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Review

Synthesis of sex hormone-derived modified steroids possessing antiproliferative activity

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ABSTRACT

During recent years intensive research has been focused on the synthesis of structurally modified steroid hormones in order to obtain compounds with beneficial biological activity such as cell-growth inhibition. Experimental results have revealed that some steroidal derivatives possess direct cytostatic effect on cancer cells in a hormone receptor-independent manner. After a brief account on the most important biological function and characteristics of the naturally occurring sex hormones in physiological and pathological conditions, structural modifications of estrane and androstane scaffolds are discussed in detail. The review covers literature publications (from 2002 to 2012) relating to the synthesis and antiproliferative activity of semisynthetic sex hormone-derived molecules containing simple or heterocyclic substituents. The compounds reviewed are divided into three main categories according to their sterane framework and the nature of substitution.

This article is part of a Special Issue entitled "Synthesis and biological testing of steroid derivatives as inhibitors".

Contents

1. Introduction

Natural sex hormones, *i.e.* androgens, estrogens and progestogens, which are produced primarily by the gonads and in smaller amounts by the adrenal glands and other tissues, exert a wide range of biological effects on the body, affecting the growth and function of the reproductive organs and the development of secondary sexual characteristics. Androgens are also the original anabolic steroids and the precursors of all estrogens, which (together with progesterone) play a major role in the regulation of the menstrual cycle and pregnancy. Thanks to their nonpolar and hydrophobic sterane framework, steroid hormones can easily penetrate through the cell membranes and interact with their specific intracellular receptors, either in the cytosol or in the nucleus of the target cells. Through this slow genomic mechanism, the ligand–receptor complex acts as a transcription factor in the nucleus, augmenting or suppressing particular transcription genes by its action on DNA [1]. Recent studies suggest, however, that the effects of steroid hormones can also be mediated by fast nongenomic mechanisms through membrane-associated receptors and signaling cascades [2]. As a result of extensive structure–activity relationship (SAR) studies, a considerable amount of information is available concerning the structural features of the intracellular receptors, the pharmacophore moiety of the ligands and hormone–receptor binding [3–5]. Besides hydrophobic interactions, hydrogen-bonding in some regions of the steroid binding pocket is also involved in the

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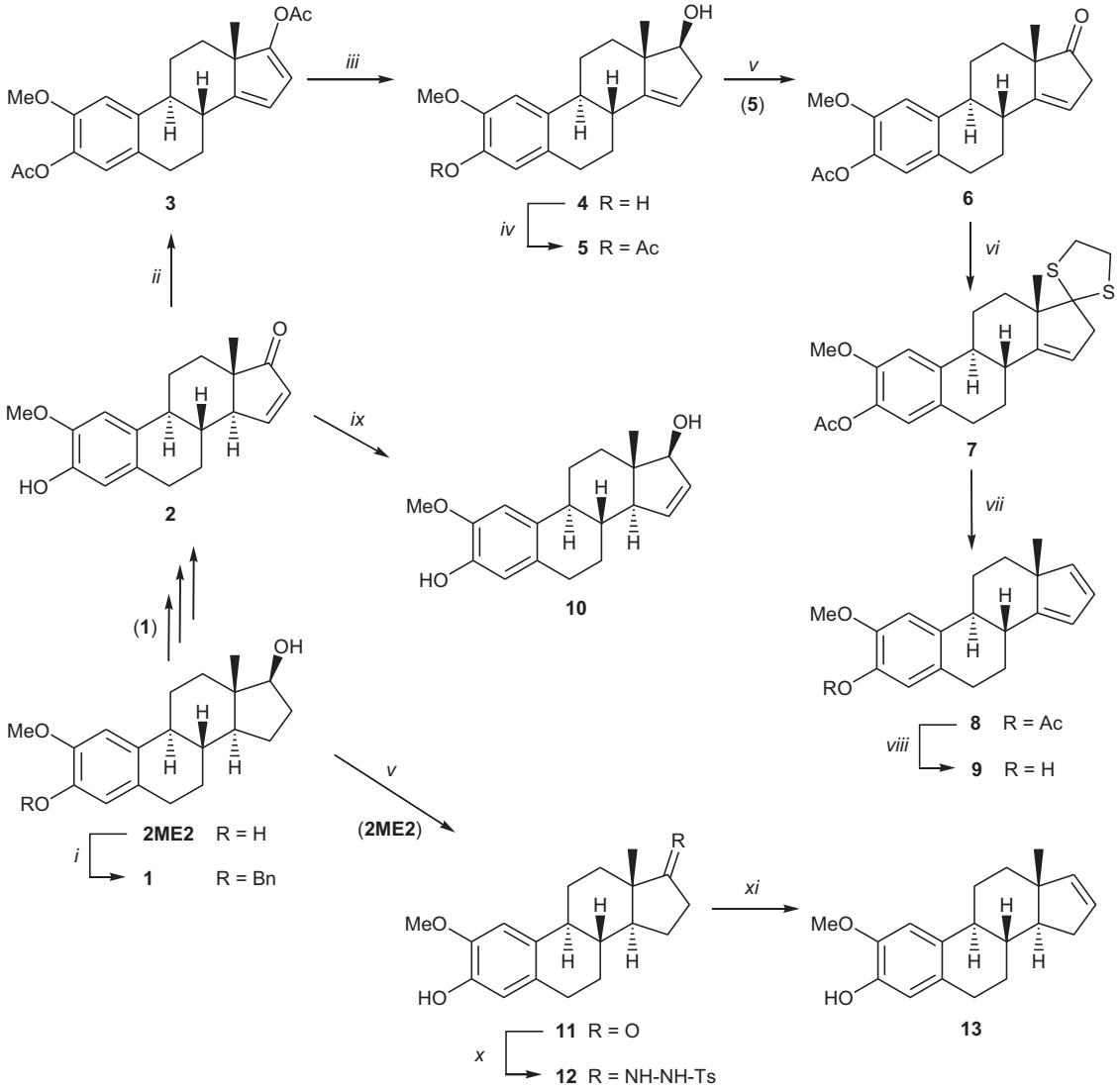
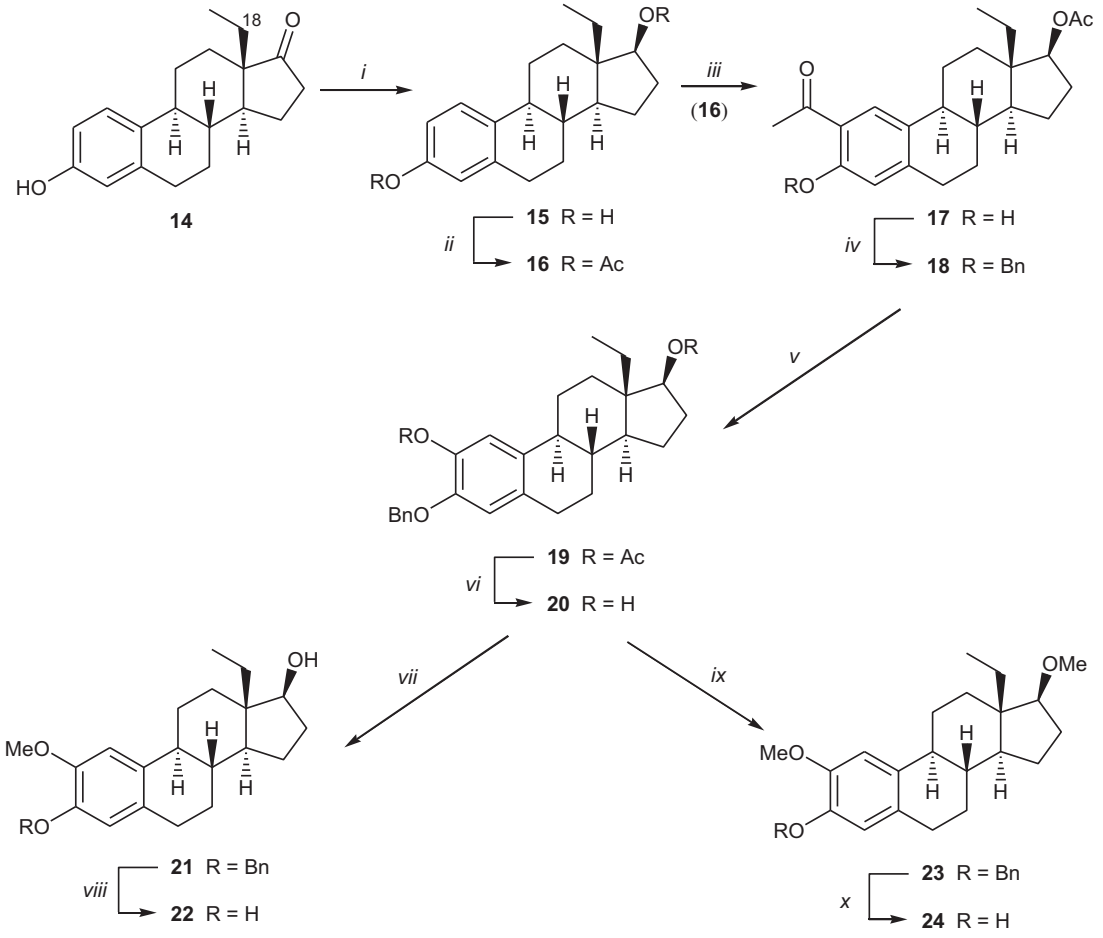


Scheme 1. Synthesis of D-ring-unsaturated analogs of **2ME2**. *Reagents and conditions*: (*i*) BnBr, K_2CO_3, DMF; (*ii*) isopropenyl acetate, Ac_2O, TsOH; (*iii*) $NaBH_4$, MeOH, THF, H_2O; (*iv*) Ac_2O, NaOH, *i*PrOH, H_2O; (*v*) Jones reagent, acetone; (*vi*) $HS(CH_2)_2SH$, $BF_3{\cdot}OEt_2$, AcOH; (*vii*) Raney Ni, acetone; (*viii*) K_2CO_3, MeOH, H_2O; (*ix*) $LiAlH_4$, Et_2O; (*x*) $TsNHNH_2$, MeOH; (*xi*) *n*BuLi, THF.

binding mechanism. All semisynthetic modifications involving the apolar sterane skeleton or the polar functional groups at C-3 and C-17 in the natural hormones may exert a significant influence on the binding affinity of the molecule.

In consequence of the important functions of steroid hormones, they and their modified analogs have been applied exogenously to humans in order to attain certain benefits in health or even to improve physical and growth performance. Estrogens and progestogens are administered as components of oral contraceptives and in hormone replacement therapy, while androgens are used to correct natural hormone deficiencies and to reduce "male menopause" symptoms such as the lack of sex drive, anxiety and depression. Furthermore, extensive effort has been devoted to the synthesis of potentially therapeutic derivatives providing enhanced anabolic potency with reduced androgenic effects, though with only modest success [4].

In addition to the modulation of normal development and maintenance of the reproductive tract, sex steroids play a crucial role in the malignant growth of these organs [6]. As steroid hormones are powerful drivers of the gene expression in hormone-dependent cancer cells, changes in the levels or activities of certain hormones can cause these cancers to cease growing, or even undergo cell death. Hormonal therapy is therefore one of the major possibilities for the medical treatment of cancer, involving manipulation of the endocrine system through the exogenous administration of steroid hormones, or drugs which inhibit the production or activity of the endogenous ligand. Thus, a number of modified steroid molecules have been described as potent inhibitors of specific enzymes involved in the biosynthesis of sex hormones, allowing their potential use in the medication of hormone-dependent diseases. Steroidal *exo*-heterocycles, for example, such as the therapeutically used abiraterone, can block androgen synthesis at an early stage by inhibiting 17α-hydroxylase/$C_{17,20}$-lyase ($P450_{17\alpha}$) and can therefore be effective in the treatment of prostate cancer [7–11]. Moreover, some drugs exert their biological activity as aromatase inhibitors by reducing estrogen production and can thus be applied in the treatment of estrogen receptor-positive (ER+) breast cancer [12,13]. Another approach in cancer therapy is the application of antagonists which bind to the receptor of a given hormone, thereby preventing its activation. However, the effectiveness of these drugs is limited by their partial agonist properties, which can cause undesirable side-effects [14]. Experimental



Scheme 2. Synthesis of 18a-homoanalogs of **2ME2**. *Reagents and conditions*: (*i*) $NaBH_4$, EtOH, H_2O; (*ii*) Ac_2O, pyridine; (*iii*) $ZrCl_4$, CH_2Cl_2; (*iv*) BnBr, K_2CO_3, DMF; (*v*) MCPBA, Na_2HPO_3, CH_2Cl_2; (*vi*) NaOH, MeOH, H_2O; (*vii*) $LiOH{\cdot}H_2O$, Me_2SO_4, THF; (*viii*) H_2, Pd/C, EtOH; (*ix*) NaH/THF, MeI; (*x*) H_2, Pd/C, EtOH.

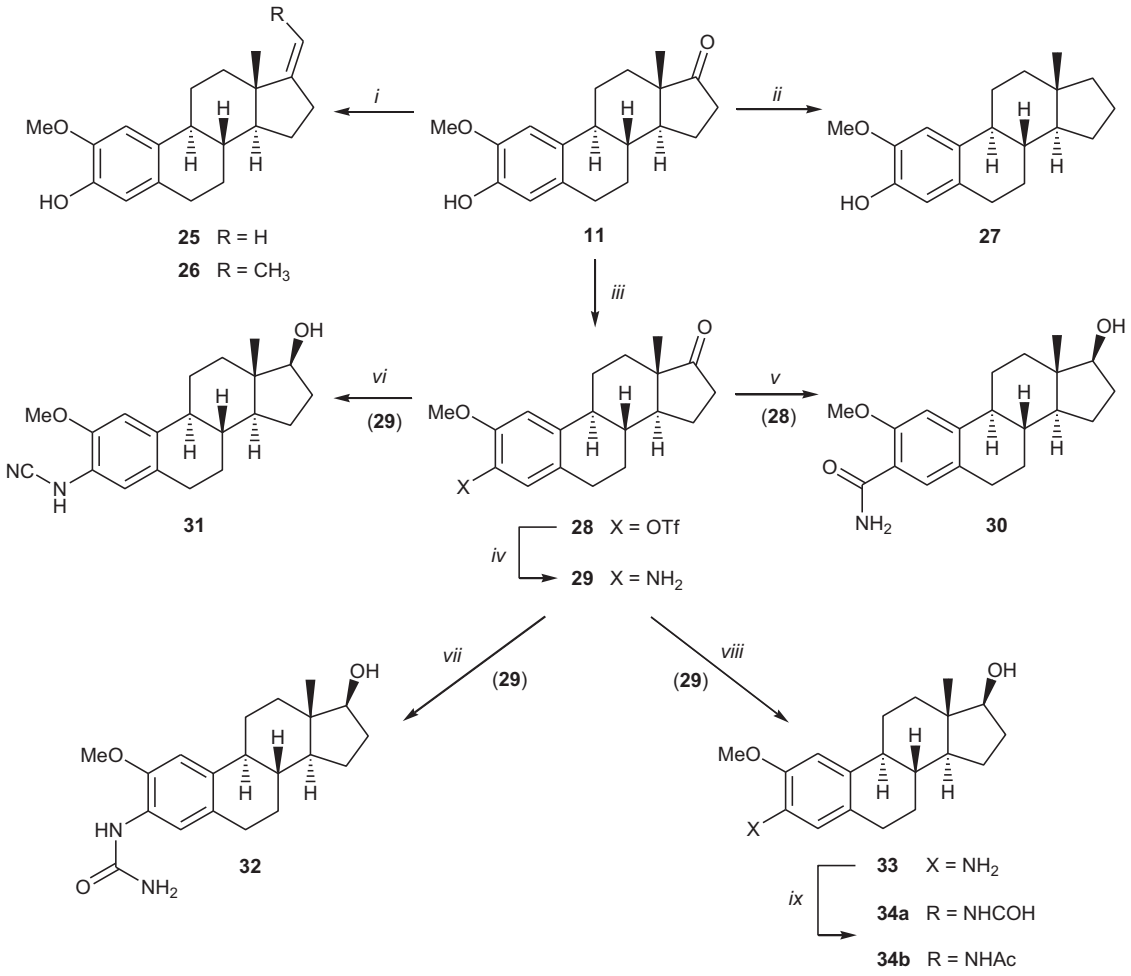
results achieved during the past few years have revealed that some steroidal derivatives play important roles in complex signal transduction mechanisms in a hormone receptor-independent manner by the inhibition of angiogenesis, tubulin polymerization and the upregulation of apoptotic pathways. The main advantage of this mode of action is that such compounds may offer a solution for the therapy of hormone-resistant cancers. The most important candidate in this class is 2-methoxyestradiol (**$2ME_2$**), an endogenous metabolite of 17β-estradiol, which has been shown to be effective in preventing tumor growth in a variety of cell lines of diverse origins [15–21]. Although the exact mechanism of action is still unclear, the unique antiproliferative and apoptotic activities of **$2ME_2$** are mediated independently of the estrogen receptors [22,23].

A major challenge in semisynthetic steroid chemistry at present is the development of novel derivatives with a biological target other than a hormone receptor, and therefore the reduction or elimination of undesirable hormonal activity. The synthetic tools for the attainment of this goal are: (a) the synthesis of molecules lacking the functionalities necessary for effective binding to the hormone receptors [24]; (b) modification of the binding ability by chemical transformation of the functional groups already present [3]; (c) steric hindrance of the substrate-receptor interaction by chemical substitution near the original groups [19]; (d) altering the primary stereostructure or the number of ring members [25]; and (e) the design of heterocyclic derivatives that are not recognized by the receptor protein in consequence of their specific structure or the fact that their geometry differs from that of the natural hormones [26]. However, some combination of the above strategies may likewise be an effective route for the development of novel derivatives with potent therapeutic activity.

The present review mainly focuses on the syntheses and brief pharmacological studies of hormone-derived molecules containing different substituents, including various side-chains or heterocyclic moieties, fused to or linked to the original sterane framework, which have been reported to exhibit an *in vitro* cell growth-inhibitory effect on malignant tumor cell lines.

2. Synthesis and antiproliferative activity of estrane-derived compounds

2ME2 has been evaluated under the name Panzem® in several clinical trials. The clinical results demonstrated that **2ME2** is metabolized principally in oxidative and conjugation processes involving oxidation of the 17-hydroxy group and glucuronidation of both the 3- and 17-hydroxy functions [27]. These transformations lead to a rapid decrease in the concentration of the active drug, resulting in its low bioavailability. Moreover, *in vitro* metabolism studies have indicated that the methoxy moiety of **2ME2** can be removed oxidatively by the action of cytochromes P450 to form 2-hydroxyestradiol, which could be estrogenic *in vivo*. Thus, appreciable efforts have been made to synthesize modified analogs with an enhanced ability to inhibit tubulin polymerization or with an antiproliferative effect, but if possible without estrogenic activity, thereby providing an improvement in the metabolic profile. In attempts to elucidate the determinants of **2ME2** activity and to



Scheme 3. Synthesis of 3- or 17-modified analogs of **2ME2**. *Reagents and conditions*: (*i*) K-*t*-amylate, $RPPh_3Br$; (*ii*) hydrazine, KOH, diethylene glycol, *n*BuOH; (*iii*) Tf_2O, pyridine, CH_2Cl_2; (*iv*) (1) $Pd(OAc)_2$, *rac*-BINAP, benzophenone imine, Cs_2CO_3, toluene, (2) 2 M HCl, THF; (*v*) (1) $Pd(OAc)_2$, *rac*-BINAP, Cs_2CO_3, dppp, HMDS, DMF, CO, (2) MeOH, then acidic workup, (3) $NaBH_4$, MeOH; (*vi*) (1) BrCN, Et_2O, CH_2Cl_2, (2) $NaBH_4$, MeOH; (*vii*) NaOCN, H_2O, AcOH; (*viii*) $LiAlH_4$ (1 M in THF); (*ix*) (1) formic acid, (2) NaOH, MeOH for **34a**; Ac_2O, NaOH (10 M aqueous) for **34b**.

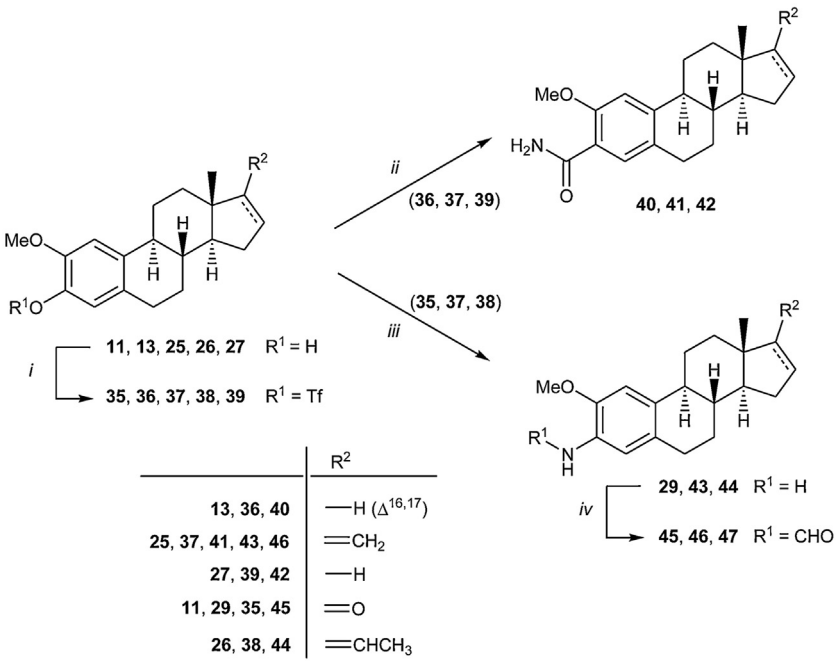
prepare more efficacious analogs, structure–activity relationship (SAR) studies have been conducted by several research groups. Most of these studies have concentrated on modifying existing substituents or incorporating new substituents in rings A and D of **2ME2**, although some B ring-expanded derivatives have also been found to exert noteworthy activity [28]. The introduction of an additional double-bond(s) into ring D of **2ME2** enhanced the *in vitro* antiproliferative activity on the MDA-MB-435 (human breast carcinoma) and SK-OV-3 (ovarian carcinoma) cell lines as compared with the parent molecule [29]. Compounds **4** and **10** were synthesized *via* well-known transformations from a common precursor **2**, available from the 3-benzyloxy analog **1** [30] of **2ME2** (Scheme 1). Interestingly, some unsaturated derivatives **9** and **13**, which lack the 17-hydroxy group, were also demonstrated to retain cell growth-inhibitory effects on both endothelial and tumor cells [31,32]. Moreover, *in vitro* metabolic stability studies have confirmed that these analogs are metabolically more stable than **2ME2** [32].

18a-Homoanalogs of **2ME2** synthesized by Rao et al. [33] from **14** *via* a multistep pathway (Scheme 2) and some of them, such as **22** and **24** were demonstrated to exhibit cytotoxic activity on MDA-MB-231 (human breast carcinoma), U87-MG (human gliomablastoma) and HUVEC (human umbilical vein endothelial) cells that was higher than or comparable to that of **2ME2**. The introduction of a Δ^{14}-bond in **22**, however, significantly lowered the biological activity.

Structural modifications including ring D homologation and aromatization of the six-membered ring D to a chrysine-type molecule were also carried out through a cumbersome synthetic sequence to furnish novel derivatives with considerable antiproliferative effects [31].

Since the 3- and 17-hydroxy groups play a crucial role in the metabolic pathways of **2ME2**, modifications of these functionalities have also been performed in order to obtain analogs with a retained or improved cell growth-inhibitory effect, but with diminished metabolic ability. The transformations depicted in Scheme 3 were carried out from 2-methoxyestrone (**11**) to furnish novel derivatives **25**–**27** lacking the 17-hydroxy moiety or containing an aminonitrile (**31**), urea (**32**), formamide (**34a**), carboxamide (**30**), amino (**33**) or acetamide (**34b**) group on C-3 [32,34]. All these compounds were found to exert diminished estrogenic activity, improved metabolic stability and increased or similar antiproliferative and antiangiogenic activity relative to **2ME2**. It was concluded that, among other factors, the H-donating ability, the π-electronic character and the size of the C-3 substituent all determine the biological activity.

The simultaneous modifications of positions 3 and 17 in **2ME2** also led to active analogs (Scheme 4) [35]. Compounds **13** and **25**–**27**, obtained from 2-methoxyestrone (**11**) by Shapiro reduction (**13**), Wittig coupling (**25** and **26**) or Wolf–Kishner reduction (**27**) (Schemes 1 and 2), were used for the syntheses. All the synthesized derivatives represented by **40**–**42** and **45**–**47** exhibited marked cell

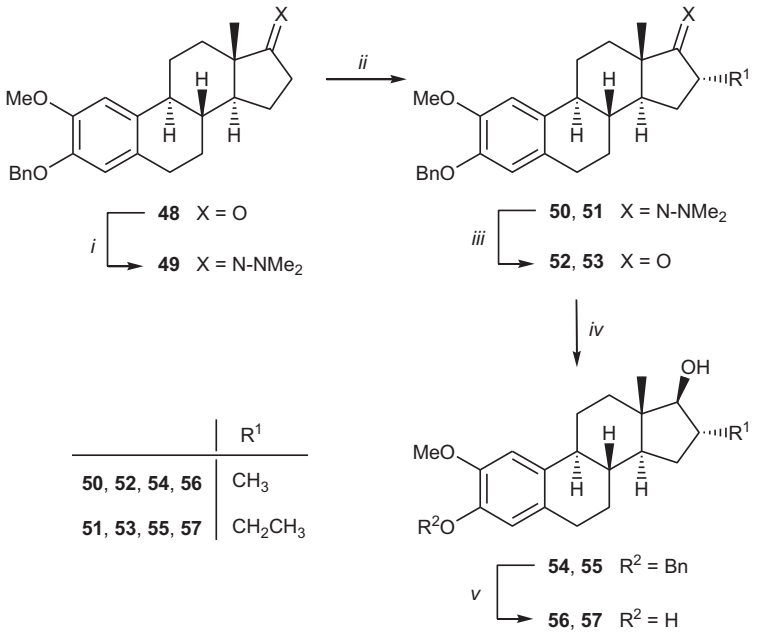


Scheme 4. Synthesis of 3,17-*bis*-modified analogs of **2ME2**. *Reagents and conditions*: (*i*) Tf_2O, pyridine; (*ii*) $PdCl_2$, dppp, CO, HMDS, DMF, MeOH, then acidic workup; (*iii*) $Pd(OAc)_2$, *rac*-BINAP, Cs_2CO_3, benzophenone imine, toluene, then 2 M HCl, THF; (*iv*) BrCN, Et_2O, CH_2Cl_2.

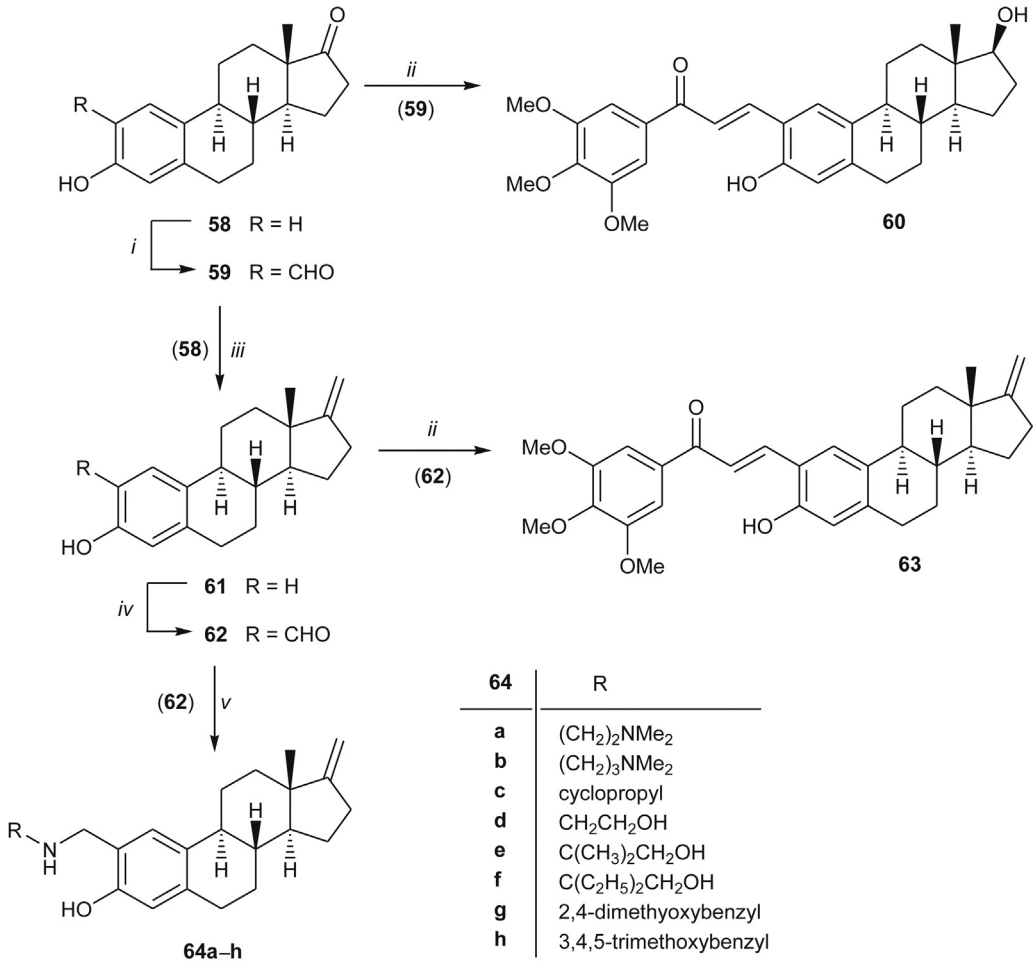
growth inhibition, but carboxamide analogs **40** and **41** were found to be 3- to 6-fold more potent in antiproliferative activity and 3- to 5-fold more potent in antiangiogenic activity as compared with **2ME2**. Moreover, all the carboxamides **40**–**42** possessed significantly better *in vivo* pharmacokinetic properties in mice than those of **2ME2**.

Some 3,17-*bis*-modified analogs of **2ME2** containing sulfamate group(s) at position(s) 3 and/or 17 were also found to display potent antiproliferative effects [36–38]. These agents inhibit steroid sulfatase, carbonic anhydrase, cancer cell proliferation and angiogenesis. Replacement of OMe with an ethyl group on C-2 led to derivatives with increased potency.

It has been demonstrated that substitution of C-16 in **2ME2** with small alkyl groups leads to active derivatives [39]. The addition of steric and/or electronic bulk at this position was assumed to prevent or slow oxidation and/or conjugation at position 17 and consequently improve the antiangiogenic and antitumor effects observed for **2ME2** *in vivo*. For the transformations, **2ME2** was first



Scheme 5. Synthesis of 16-modified analogs of **2ME2**. *Reagents and conditions*: (*i*) NH_2NMe_2, EtOH, DMF; (*ii*) *n*BuLi, THF, R^1I; (*iii*) $CuCl_2{\cdot}2H_2O$; THF/H_2O; (*iv*) $LiAlH_4$; THF; (*v*) H_2, Pd/C, EtOAc.



Scheme 6. Synthesis of 2-modified and 2,17-bis-modified analogs of **2ME2**. *Reagents and conditions*: (*i*) (1) $NaBH_4$, THF, MeOH, (2) EtMgBr, $(CH_2O)_n$, THF, HMPA; (*ii*) $ArCOCH_3$, MeOH, KOH; (*iii*) $CH_3P^+Ph_3I^-$, NaH, DMSO; (*iv*) EtMgBr, $(CH_2O)_n$, THF, HMPA; (*v*) (1) RNH_2, Na_2SO_4, DMF, (2) $NaBH_4$, MeOH.

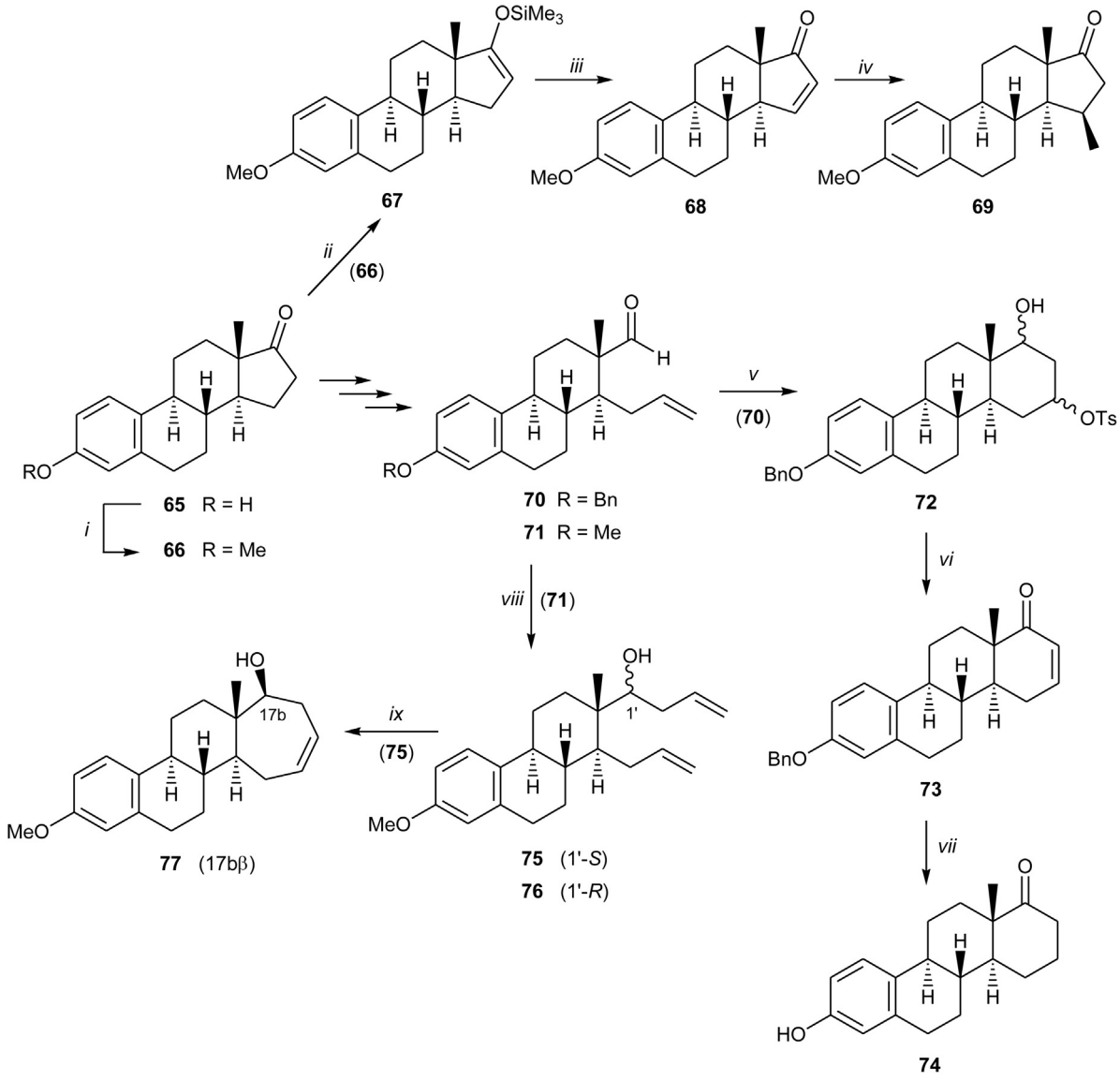
protected as benzyl ether with K_2CO_3/benzyl bromide in ethanol, and then oxidized to 3-*O*-benzyl-2-methoxyestrone (**48**) under Swern conditions [40]. The 16-substituted compounds **56** and **57** were prepared from **48** as starting material in multistep pathways (Scheme 5). Methyl and ethyl substitution were well tolerated for antiproliferative activity against MDA-MB-231 breast tumor cells, but **56** was found to be more estrogenic than **2ME2**.

Since steric and electronic factors at position 2 are prominent contributors to the cytotoxicity and the antitubulin activity of **2ME2** and its analogs [41], certain 2-substituted and 2,17-*bis*-modified analogs were prepared in an effort to improve the biological activity of **2ME2** (Scheme 6) [42]. Among the various synthesized compounds, some chalcone (**60**, **63**) and 2-alkylamino derivatives (**64a**–**h**) were found to exhibit *in vitro* antiproliferative activities comparable with that of the parent analog against four human cancer cell lines [HCT-116 (colon), NCIH-460 (lung), U-251 (glioma), MB-435 (breast)].

The above-mentioned results led to further investigations being directed toward the synthesis of novel estrane-type antiproliferative agents. Thus, 15β-methylestrone-3-methyl ether (**69**), available from estrone (**65**), was demonstrated to inhibit the viability of MGC-803 (human gastric) cancer cells, similarly to doxorubicin (Scheme 7) [43]. The key step of the reaction sequence involved the stereoselective conjugate addition of a methyl group derived from Me_3Al into **68** under CuBr catalysis. Zupko et al. have reported that a D-ring-expanded analog of estrone, compound **74**, which can be synthesized in three steps from a D-secoestrone-aldehyde **70**, exerts a cytostatic effect on HeLa (human cervix epitheloid carcinoma) cells that is similar to that of cisplatin, but its selectivity toward noncancerous cells is significantly better than that of the reference compound [44]. Moreover, *in vitro* pharmacological studies confirmed modulation of the cell cycle progression through the promotion of several steps of apoptosis, and the lack of significant estrogenic activity [25]. D-Dihomologation by Barbier allylation – ring-closing metathesis, however, led to a derivative **77** with merely moderate antiproliferative activity [45].

3. Synthesis and antiproliferative activity of androstane-derived compounds

As compared with the estrane-derived semisynthetic molecules, for fewer examples are to be found in the literature as concerns the synthesis of simpler substituted androstane derivatives that exert cytostatic activity. Although dehydroepiandrosterone (**78**), the most abundant adrenal androgenic steroid in young adult humans, and its endogenous metabolites have been demonstrated to possess chemopreventive and antiproliferative effects [46,47], clarification of the exact mechanisms of their action is still required. Nevertheless, intensive searches for more potent and less androgenic androstane derivatives are ongoing. In this respect, the synthetic 3-ester **80** and 3-ether analog **81** of **78** have been found to exert promising antiproliferative activity toward a prostate cancer cell line (DU-145) associated with a better antiandrogenic effect



Scheme 7. Synthesis of modified estrone derivatives with antiproliferative activity. *Reagents and conditions*: (*i*) $Bu_4N^+I^-$, MeI, CH_2Cl_2, NaOH; (*ii*) $(iPr)_2NH$, THF, *n*BuLi, Et_3N, TMSCl; (*iii*) $Pd(OAc)_2$, CH_3CN, CH_2Cl_2; (*iv*) Me_3Al, CuBr, TMSCl, THF; (*v*) *p*-TsOH, CH_2Cl_2; (*vi*) Jones reagent, acetone; (*vii*) H_2, Pd/C, EtOAc; (*viii*) allyl bromide, Zn, THF; (*ix*) (NHC)$(PCy_3)Cl_2Ru$=CHR, CH_2Cl_2.

than that of the reference control finasteride (Scheme 8) [48]. Djurendić et al. [49] investigated the cell growth-inhibitory effects of some D-homolactone derivatives of **78**, and a 6-nitro compound **84** proved to show high selectivity toward a prostate cancer cell line (PC-3) and low toxicity against normal fetal fibroblasts (MRC-5).

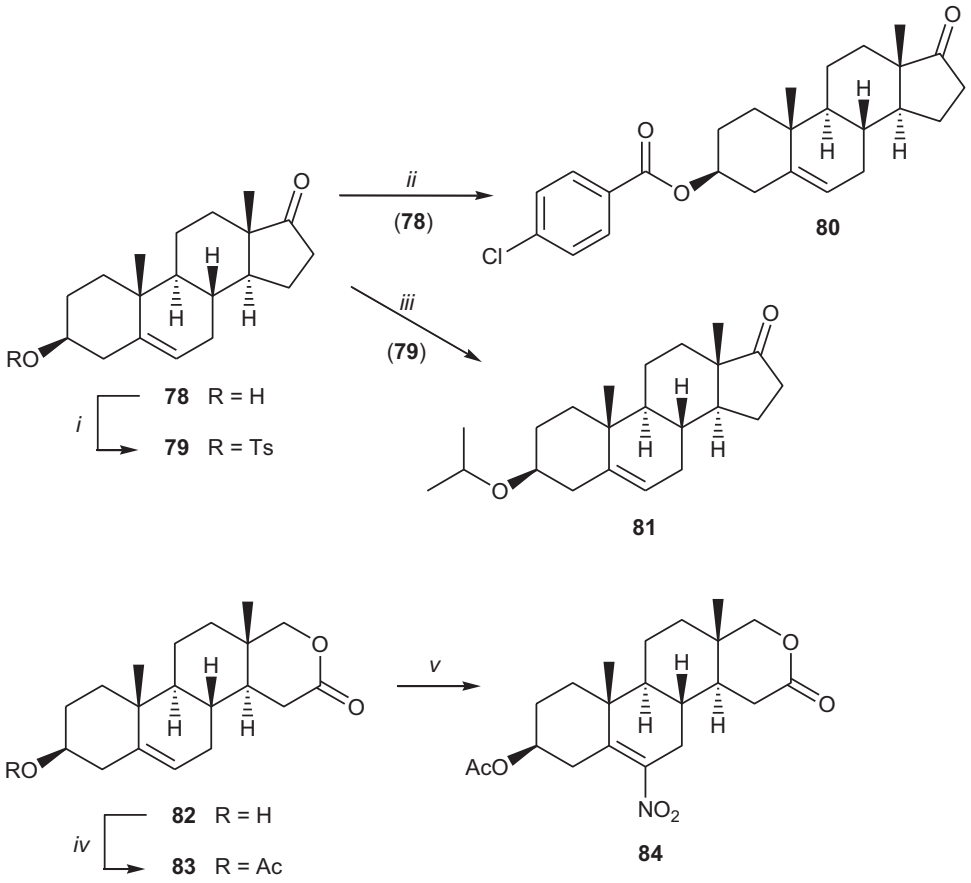
Some 7α-methyl-substituted Δ^4-3-ones (**88** and **94**), synthesized in a highly stereoselective manner from their dienone precursors (**87** and **93**) by 1,6-conjugate addition, were reported to exhibit similar cytostatic activities on gastric cancer cell line MGC-803 to that of the reference doxorubicin (Scheme 9) [43].

4. Synthesis and antiproliferative activity of hormone-derived heterocyclic compounds

Besides the introduction of simple substituents onto the sterane skeleton, the construction of heterocyclic moieties either connected to or condensed with the original framework offers another possibility of obtaining analogs possessing antitumor activity. The most frequent synthetic modifications are performed on C-3, C-17 or C-20, or on the carbon atoms adjacent to the already substituted C-3, C-17 or C-20 due to the simple implementation. Substitution at other positions of the sterane skeleton has proved to be more difficult, necessitating several reaction steps, and is therefore rarely applied. Steroidal heterocycles containing various numbers of ring nitrogen atoms will be discussed bellow.

4.1. Steroidal heterocycles containing one ring nitrogen atom

Compounds that inhibit the enzyme aromatase have found applications in the treatment of advanced ER+ breast cancer. In recent years, highly potent and specific aromatase inhibitors have been developed [50,51]. A nonsteroidal inhibitor of aromatase, rogletimid (3-ethyl-3(4-pyridyl)piperidine-2,6-dione), contains a pyridine ring as an important constitutive element [52]. Accordingly steroidal 17-picolyl **96** and 17-picolinylidene **97** and **98** derivatives were synthesized from dehydroepiandrosterone (**78**), and their Oppenauer oxidation led to the corresponding Δ^4-3-ketosteroids **100** and **103** (Scheme 10), which similarly proved to be



Scheme 8. Synthesis of modified dehydroepiandrosterone analogs with antiproliferative activity. *Reagents and conditions*: (*i*) *p*-TsCl, pyridine; (*ii*) 4-chlorobenzoyl chloride, pyridine; (*iii*) *i*PrOH; (*iv*) Ac_2O; pyridine; (*v*) HNO_3, $NaNO_2$, Et_2O.

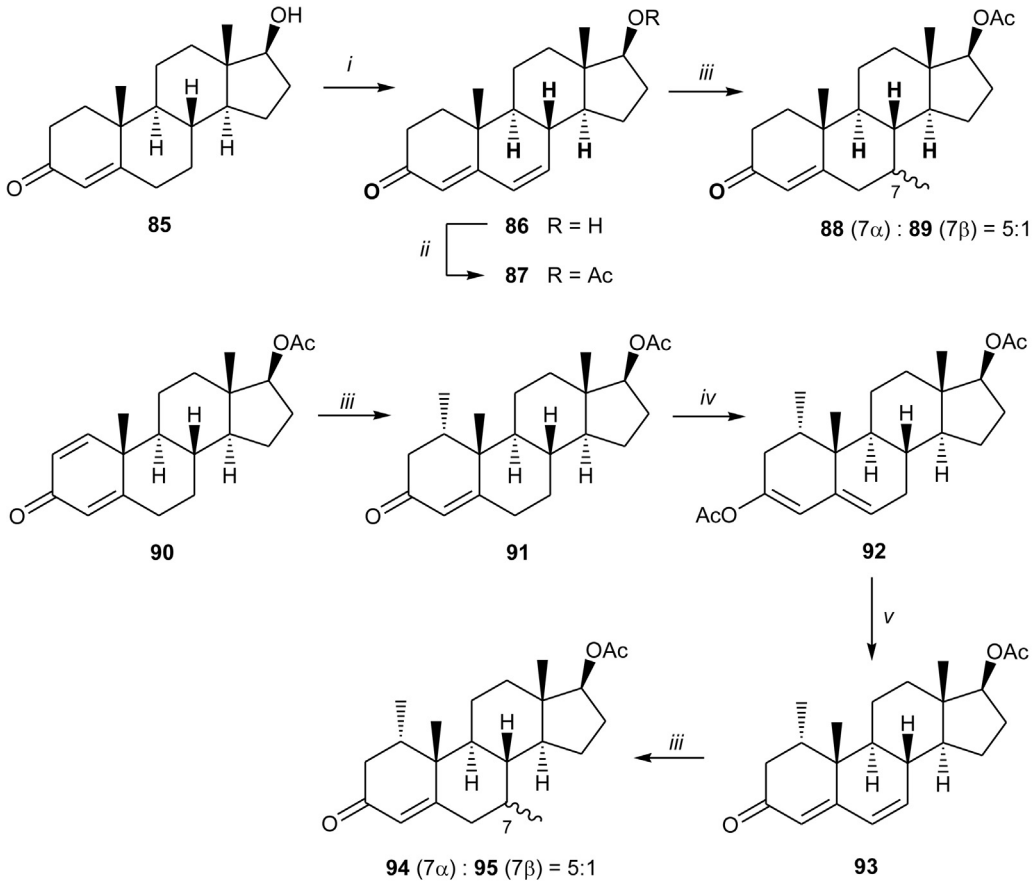
aromatase inhibitors [53]. In investigations of the relation between the aromatase inhibitory effect and the antitumor activity, therefore, some modified derivatives were tested on three tumor cell lines: MCF-7 (ER+ human breast adenocarcinoma), MDA-MB-231 (ER− human breast adenocarcinoma) and PC3 (prostate cancer). Both the *N*-oxides **99** and **104** prepared from **97** and **103**, respectively, by oxidation with *m*-chloroperoxybenzoic acid (MCPBA), and also the starting material **96** were found to exert strong antiproliferative activity against PC3, with IC_{50} values in the range 0.55–10 μM, while the 4β,5β-epoxide **102** obtained from **100** on reaction with H_2O_2 in alkaline medium displayed a marked cell growth-inhibitory effect against MDA-MB-231 [54]. It additionally emerged that the 16-keto analogs of **97** and **98** [55,56] effectively inhibited the proliferation of HeLa, FemX (human melanoma) and K562 (human myelogenous leukemia) cells [53].

Besides picolyl derivatives, various steroidal isoxazolidines and isoxazoles were synthesized and investigated for their antiproliferative activity. The thermally induced intramolecular 1,3-dipolar cycloadditions of a D-secopregnene aldehyde (**105**) (obtained from pregnadienolone acetate [57]) with *N*-substituted hydroxylamines furnished *N*-functionalized isoxazolidines **107** diastereoselectively, *via* the corresponding alkenyl nitrones **106** (Scheme 11). When the cytostatic effects of the 3-deacetylated compounds **108** were tested *in vitro*, the *N*-benzyl-substituted derivative (R^2 = Bn) displayed the highest activity on HeLa cells, with an IC_{50} value comparable to that of the reference cisplatin [58].

Steroidal 17α-isoxazoles in the estrane series were also prepared by 1,3-dipolar cycloaddition (Scheme 12). The CuI-catalyzed regioselective ring closures of different aryl nitrile oxides with mestranol (**109**) afforded the *exo*-heterocyclic products **110** in good to excellent yields [59,60]. These novel isoxazolyl derivatives, which lack an estrogenic effect [61], were subjected to *in vitro* pharmacological studies in order to investigate their antiproliferative effects on three malignant human gynecological cell lines. The fluorinated analog **110** (R = *p*-F) and the *p*-nitro-substituted derivative **110** (R = *p*-NO_2) proved to be the most promising compounds as concerns cell growth inhibition.

4.2. Steroidal heterocycles containing two ring nitrogen atoms

Investigations of the antiproliferative activities of this type of compounds have mainly focused on derivatives containing a pyrazoline moiety. An efficient and facile synthesis of 17β-pyrazolinyl derivates was reported by Banday et al. [62]. The reaction sequence involved the conversion of pregnenolone (**111**) to benzylidene derivatives **112** with (substituted) benzaldehyde(s), and the subsequent ring closures of **112** with hydrazine in the presence of acetic acid (Scheme 13). Although the newly formed stereogenic center at C-5′ can allow the formation of two epimers (**113** and **114**), the products were tested as the unseparated mixtures on seven human cancer cell lines [HT-29, HCT-15 (colon), 502,713 (colon), HOP-62, A-549 (lung), MCF-7 (breast) and SF-259 (CNS)]. The IC_{50} values of the diastereomeric products (**113** + **114**) indicated that some of the compounds (R = *m*-F, *p*-F, *o*-CH_3, *m*-CH_3 and *p*-OMe) exerted significant cytotoxic activity, especially against the colon cell lines. Moreover, the cytotoxicity of the analogs for certain cancer cell lines varied more than 100-fold on change of the position of a given substituent on the aromatic ring (*e.g.* R = *o*-OMe *vs.* R = *p*-OMe). Following the synthetic route indicated above, Iványi et al. prepared further pyrazolinyl derivatives, which could be separated,



Scheme 9. Synthesis of some methyl-substituted testosterone derivatives. *Reagents and conditions*: (*i*) tetrachlorobenzoquinone, *t*BuOH; (*ii*) Ac_2O, DMAP, CH_2Cl_2; (*iii*) Me_3Al, CuBr, TMSCl, THF; (*iv*) Ac_2O, AcCl; (*v*) NBS, LiBr, Li_2CO_3.

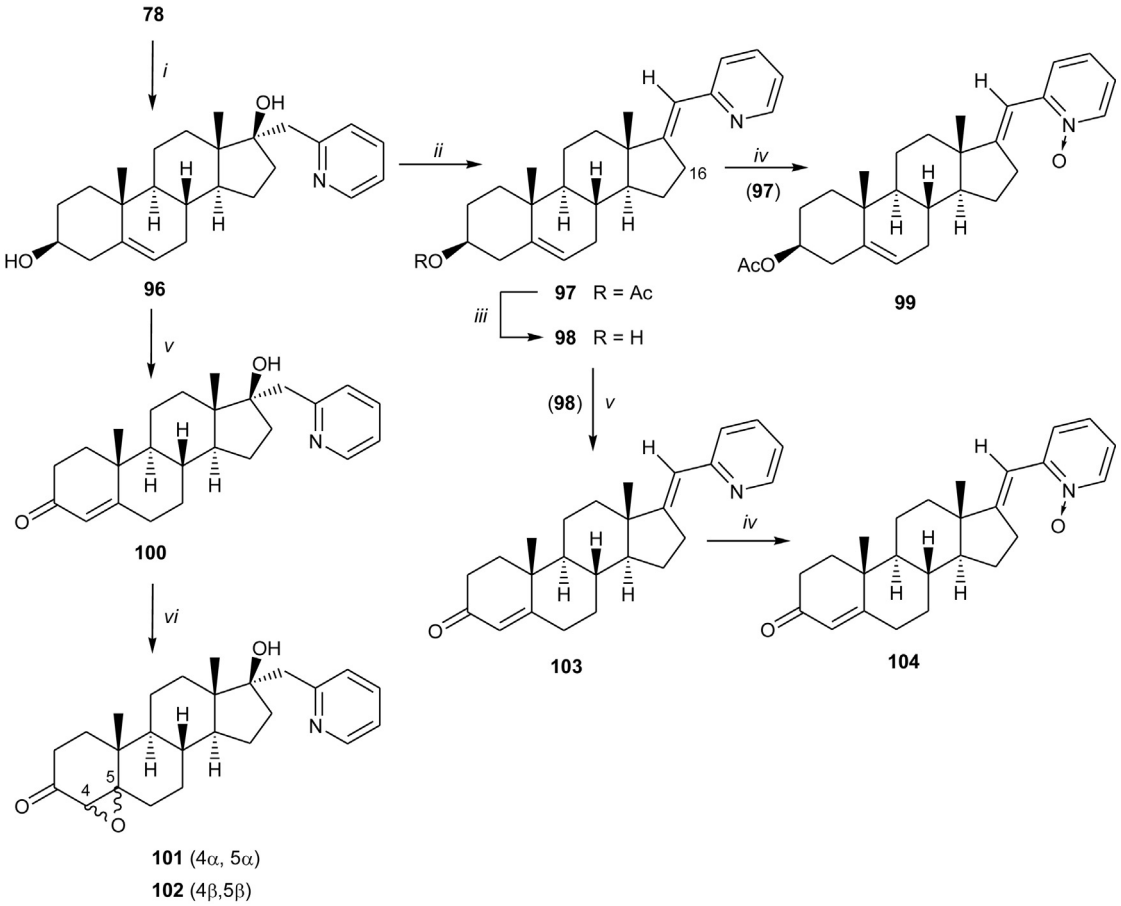
but only in their acetylated forms (**115** and **116**) [63]. The deacetylated isomers **113** and **114** were tested separately on four cancer cell lines: HeLa (cervix), MCF7 (breast), A2780 (ovarian) and A431 (skin), but their antiproliferative potencies were not comparable with those of the reference cisplatin. However, it should be noted that substantial differences were found in the *in vitro* results on the diastereomeric pairs: the 5′*S* epimers **114** were more potent than their 5′*R* counterparts **113**.

As regards the six-membered *N*,*N*-heterocycles, Poirier et al. [64,65] reported the synthesis of a large set of 2β-(*N*-substituted piperazino)-5α-androstane derivatives and several compounds were found to inhibit the proliferation of HL-60 human leukemia cells.

Besides simple heteroring substitution, another possibility for the design of cytostatic steroidal agents is the construction of molecules structurally similar to natural steroid alkaloids. Plants are among the most varied and promising sources of new anticancer drugs, as they contain compounds which are produced by specific enzyme-catalyzed processes that differ significantly from those in animals and humans. Some of these alkaloid analogs are known to have cytotoxic properties [66] or to act as chemosensitizers in multidrug resistance [67]; consequently, structurally related compounds may have similar or even improved biological activity. In this respect, an efficient approach has been reported to novel androstene-fused arylpyrazolines that can be regarded as synthetic solanidine analogs, and these compounds were tested for their antiproliferative activities [26,68]. The highly diastereoselective Lewis acid-induced intramolecular 1,3-dipolar cycloadditions of alkenylphenylhydrazones **117** obtained from D-secopregnene aldehyde **105** resulted in D-ring-fused products **118** in good to excellent yields (Scheme 14). The ability of phenylhydrazones **117** to undergo cyclization was found to be affected significantly by the electronic features of the substituents (R^1) on the aromatic moiety. Both the experimental findings and theoretical calculations indicated that the cycloadditions follow a stepwise rather than a pure concerted mechanism. The antiproliferative activities of the pyrazoline derivatives **118** and **119** were tested *in vitro* on three malignant human cell lines (HeLa, MCF7 and A431): the microculture tetrazolium assay (MTT) revealed that several compounds exerted marked cell growth-inhibitory effects. The highest cytostatic activities, displayed by the *p*-methoxyphenylpyrazoline derivative **119** (R^1 = *p*-OMe), were better than those of cisplatin. Moreover, this compound inhibited the proliferation of normal human fibroblast cells (MRC-5) at a one order of magnitude higher concentration (IC_{50} = 17.01 μM) than for HL-60 leukemia cells (IC_{50} = 1.27 μM), reflecting its efficiently selective cytotoxic activity [69]. Further pharmacological studies indicated that **119** (R^1 = *p*-OMe) promoted a marked disturbance in the cell cycle, and induced the manifestation of the markers of early apoptosis and secondary necrosis, which was in part a consequence of its inhibitory effect on the activity of ribonucleotide reductase.

4.3. Steroidal heterocycles containing three ring nitrogen atoms

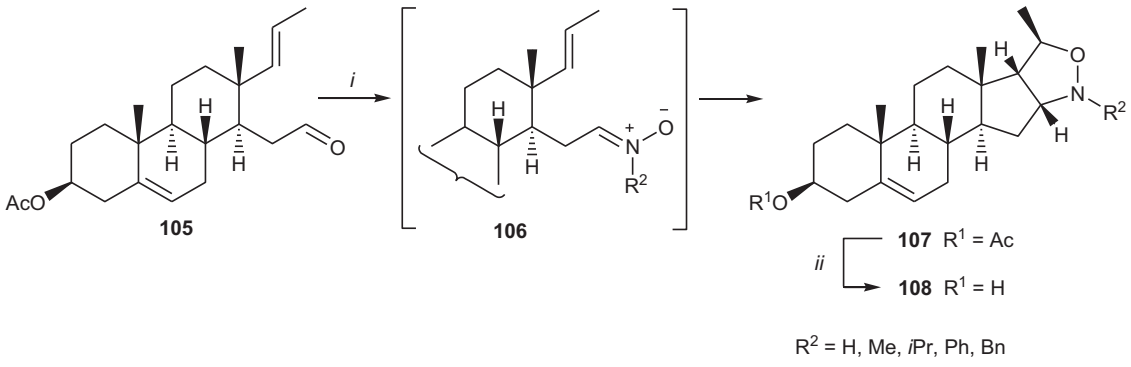
Among the heterocyclic systems, triazole often demonstrates some special activity when it is introduced into biological active compounds [70–72]. Moreover, the basicity and hydrophilicity of this moiety might alter the pharmacological function of a steroid. With regard to these facts, therefore, a number of steroidal triazoles have been synthesized during the past few years, through



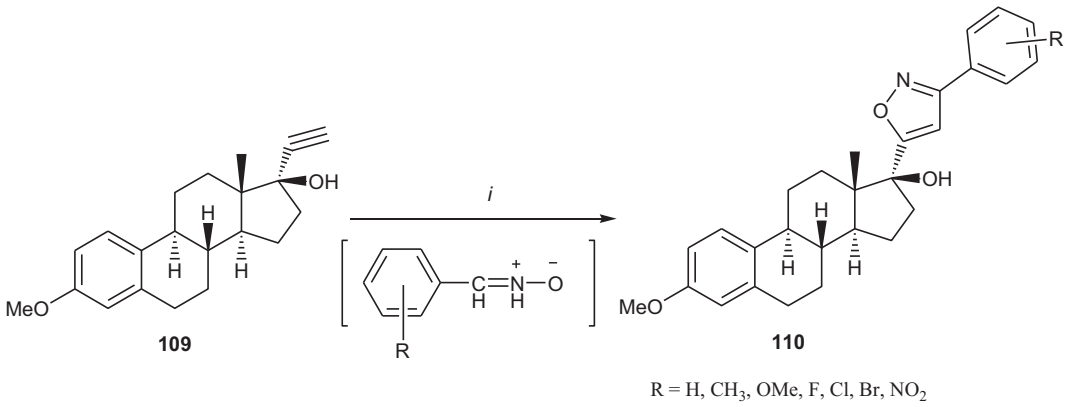
Scheme 10. Synthesis of some picolyl-substituted derivatives. *Reagents and conditions*: (*i*) α-$PyCH_2Li$, THF; (*ii*) Ac_2O; (*iii*) KOH, MeOH; (*iv*) MCPBA, CH_2Cl_2, $NaHCO_3$; (*v*) $Al(tBuO)_3$, cyclohexanone; (*vi*) H_2O_2, NaOH, MeOH.

a click chemistry approach. The synthetic routes involve the introduction of an azido group into any position of the sterane framework and the subsequent ring closure of the steroidal azide with a terminal acetylene in the presence of a Cu(I) source. Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) is convenient for the regioselective construction of 1,4-disubstituted triazoles in which the heterocycle is attached directly to the steroid nucleus through a nitrogen atom, or these two moieties are connected to each other through a linker. The reaction has further advantageous benefits, such as high yields of the desired products, tolerability to a variety of common parameters (functional groups, solvents, pH and temperature), the lack of by-products and insensitivity to the steric and electronic properties of the reactants [73–75].

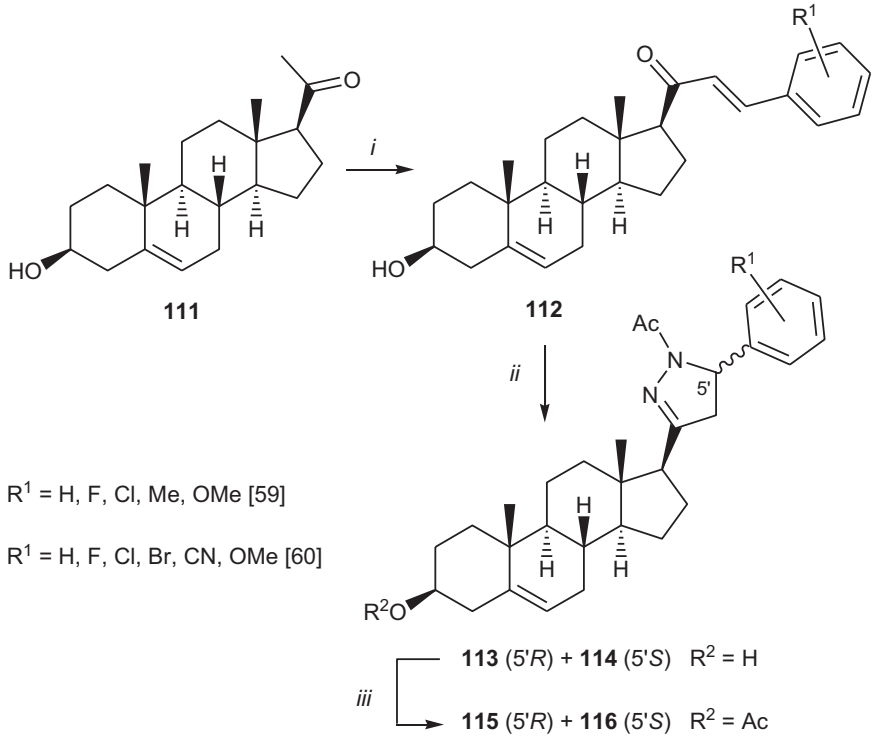
The simplest procedures for the formation of steroidal azides are either the modification of extant functional groups (C-3, C-17 or C-20) in the original hormone analog or substitution at adjacent positions (C-2, C-16 or C-21). Thus, Bandy et al. [76] recently reported a facile synthesis of 21-triazolyl derivatives of pregnenolone by using an *in situ* one-pot CuAAC (Scheme 15). In this case the triazolyl moiety is attached to the steroid nucleus through a methylene ketone linkage. The synthesis started from pregnenolone (**111**), which was treated with Br_2/MeOH in the dark to furnish 21-bromopregnenolone (**120**) in good yield. The *in situ* two-step conversion of **120** in the presence of NaN_3, $CuSO_4$, sodium ascorbate and the appropriate alkyne in a 1:1 mixture of *tert*-butanol and water resulted in 21-triazolylpregnenolone derivatives **121a–g**. These compounds were screened *in vitro* for



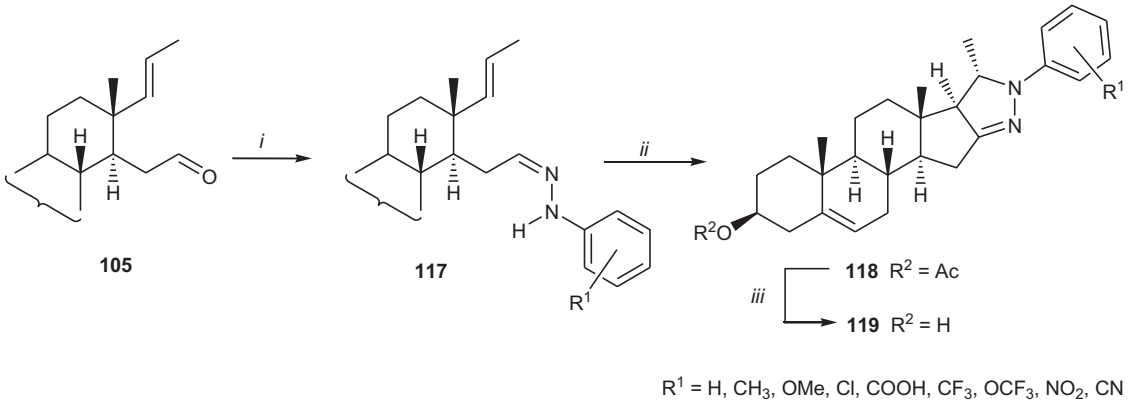
Scheme 11. Synthesis of D-ring-fused isoxazolidines by thermally induced 1,3-dipolar cycloaddition. *Reagents and conditions*: (*i*) R^2NHOH, NaOAc, MeOH; (*ii*) KOH, MeOH.



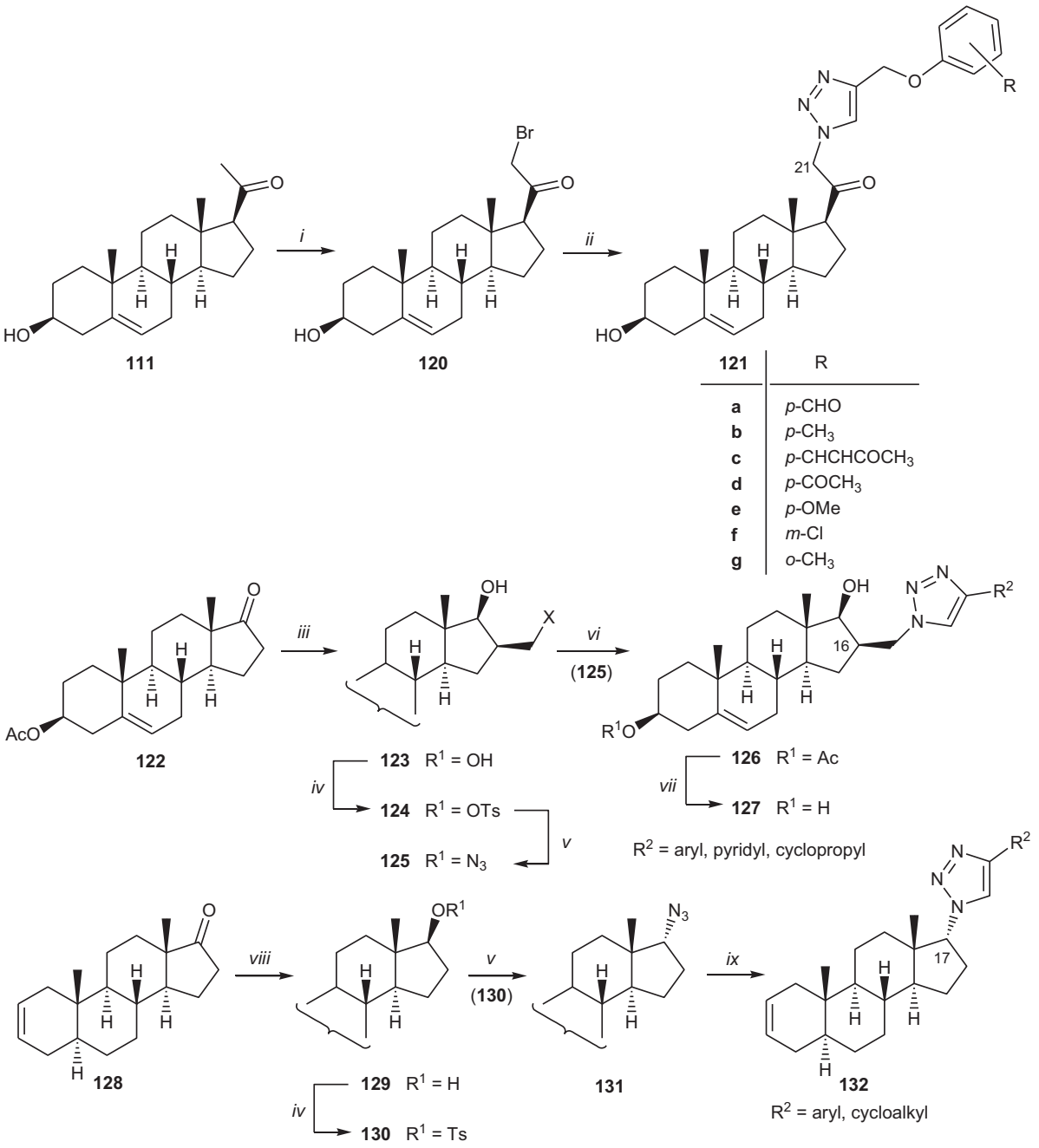
Scheme 12. Synthesis of 17α-isoxazoles by Cu(I)-catalyzed 1,3-dipolar cycloaddition. *Reagents and conditions*: (*i*) CuI, Ph_3P, DIPEA, toluene.



Scheme 13. Synthesis of 17-*exo*-pyrazolinyl derivatives. *Reagents and conditions*: (*i*) (R^1)benzaldehyde, EtOH, NaOH; (*ii*) AcOH, hydrazine hydrate; (*iii*) Ac_2O, pyridine.



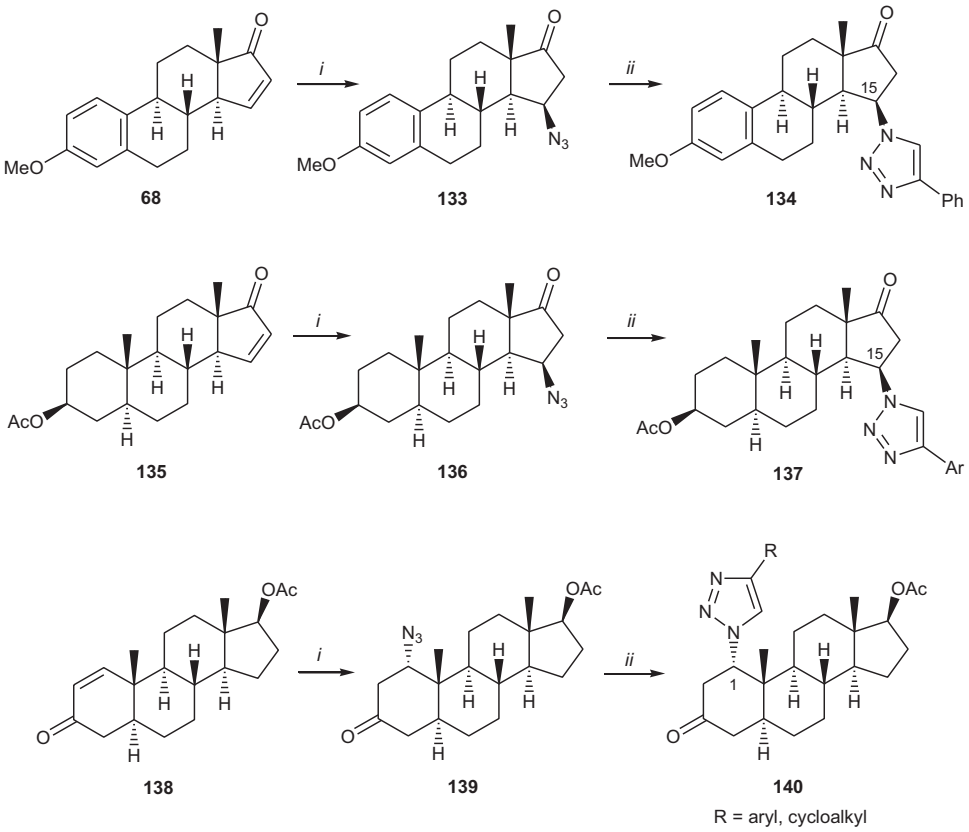
Scheme 14. Synthesis of D-ring-fused pyrazolines. *Reagents and conditions*: (*i*) (R^1)phenylhydrazine, MeOH; (*ii*) $BF_3{\cdot}OEt_2$, CH_2Cl_2; (*iii*) KOH, MeOH.



Scheme 15. Construction of triazolyl moieties at the site of an already existing functional group (C-17) or in nearby (C-16 or C-21) positions. *Reagents and conditions*: (*i*) HBr, Br_2, MeOH; (*ii*) NaN_3, $CuSO_4{\cdot}5H_2O$, Na ascorbate, *t*BuOH, H_2O, $HC{\equiv}CCH_2OC_6H_4R$; (*iii*) (1) ethyl formate, NaOMe, toluene, (2) Ac_2O, pyridine, (3) KBH_4, EtOH; (*iv*) TsCl, pyridine; (*v*) NaN_3, DMF; (*vi*) $R^2C{\equiv}CH$, $CuSO_4{\cdot}5H_2O$, Na ascorbate, CH_2Cl_2, H_2O; (*vii*) KOH, MeOH; (*viii*) KBH_4, MeOH, CH_2Cl_2; (*ix*) $R^2C{\equiv}CH$, CuI, Ph_3P, CH_2Cl_2.

their activity against seven human cancer cell lines, *i.e.* DU-145, PC-3 (prostate), SF-295 (CNS), HCT-15, 502713 (colon), HEP-2 (liver) and A-549 (lung), using the sulforhodamine B assay. The IC_{50} values revealed that **121e** (R = *p*-OMe) exerted significant cytotoxicity against the PC-3, HCT-15 and 502713 cell lines, while the other derivatives were found to be effective against all these diverse cancer cell lines.

Kádár et al. [77] recently prepared 1,2,3-triazolyl derivatives in the androstane series in which the heterocycle is connected to C-16 of the sterane framework through a methylene group. The Claisen condensation of 3β-acetoxyandrost-5-en-17-one (**122**) with ethyl formate in the presence of NaOMe and the subsequent acetylation/reduction sequence led to three 3β-acetoxy-16-hydroxymethyl-17-diols, from which the 16β-hydroxymethyl-17β-hydroxy isomer **123** was separated (Scheme 15). After conversion of the primary hydroxyl group of **123** to *p*-toluenesulfonate derivative **124**, nucleophilic substitution with NaN_3 was carried out to furnish the corresponding azide **125**. Various aryl, heteroaryl and cycloalkyl-substituted triazoles **126** were then synthesized by the CuACC of **125** with various terminal alkynes, $CuSO_4{\cdot}5H_2O$ serving as Cu(I) source in reductive medium. For the cyclizations, a mixture of dichloromethane as solvent and water as co-solvent was employed to eliminate the need for ligands and to simplify the reaction protocol [78]. The antiproliferative activities of the deacetylated analogs (**127**) were determined *in vitro* with the MTT assay on three gynecological cell lines (HeLa, MCF-7 and A2780). Compound **127**, containing a 3-methylphenyl group on the triazolyl moiety, was found to exert a marked cell



Scheme 16. Construction of triazolyl moieties at unconventional (C-1 and C-15) positions. *Reagents and conditions*: (*i*) NaN_3, AcOH, THF; (*ii*) (1) KBH_4, MeOH, (2) PhC≡CH (for **134**), ArC≡CH (for **137**) or RC≡CH (for **140**), CuI, Ph_3P, DIPEA, (3) Jones reagent, acetone.

growth-inhibitory effect on A2780 cells, while the 2-pyridyl derivative proved effective on HeLa cells.

The preparation of novel 17α-triazoles in which the heterocycle is attached directly to ring D of the sterane core has also been reported recently [24]. For the CuAACs, steroidal 17α-azide **131**, readily available from 5α-androst-2-en-17-one (**128**) in a three-step pathway, was used as starting material (Scheme 15). Stereoselective reduction of the 17-keto group, leading to **129**, was followed by tosylation to give **130**, which then underwent facile S_N2 substitution with NaN_3 to furnish the corresponding 17α-azido compound **131**. The ring closures of **131** with different terminal acetylenes were carried out in refluxing dichloromethane with CuI as catalyst in the presence of Ph_3P. Although determination of the affinities for the hormonal receptors did not fall within the scope of the work, in the absence of a keto functional group at position C-3, the newly prepared triazolyl derivatives were considered not to possess an androgenic effect. The *in vitro* pharmacological tests indicated that **132**, containing a cyclopropyl group (R^2) on its heterocycle, possessed the highest activity, causing 82–98% growth inhibition on all malignant cell lines (HeLa, MCF7 and A431) at 30 μM, and was therefore comparable to the reference compound cisplatin. Interestingly, the similar transformations in the estrane series led to 17α-triazoles that generally exhibited lower pharmacological activities.

Further triazolyl derivatives were synthesized in both the estrane and the androstane series, in which the triazolyl moiety was situated at the unconventional C-15 position [79]. First, an azido group was introduced stereoselectively onto position 15β of sterane skeleton of the unsaturated ketones **68** and **135** by the 1,4-Michael addition of azoimide [80], generated *in situ* from NaN_3 and acetic acid, to afford azidoketones **133** and **136** in good yields (Scheme 16). After subsequent reduction of the 17-keto group in order to avoid elimination, the resultant steroidal *cis*-azidoalcohols were reacted with different terminal alkynes. The best conversions were observed on the use of a catalytic amount of CuI with Ph_3P as stabilizing ligand and excess *N*,*N*-diisopropyl ethylamine (DIPEA) as amine base in refluxing toluene. The following Jones oxidation afforded the corresponding 17-keto 15β-triazolyl derivatives **134** and **137** in both the estrane and the androstane series. The antiproliferative activities of the synthesized compounds were determined by using three malignant adherent cell lines (HeLa, MCF7 and A431) in the MTT assay, in comparison with cisplatin as positive control. 15β-Triazolylestrone methyl ether (**133**) and its 17-hydroxy analog exhibited marked antiproliferative effects against A431 cells, while the 5α-androstane counterpart (**137**) exerted similar or higher activities on HeLa and MCF7 cells than these of cisplatin. Since substantial differences were not detected between the IC_{50} values of the different aryl-substituted derivatives of **137**, it was concluded that the substituent on the triazolyl ring does not have a crucial impact on the anticancer capacities of this skeleton. Further biochemical and morphological results indicated that the initiation of apoptosis predominated in the antiproliferative action of the tested compounds.

After a systematic study of D-ring-substituted triazolyl compounds, a number of analogous derivatives were prepared in which the triazoles were connected to ring A [81]. Starting from unsaturated ketone **138**, an azido group was introduced onto position 1 of the sterane skeleton by Michael addition, which resulted stereoselectively in the 1α-azido isomer **139** due to the steric bulk of the neighboring angular methyl group. 1α-Triazolyl-17-oxo derivatives **140** were obtained in a rather roundabout way following the reduction– CuAAC–oxidation sequence. These derivatives exerted outstanding cytotoxic activity on HeLa cells, characterized by IC_{50} values between 1 and 2 μM, *i.e.* one order of magnitude lower than

that of the reference cisplatin. On the other hand, the cell lines MCF7 and A2780 seemed to be much less sensitive to **140** than to cisplatin.

5. Summary

The routes available to date for the synthesis of sex hormone-derived steroidal compounds that display marked cell growth-inhibitory effects on one or more cancer cell lines of diverse origins have been discussed. The results achieved so far demonstrate that the introduction of either simple substituents or heterocyclic moieties may alter the biological activities of the parent compounds significantly, and the application of suitable modifications can eliminate the undesirable hormonal properties. Thus, both structure-based drug design and a more random search for effective derivatives appear to deserve attention in the quest for novel steroidal anticancer agents.

Acknowledgments

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