acyclic nitrogen-containing derivatives on the A-ring, heterocycle-fused steroids have also been reported as anticancer agents. Cephalostatin 1 (Fig. 8 [Figure 8: see original paper], compound 43) [25], a remarkably cytotoxic bis-steroidal pyrazine isolated from the marine tube worm *Cephalodiscus gilchristi*, has become an important target for total synthesis and structural modification in drug discovery. Tian' s lab demonstrated an efficient and practical synthesis of 43 using natural tetraol and lactone instead of traditional pregnenolone or epiandrostenone [26]. Pettit simplified its E and F rings by replacement at C-17 with -pyrone or dihydro- -pyrone, synthesizing two bis-steroidal pyrazine pyrones (44, 45) in 8 steps that showed moderate anti-proliferative activities in murine P388 lymphocytic leukemia cells with corresponding 50% effective doses (ED50) of 25–57 M [27].

Fig. 8. Chemical structures of compounds 43–45

2.2 Modification on the B-ring

Due to the unique [6-6-6-5]-fused ring system, steroidal compounds display diverse biological activities and play important roles in living systems, with many unusual and interesting steroidal drugs reported. However, relatively few liter-

ature reports address B-ring modifications as anticancer agents.

Cui et al. prepared several series of substituted 5(6→7) abeo-sterols (Fig. 9 [Figure 9: see original paper], compounds 46, 47) [28] and 5(6→7) abeo-cholesterol derivatives (48–51) [29], investigating their anti-proliferative activity against various cancer cells. SAR studies indicated that a cholesterol-type side chain was essential for cytotoxicity, particularly when a thiosemicarbazone group was substituted at the C-6 position. While elimination of the 5-hydroxyl group had no obvious effect on cytotoxic function, removal of the hydroxyl at C-3 markedly decreased anti-proliferative activity. The most potent analogue 48 exhibited excellent cytotoxic activity with an IC50 value of 4.0 M against Bel-7404 cells (compared to cisplatin, IC50: 23 M). Steroidal N-methylthiazole derivatives (52, 53) showed distinct anti-proliferative activity against A549 and HEPG2 cells, while N-phenylthiazole substituted compounds (54, 55) displayed selective cytotoxicity against HeLa cells and were almost inactive toward HEK293T cells [30]. They also studied steroidal semicarbazones (56–58), thiosemicarbazones (59), and hydrazones, finding these compounds exhibited significant inhibitory activity against Bel-7404 cells with IC50 values of 4.2–15.0 M (cisplatin, IC50: 11.6 M) [31]. The steroidal O-benzyloxime ether with 6-thiosemicarbazone (60) [32] displayed excellent anti-proliferative activity against CNE-2 cancer cells with an IC50 value of 5.7 M.

Fig. 9. Chemical structures of compounds 46–60

Steroidal 5,6-fused benzothiazines (Fig. 10 [Figure 10: see original paper], compounds 61–63) were also reported by Shamsuzzaman as cancer chemotherapeutic agents [33,34], prepared from cholest-5-en-7-one derivatives in high yield. Molecular docking studies with the DNA duplex of sequence d(CGCGAATTCGCG) dodecamer (PDB ID: 1BNA) indicated that electrostatic and hydrophobic interactions between nucleotide base pairs and the amino group in the compounds were the main contributors to high activity.

Fig. 10. Chemical structures and docking simulations for compounds 61–63

2.3 Modification on the C-ring

Introduction of nitrogen atoms into steroidal molecules through heteroatom incorporation affects chemical properties and often alters biological activities, such as reducing acute toxicity, enhancing enzyme inhibition, or improving antitumor activity [35,36].

Hanson used different lengths of triazole linkers to connect 11 -(4-substituted oxyphenyl)estradiols with geldanamycin components, finding that the final antiestrogen-geldanamycin conjugates (Fig. 11 [Figure 11: see original paper], compounds 64, 65) [37] retained significant anti-proliferative activity against two breast cancer cell lines with respective IC50 values below 102 nM. These results indicated that further modifications in both ER-targeting strategies and linking groups were needed to achieve greater potency and selectivity in

therapeutic drug delivery.

Fig. 11. Chemical structures of compounds 64–65

Metapristone (Fig. 12 [Figure 12: see original paper], compound 67) is the major active metabolite of mifepristone (66). Shao and co-workers confirmed it as a promising cancer metastatic chemopreventive agent with modest cytostatic effects including cell cycle arrest, mitochondrial membrane potential disruption, and apoptosis induction in human colorectal cancer HT-29 cells [38]. Computational docking provided evidence that both drugs exert their pharmacological effects through key hydrogen-bonding contacts in the same hydrophobic binding cavity composed of Gln570, Arg611, and Gln642. Chen' s method improved the N-demethylation conditions for large-scale preparation of 66, achieving significantly higher yields (97%) using LiOAc as a suitable base [39], enabling gram-scale synthesis for further clinical trials.

Fig. 12. Chemical structures of compounds 66–67

Most breast cancers are initially hormone-dependent diseases, so structural modifications of steroidal hormones have led to steroidal inhibitors targeting aromatase or steroid sulfatase for treatment of estrogen-dependent (ER+) breast cancers [40]. Mernyak found that D-secooximes of 13 - and 13 -estrones (Fig. 13 [Figure 13: see original paper], compounds 68–70) displayed high in vitro antiproliferative potential against multiple cancer cell lines, with IC50 values in the low submicromolar range [41]. Recently, several p-alkylbenzyl-substituted triazoles in the 13 - and 13 -D-secoestrone series (71–74) [42] were synthesized and found to selectively exert high cytostatic action against A2780 cells with IC50 values of 1 M. These secosteroid triazoles could effectively suppress estrone to 17 -estradiol conversion with IC50 values in the low micromolar range by inhibiting human 17 -hydroxysteroid dehydrogenase type 1 activity.

Fig. 13. Chemical structures of compounds 68–74

2.4 Modification on the D-ring

Investigation of SAR for D-ring modifications on the steroidal skeleton has attracted considerable interest from medicinal chemists. Due to the lack of proper directing groups, substitution on the steroidal D-ring (e.g., introduction of an azido group) has proven more difficult [43].

Zupko and co-workers reported the stereoselective synthesis of 15 -triazolyl-5 -androstanes (Fig. 14 [Figure 14: see original paper], compounds 75–78) from 1 -azidoandrostanes, which showed noteworthy in vitro cytostatic effects against HeLa, MCF-7, and A431 cell lines with IC50 values of 1.69–8.40 M [44]. Yu' s group synthesized steroidal spiro-pyrrolidinyl oxindoles (79, 80) with good antiproliferative activities (IC50: 0.71–4.33 M against SMMC-7721 and MCF-7, respectively), which exerted anticancer effects by inducing cellular early apoptosis and arresting the cell cycle at G2/M phase in a concentration- and time-dependent manner [45].

Fig. 14. Chemical structures of compounds 75–80

Mernyak prepared trans-16-triazolyl-13 -methyl-17-estradiol derivatives (Fig. 15 [Figure 15: see original paper], compounds 81–84) in 2015 [46,47], studying SAR for stereochemical configurations of various substituents at C-16 and C-17. The 16 ,17 isomers generally proved most potent across all tested cancer cell lines, particularly when substituted with p-alkyl groups on the triazolyl-phenyl moiety, displaying outstanding activities.

Fig. 15. Chemical structures of compounds 81–84

To study SAR of novel dehydroepiandrosterone derivatives containing triazole or pyrazole rings at C-17, Cabeza prepared these heterocycle-substituted steroids (Fig. 16 [Figure 16: see original paper], compounds 85–87) through multi-step organic reactions in high yields [48]. Biological evaluation indicated that triazole rings at C-17 showed much higher cytotoxic activity compared to pyrazole rings at the same position, possibly attributed to the greater number of hydrogen bond acceptors in triazole systems.

Subsequently, Banday reported a series of novel D-ring substituted isoxazoline and oxazoline derivatives of dehydroepiandrosterone and pregnenolone (88–91) as potential anti-prostate cancer agents [49]. He and co-workers also designed and synthesized 17-(2' ,5' -disubstituted-oxazolyl)-androsta-4,16-dien-3-one derivatives via gold-catalyzed steroidal alkyne oxidation from 4-androstene-3,17-dione (92–95) [50]; most exhibited potent antitumor activities with IC50 values of 3.0–25.5 mol/L against the MCF-7 cell line. To further study SAR of steroidal pyrazole analogs, Shi and co-workers modified 17 -pyrazolyl steroid derivatives through a concise route from pregnenolone [51]. Compound 96 exhibited excellent cytotoxicity against A549 with an IC50 value of 0.91 M, suggesting that 4-chlorophenyl and NH groups were important for enhanced anti-proliferative effects.

Fig. 16. Chemical structures of compounds 85–96

To investigate anticancer effects of D-ring fused steroidal heterocycles, Elmegeed' s group synthesized several pyridazino-, pyrimido-, quinazolo-, oxirano-, and thiazolo-steroid derivatives [52]. Both the acetonitrilothiazolyl androstane derivative (Fig. 17 [Figure 17: see original paper], compound 97) and its copper complex (98) exhibited greater inhibitory influence on MCF-7 growth than the reference drug doxorubicin (Dox) after 48 h incubation.

Liu and coworkers also prepared a series of steroidal[17,16-d]thiazole, steroidal[1,2-b]pyridine, and steroidal[17,16-d]thiazole[2,1-b]imidazo products through a convenient and productive method [53]. They found that imine-substituted steroidal[17,16-d]thiazole (99) had potent cytotoxicity against MGC-803 with an IC50 value of 3.75 M. Most amino-substituted steroidal[17,16-d]thiazole showed relatively better activity against three cell lines, with the most active compound 100 strongly inhibiting EC109 cell proliferation with an IC50 value of 0.196 M.

Fig. 17. Chemical structures of compounds 97–100

2.5 Other modifications

E/F-spiroacetal ring-modified steroids have been identified as useful templates for antitumor agent design and represent challenging subjects for novel synthetic methodology development. In 2014, Negi reported synthesis of furostane derivatives from diosgenin by opening the F-spiroacetal ring using sodium cyanoborohydride in AcOH at room temperature [54]; the final steroidal aldoxime (Fig. 18 [Figure 18: see original paper], compound 101) and oxime acetate (102) derivatives strongly inhibited proliferation of five cancer cell lines similarly to tamoxifen. Wang' s group synthesized a range of aza-sapogenin derivatives via a facile route from natural sarsasapogenin [55]; pharmacological results showed most products displayed excellent selective cytotoxicity toward cancer cell lines. Compared to bromo or morpholinyl substituents at C-3 and C-26 positions, C-3/C-26 amino derivatives produced much better cytotoxic effects, with compound 103 (IC50: 0.56 M against A375-S2 cells; 0.72 M against HT1080 cells) and compound 104 (IC50: 1.70 M against A549 cells) exhibiting significant cytotoxic activity.

Fig. 18. Chemical structures of compounds 101–104

Using diosgenin as starting material, Fan' s group also designed and synthesized a series of cytotoxic F-spiroacetal ring-opening products substituted by imidazole hydrobromide (Fig. 19 [Figure 19: see original paper], compounds 105–108) with IC50 values of 2.99–8.94 M against K562 or A549 cells [20,21]. After further modification, A- and F-ring opening steroid (109) [56] was found to possess pharmacological activity similar to Taxol, suggesting that introducing more bulky hydrophilic groups on A- and D-rings might be beneficial for antitumor effects.

Fig. 19. Chemical structures of compounds 105–109

Barrera' s group synthesized two pregnane derivatives bearing a triazole (Fig. 20 [Figure 20: see original paper], compound 110) or imidazole (111) moiety at the C-21 position using commercial 16-dehydropregnenolone acetate as starting material [57]; both compounds inhibited proliferation of prostate cancer (PC-3), breast cancer (MCF7), and lung cancer (SK-LU-1) cell lines in a dose-dependent manner. Compound 111 showed antagonist effects on the progesterone receptor while completely discarding androgen receptor or vitamin D receptor pathways as possible mechanisms of anti-proliferative action, suggesting that steroidal modification by introducing imidazole and triazole moieties deserves further investigation.

Fig. 20. Chemical structures of compounds 110 and 111

Cui' s group reported a series of dehydroepiandrosterone-17-hydrazone derivatives possessing various aromatic heterocycle structures in the 17-side chain of the steroidal nucleus (Fig. 21 [Figure 21: see original paper], compounds 112–

114) [58,59], which showed distinct anti-proliferative activity against some cancer cells in vitro through apoptosis induction. Compared to 112 containing a quinoline structure (IC50: 1.0 M in SGC7901 cells), the indole-substituted product 113 showed selective cytotoxicity against HeLa cells with an IC50 value of 5.0 M.

Fig. 21. Chemical structures of compounds 112–114

3. Conclusion

Although many chemotherapy drugs are currently on the market, consequent side effects and tumor metastasis seriously affect drug efficiency [2]. Recent research indicates that more than 60% of current anticancer chemotherapeutic drugs used clinically were initially developed from natural products [2,60]. Steroids are abundant in nature with structural diversity and broad bioactivities, and have been widely used as antitumor agents for a long period, including phytosterols [4,61], diosgenin [62], and cardiotonic steroids [63]. Chemical modification of natural steroids has proven to be an effective approach for developing anticancer agents, with many lead compounds including OSW-1 [64] and 2-methoxyestradiol [65] identified as potential therapeutic agents for cancer therapy. These examples demonstrate that introducing heteroatoms such as oxygen and nitrogen substantially alters the chemical and biological properties of steroids. Therefore, the potential development of heterosteroids as anticancer agents, particularly azasteroids [6,35], has gained increasing attention in medicinal chemistry due to unique heteroatom effects [66].

This review summarized recent progress on introducing anticancer bioactive pharmacophores to steroid scaffolds to improve drug efficiency and selectivity, reporting synthetic methods and SAR studies for nitrogen mustard hybrids, N-heterocycle-substituted or fused steroids. Classic nitrogenous chains (such as amide, imine, oxime, hydrazone, etc.) and 5/6-membered nitrogen heterocycles proved to be effective pharmacophores for enhancing antitumor effects in steroids. SAR investigations also indicated that the superior biological activity of these nitrogenous steroids compared to their precursors was attributable to bulky stereoconfiguration, aromaticity, and the ability to form hydrogen bonds with biological macromolecules. Hopefully, this review will provide valuable reference and suggestions for scientists and medicinal chemists in the successful development of nitrogenous steroidal derivatives as novel antitumor drugs.

References

(1) Chen, W. Q.; Zheng, R. S.; Zeng, H. M.; Zhang, S. W.; Zhao, P.; Hao, J. Trend analysis and projection of cancer incidence in China between 1989 and 2008. Chin. J. Clin. Oncol. 2012, 34, 517–524.

(2) Singh, A.; Settleman, J. EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. Oncogene 2010, 29, 4741–

4751.

(3) Hua, F.; Shang, S.; Hu, Z. W. Seeking new anti-cancer agents from autophagy-regulating natural products. J. Asian Nat. Prod. Res. 2017, 19, 305–313.

(4) Shahzad, N.; Khan, W.; Md, S.; Ali, A.; Saluja, S. S.; Sharma, S.; Al-Allaf, F. A.; Abduljaleel, Z.; Ibrahim, I.A.; Abdel-Wahab, A. F.; Afify, M. A.; Al-Ghamdi, S. S. Phytosterols as a natural anticancer agent: current status and future perspective. Biomed. Pharmacother. 2017, 88, 786–794.

(5) Man, S.; Gao, W.; Zhang, Y.; Huang, L.; Liu, C. Chemical study and medical application of saponins as anti-cancer agents. Fitoterapia 2010, 81, 703–714.

(6) Bansal, R.; Acharya, P. C. Man-made cytotoxic steroids: exemplary agents for cancer therapy. Chem. Rev. 2014, 114, 6986–7005.

(7) Saha, P.; Debnath, C.; Bérubé, G. Steroid-linked nitrogen mustards as potential anticancer therapeutics: a review. J. Steroid Biochem. Mol. Biol. 2013, 137, 271–300.

(8) Xiao, Z.; Morris-Natschke, S. L.; Lee, K. H. Strategies for the optimization of natural leads to anticancer drugs or drug candidates. Med. Res. Rev. 2016, 36, 32–91.

(9) Cabeza, M.; Bratoeff, E.; Heuze, I.; Rojas, A.; Terán, N.; Ochoa, M.; Ramírez-Apan, T.; Ramírez, E.; Pérez, V.; Gracia, I. New progesterone derivatives as inhibitors of 5alpha-reductase enzyme and prostate cancer cell growth. J. Enzyme Inhib. Med. Chem. 2006, 21, 371–378.

(10) Roy, J.; Maltais, R.; Jegham, H. D. Libraries of 2 -(N-substituted piperazino)-5 -androstane-3 ,17 -diols: chemical synthesis and cytotoxic effects on human leukemia HL-60 cells and on normal lymphocytes. Mol. Divers. 2011, 15, 317–339.

(11) Jegham, H.; Maltais, R.; Dufour, P.; Roy, J.; Poirier, D. Solid-phase chemical synthesis and in vitro biological evaluation of novel 2 -piperazino-(20R)-5 -pregnane-3 ,20-diol N-derivatives as antileukemic agents. Steroids 2012, 77, 1403–1418.

(12) Maltais, R.; Hospital, A.; Delhomme, A.; Roy, J.; Poirier, D. Chemical synthesis, NMR analysis and evaluation on a cancer xenograft model (HL-60) of the aminosteroid derivative RM-133. Steroids 2014, 82, 68–76.

(13) Ayan, D.; Maltais, R.; Hospital, A.; Poirier, D. Chemical synthesis,

cytotoxicity, selectivity and bioavailability of 5 -androstane-3 ,17 -diol derivatives. Bioorg. Med. Chem. 2014, 22, 5847–5859.

(14) Perreault, M.; Maltais, R.; Dutour, R.; Poirier, D. Explorative study on the anticancer activity, selectivity and metabolic stability of related analogs of aminosteroid RM-133. Steroids 2016, 115, 105–113.

(15) Lu, X. F.; Yang, Z.; Huang, N. Y.; He, H. B.; Deng, W. Q.; Zou, K. Synthesis and cytotoxic activities of 2-substituted (25R)-spirostan-1,4,6-triene-3-ones via ring-opening/elimination and 'click' strategy. Bioorg. Med. Chem. Lett. 2015, 25, 3726–3729.

(16) Zivkovic, M. B.; Matic, I. Z.; Rodic, M. V.; Novakovic, I. T.; Sladic, D. M.; Krstic, N. M. Synthesis, characterization and in vitro cytotoxic activities of new steroidal thiosemicarbazones and thiadiazolines. RSC Adv. 2016, 6, 34312–34333.

(17) Ma, B.; Xiao, Z. Y.; Chen, Y. J.; Lei, M.; Meng, Y. H.; Guo, D. A.; Liu, X.; Hu, L. H. Synthesis and structure-activity relationships study of cytotoxic bufalin 3-nitrogen-containing-ester derivatives. Steroids 2013, 78, 508–512.

(18) Lei, M.; Xiao, Z.; Ma, B.; Chen, Y.; Liu, M.; Liu, J.; Guo, D.; Liu, X.; Hu, L. Synthesis and biological evaluation of bufalin-3-yl nitrogen-containing carbamate derivatives as anticancer agents. Steroids 2016, 108, 56–60.

(19) Chen, H.; Lei, M.; Ma, B.; Liu, M.; Guo, D.; Liu, X.; Hu, L. Synthesis and cytotoxicity evaluation of 4' -amino-4' -dehydroxyloleandrin derivatives. Fitoterapia 2016, 113, 85–90.

(20) Jiang, H. P.; Wu, Y. K.; Zheng, W.; Zeng C. L.; Fu, W. W.; Fan, J. Z. Structure-activity relationship of diosgenin derivatives as Bcl-2 antagonists. Acta Pharm. Sin. 2011, 46, 539–547.

(21) Ding, X. Y.; He, G.; Jiang, H. P.; Wan, J. F.; Fan J. Z. Novel derivatives of diosgenin: design, synthesis and anti-tumor activity. Acta Pharm. Sin. 2012, 47, 479–485.

(22) Liu, J.; Ma, S. N.; Zhang, X.; Jin, M. N.; Jin, M. H.; Kong, D.; Qin, N.; Duan, H. Q. Synthesis and antimetastatic effect of E-salignone. Chem. Nat. Compd. 2014, 50, 697–701.

(23) Wang, H. L.; Qin, N.; Liu, J.; Jin, M. N.; Zhang, X.; Jin, M. H.; Kong, D.; Jiang, S. D.; Duan, H. Q. Synthesis and antimetastatic effects of E-salignone amide derivatives. Drug Dev. Res. 2014, 75, 76–87.

(24) Qin, N.; Jia, M.; Wu, X. R.; Shou, X. A.; Liu, Q.; Gan, C. C.; Jin, M. N.; Yu, Y.; Duan, H. Q. Synthesis and anti-metastatic effects of pregn-17(20)-en-3-amine derivatives. Eur. J. Med. Chem. 2016, 124, 490–499.

(25) Pettit, G. R.; Moser, B. R.; Herald, D. L.; Knight, J. C.; Chapuis, J. C.; Zheng, X. The cephalostatins. 23. conversion of hecogenin to a steroidal 1,6-dioxaspiro[5.5]nonane analogue for cephalostatin 11. J. Nat. Prod. 2015, 78, 1067–1072.

(26) Shi, Y.; Jia, L. Q.; Xiao, Q.; Lan, Q.; Tang, X. H.; Wang, D. H.; Li, M.; Ji, Y.; Zhou, T.; Tian, W. S. A practical synthesis of cephalostatin 1. Chem. Asian J. 2011, 6, 786–790.

(27) Pettit, G. R.; Moser, B. R.; Mendonca, R. F.; Knight, J. C.; Hogan, F. The cephalostatins. 22. synthesis of bis-steroidal pyrazine pyrones. J. Nat. Prod. 2012, 75, 1063–1069.

(28) Gan, C. F.; Fan, L. H.; Cui, J. G.; Huang, Y. M.; Jiao, Y. X.; Wei, W. X. Synthesis and in vitro antiproliferative evaluation of some ring B abeo-sterols. Steroids 2012, 77, 1061–1068.

(29) Gan, C. F.; Lin, Q. F.; Cui, J. G.; Feng, J. D.; Guo, J. N.; Liao, H. Y.; Huang, Y. M. Synthesis and in vitro antiproliferative evaluation of some novel B-norcholesterols. Steroids 2014, 79, 37–43.

(30) Huang, Y. M.; Qi, B. B.; Cui, J. G.; Gan, C. F.; Yang, C. H.; Liu, C.; Chen, S.; Shi, H. X. Synthesis and evaluation of some new B-norcholesteryl thiazole compounds as anticancer agents. Chin. J. Org. Chem. 2016, 36, 1602–1610.

(31) Gan, C. F.; Cui, J. G.; Su, S. Y.; Lin, Q. F.; Jia, L. Y.; Fan, L. H.; Huang, Y. Synthesis and antiproliferative activity of some steroidal thiosemicarbazones, semicarbazones and hydrozones. Steroids 2014, 87, 99–107.

(32) Huang, Y. M.; Su, S. Y.; Jia, L. Y.; Gan, C. F.; Lin, Q. F.; Kong, E. B.; Cui, J. G. Synthesis and antiproliferative evaluation of some steroidal oxime ether. Chin. J. Org. Chem. 2014, 34, 1816–1828.

(33) Shamsuzzaman; Dar, A. M.; Khan, Y.; Sohail, A. Synthesis and biological studies of steroidal pyran based derivatives. J. Photochem. Photobiol. B 2013, 129, 36–47.

(34) Shamsuzzaman; Dar, A. M.; Khanam, H.; Gatoo, M. A. Anticancer and antimicrobial evaluation of newly synthesized steroidal 5,6 fused

benzothiazines. Arab. J. Chem. 2014, 7, 461–468.

(35) Ibrahim-Ouali, M.; Rocheblave, L. Recent advances in azasteroids chemistry. Steroids 2008, 73, 375–407.

(36) Abdu-Allah, H. H.; Chang, T. T.; Li, W. S. Synthesis of B- and C-ring-modified lithocholic acid analogues as potential sialyltransferase inhibitors. Steroids 2016, 112, 54–61.

(37) Adam, H. J.; Hanson, R. N.; Amolins, M.; Mihelcic, J. M.; Blagg, B. S. Synthesis and preliminary evaluation steroidal antiestrogen-geldanamycin conjugates. Bioorg. Med. Chem. Lett. 2013, 23, 3635–3639.

(38) Wang, J. C.; Chen, J. Z.; Wan, L. Y.; Shao, J. W.; Lu, Y. S.; Zhu, Y. W.; Ou, M. R.; Yu, S. H.; Chen, H. J.; Jia, L. Synthesis, spectral characterization, and in vitro cellular activities of metapristone, a potential cancer metastatic chemopreventive agent derived from mifepristone (RU486). AAPS J. 2014, 16, 289–298.

(39) Wu, J. L.; Yu, X. M.; Liu, J.; Lin, Y. Q.; Gao, Y.; Jia, L. Y.; Chen, H. J. Synthesis of metapristone through an efficient N-demethylation of mifepristone. RSC Adv. 2016, 6, 7195–7197.

(40) Chuffa, L. G.; Lupi-Júnior, L. A.; Costa, A. B.; Amorim, J. P.; Seiva, F. R. The role of sex hormones and steroid receptors on female reproductive cancers. Steroids 2017, 118, 93–108.

(41) Mernyak, E.; Fiser, G.; Szabo, J.; Bodnar, B.; Schneider, G.; Kovacs, I.; Ocsovszki, I.; Zupko, I.; Wolfling, J. Synthesis and in vitro antiproliferative evaluation of D-secooxime derivatives of 13 - and 13 -estrone. Steroids 2014, 89, 47–55.

(42) Szabo, J.; Bacsa, I.; Wölfling, J.; Schneider, G.; Zupko, I.; Varga, M.; Herman, B.; Kalmar, L.; Szecsi, M.; Mernyak, E. Synthesis and in vitro pharmacological evaluation of N-[(1-benzyl-1,2,3-triazol-4-yl) methyl]-carboxamides on D-secoestrone scaffolds. J. Enzyme Inhib. Med. Chem. 2016, 31, 574–579.

(43) Cepa, M. M.; Tavares, S. E. J.; Correia-da-Silva, G.; Roleira, F. M.; Teixeira, N. A. Structure-activity relationships of new A,D-ring modified steroids as aromatase inhibitors: design, synthesis, and biological activity evaluation. J. Med. Chem. 2005, 48, 6379–6385.

(44) Kadar, Z.; Molnar, J.; Schneider, G.; Zupko, I.; Frank, E. A facile 'click' approach to novel 15b-triazolyl-5a-androstane derivatives, and an evaluation of their antiproliferative activities in vitro. Bioorg. Med.

Chem. 2012, 20, 1396–1402.

(45) Yu, B.; Shi, X. J.; Qi, P. P.; Yu, D. Q.; Liu, H. M. Design, synthesis and biological evaluation of novel steroidalspiro-oxindoles as potent antiproliferative agents. J. Steroid Biochem. Mol. Biol. 2014, 141, 121–134.

(46) Mernyak, E.; Kovacs, I.; Minorics, R.; Sere, P.; Czegany, D.; Sinka, I.; Wolfling, J.; Schneider, G.; Ujfaludi, Z.; Boros, I.; Ocsovszki, I.; Varga, M.; Zupko, I. Synthesis of trans-16-triazolyl-13 -methyl-17-estradiol diastereomers and the effects of structural modifications on their in vitro antiproliferative activities. J. Steroid Biochem. Mol. Biol. 2015, 150, 123–134.

(47) Schneider, G.; Gorbe, T.; Mernyak, E.; Wolfling, J.; Holczbauer, T.; Czugler, M.; Sohar, P.; Minorics, R.; Zupko, I. Synthesis of novel 17-(5 -iodo) triazolyl-3-methoxyestrane epimers via Cu(I)-catalyzed azide-alkyne cycloadditon, and an evaluation of their cytotoxic activity in vitro. Steroids 2015, 98, 153–165.

(48) Garrido, M.; Cabeza, M.; Cortes, F.; Gutierrez, J.; Bratoeff, E. Cytotoxic effect of novel dehydroepiandrosterone derivatives on different cancer cell lines. Eur. J. Med. Chem. 2013, 68, 301–311.

(49) Banday, A. H.; Giri, A. K.; Parveen, R.; Bashir, N. Design and synthesis of D-ring steroidal isoxazolines and oxazolines as potential antiproliferative agents against LNCaP, PC-3 and DU-145 cells. Steroids 2014, 87, 93–98.

(50) Li, L. X.; Xie, L. Y.; Wang, J.; He, W. M.; Xiang, J. N. Synthesis anti-tumor activity 17-(2 ,5 -disubstituted-oxazolyl)-androsta-4,16-dien-3-one derivatives. Chin. J. Org. Chem. 2014, 34, 1864–1869.

(51) Li, J.; Zhao, X.; Li, L.; Yuan, Z.; Tan, F.; Shi, B.; Zhang, J. Design, synthesis and cytotoxic activity of a novel series of steroidal phenylpyrazoles. Steroids 2016, 107, 45–54.

(52) Elmegeed, G. A.; Khalil, W. K.; Mohareb, R. M.; Ahmed, H. H.; Abd-Elhalim, M. M.; Elsayed, G. H. Cytotoxicity and gene expression profiles of novel synthesized steroid derivatives as chemotherapeutic anti-breast cancer agents. Bioorg. Med. Chem. 2011, 19, 6860–6872.

(53) Zhang, B. L.; Song, L. X.; Li, Y. F.; Li, Y. L.; Guo, Y. Z.; Zhang, E.; Liu, H. M. Synthesis and biological evaluation of dehydroepiandrosterone-fused thiazole, imidazo[2,1-b]thiazole, pyridine steroidal analogues. Steroids 2014, 80, 92–101.

(54) Hamid, A. A.; Hasanain, M.; Singh, A.; Bhukya, B.; Omprakash; Vasudev, P. G.; Sarkar, J.; Chanda, D.; Khan, F.; Aiyelaagbe, O. O.; Negi, A. S. Synthesis of novel anticancer agents through opening of spiroacetal ring of diosgenin. Steroids 2014, 87, 108–118.

(55) Yin, Y.; Zhao, X. C.; Wang, S. J.; Gao, P. Y.; Li, L. Z.; Ikejima, T.; Song, S. J. Synthesis and biological evaluation of novel sarsasapogenin derivatives as potential anti-tumor agents. Steroids 2015, 93, 25–31.

(56) Liao, P. F.; Dong, J. H.; Fan, J. Z. Synthesis of diosgenin derivatives based on structure modification of its A and F ring and preliminary study of their antitumor activity. West China J. Pharm. Sci. 2013, 28, 364–371.

(57) Silva-Ortiz, A. V.; Bratoeff, E.; Ramírez-Apan, M. T.; García-Becerra, R.; Ordaz-Rosado, D.; Noyola-Martínez, N.; Castillo-Bocanegra, N.; Barrera, D. Synthesis and biological activity of two pregnane derivatives with a triazole or imidazole ring at C-21. J. Steroid Biochem. Mol. Biol. 2016, 159, 8–18.

(58) Cui, J. G.; Liu, L.; Zhao, D. D.; Gan, C. F.; Huang, X.; Xiao, Q.; Qi, B. B.; Yang, L.; Huang, Y. M. Synthesis, characterization and antitumor activities of some steroidal derivatives with side chain of 17-hydrazone aromatic heterocycle. Steroids 2015, 95, 32–38.

(59) Cui, J. G.; Zhao, D. D.; He, D. M.; Huang, Y. M.; Liu, Z. P.; Lin, Q. F.; Shi, H. X.; Gan, C. F. Synthesis of dehydroepiandrosteronyl thiazole derivatives and their antiproliferative evaluation. Chin. J. Org. Chem. 2016, 36, 630–637.

(60) Kinghorn, A. D.; DE Blanco, E. J.; Lucas, D. M.; Rakotondraibe, H. L.; Orjala, J.; Soejarto, D. D.; Oberlies, N. H.; Pearce, C. J.; Wani, M. C.; Stockwell, B. R.; Burdette, J. E.; Swanson, S. M.; Fuchs, J. R.; Phelps, M. A.; Xu, L.; Zhang, X.; Shen, Y. Y. Discovery of anticancer agents of diverse natural origin. Anticancer Res. 2016, 36, 5623–5637.

(61) Woyengo, T. A.; Ramprasath, V. R.; Jones, P. J. Anticancer effects of phytosterols. Eur. J. Clin. Nutr. 2009, 63, 813–820.

(62) Raju, J.; Mehta, R. Cancer chemopreventive and therapeutic effects of diosgenin, a food saponin. Nutr. Cancer 2009, 61, 27–35.

(63) Mijatovic, T.; Van Quaquebeke, E.; Delest, B.; Debeir, O, Darro F.; Kiss, R. Cardiotonic steroids on the road to anti-cancer therapy. Biochim. Biophys. Acta 2007, 1776, 32–57.

(64) Tang, Y.; Li, N.; Duan, J. A.; Tao, W. Structure, bioactivity, and chemical synthesis of OSW-1 and other steroidal glycosides in the genus Ornithogalum. Chem. Rev. 2013, 113, 5480–5514.

(65) Kumar, B. S.; Raghuvanshi, D. S.; Hasanain, M.; Alam, S.; Sarkar, J.; Mitra, K.; Khan, F.; Negi, A. S. Recent advances in chemistry and pharmacology of 2-methoxyestradiol: an anticancer investigational drug. Steroids 2016, 110, 9–34.

(66) Mazurek, A. Electron donor acceptor descriptors of the single and double bonded substituent and heteroatom incorporation effects. A review. Acta Pol. Pharm. 2016, 73, 269–283.

Note: Figure translations are in progress. See original paper for figures.