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Review

Synthesis and Pharmacological Properties of Modified A- and D-Ring in Dehydroepiandrosterone (DHEA): A Review

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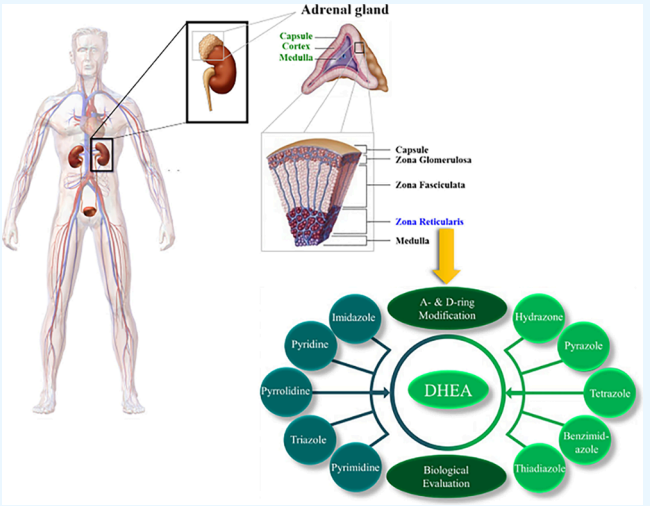
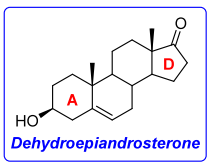
ABSTRACT: Dehydroepiandrosterone (3β-hydroxyandrost-5-en-17-one) (DHEA) is a naturally occurring steroid hormone primarily produced in the zona reticularis of the human adrenal glands. It serves as a crucial precursor for sex hormones, such as testosterone, estradiol, and androstenedione. Recent findings indicate that DHEA serves as the primary source of sex steroids in women during both pre- and postmenopausal stages. Additionally, a decline in DHEA levels with age is linked to various hormone-deficiency symptoms. Despite the wide array of biological activities that make DHEA a valuable polycyclic natural steroid, particularly for pharmaceutical and cosmetic applications, reports suggest that oral DHEA has limited clinical effect. Thus, A- and D-ring modified DHEA are synthesized and their biological activities are carried out by different research groups and enhanced biological activity reported in the literature. Here, in this review, we have tried to cover all of the synthetic routes and biological studies of modified A- and D-ring DHEA from 2015 to mid-2022.



1. INTRODUCTION

Steroids are naturally occurring polycyclic molecules with a broad spectrum of biological and physiological activities and are mostly found in plants, animals, and humans.[1] So they have been used as physiological regulators, hormones, anesthetic agents, antitumors, and cardiotonic. These make the steroidal drugs high-impact classes of pharmaceuticals. Recent studies revealed that more than 60% of anticancer drugs are formulated from natural sources.[2] Even a small modification in the steroidal skeleton especially the incorporation of heterocycles, replacing one or more carbon atoms with heteroatoms, change in the steroidal side chain, and substitution of the steroidal skeleton[3] results in a great alteration in biological activity which further responsible for the development of novel synthetic derivatives with antibacterial, antiproliferate, anti-inflammatory, and anticancer activity.[4]



Dehydroepiandrosterone

Dehydroepiandrosterone (DHEA), also known as androsterone, is a naturally occurring endogenous steroid hormone precursor produced in the adrenal gland, the gonads, and the brain which is the most abundant hormone in humans. It has aroused many researchers' interest in recent years because they have been evaluated as potential antiaging, antibacterial, anticancer, antiviral agents, assimilation of protein, and CYP17 inhibitors.[5,6] Moreover, it has been proven that DHEA is useful in the protest against heart disease and diabetes, management of obesity, enhancing the immune system, and reducing cholesterol.[7] Also, there are reports on the antioxidant and anti-inflammatory properties of DHEA.

In this review, we aimed to analyze the literature and summarize the modified synthetic procedure on the A- and D-ring of DHEA to alter and enhance its biological activity by the different research groups to bring it to one place. This makes this review unique among reviews in this field. To the best of our knowledge, no other review in this field has focused on the synthetic aspect of the ring modification of DHEA; instead, only one exclusive review on its biological perspectives was published

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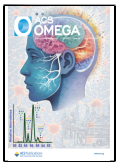


Scheme 1. Synthesis of DHEA-17-Hydrazone Derivatives 3−11

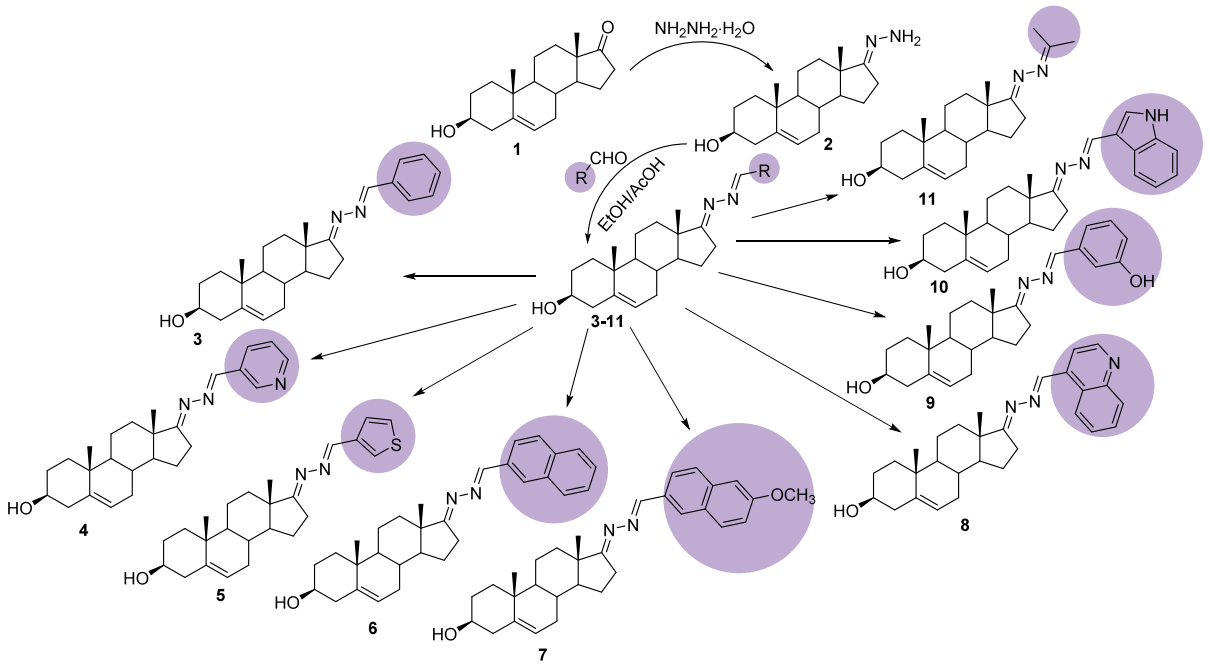
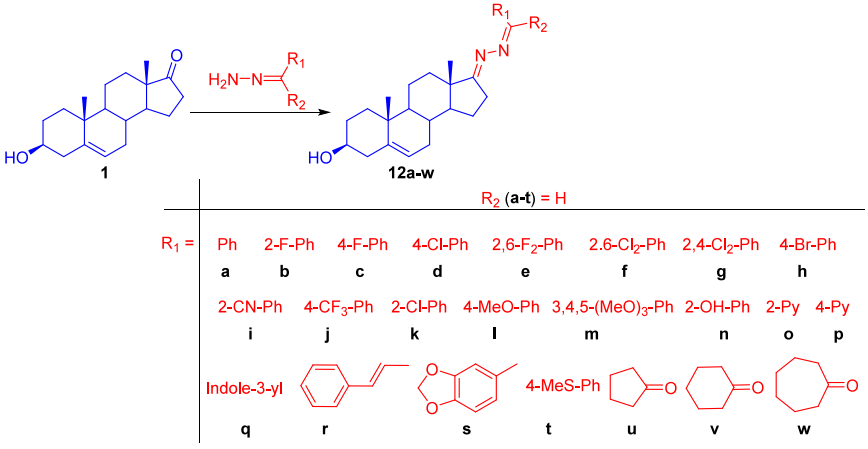


Table 1. IC_{50}[a] (μM) Values of DHEA-17-Hydrazone Derivatives against Various Cancer Cell Lines

s. no.	compounds	IC_{50}[a] (μM)			
		HeLa	HT-29	Bel-7404	SGC-7901
1.	**3**	69.2	12.8	15.3	15.3
2.	**4**	43.5	20.5	33.2	21.9
3.	**5**	35.4	29.2	33.9	40.4
4.	**6**	20.0	17.2	23.2	13.6
5.	**7**	46.8	12.7	33.2	46.8
6.	**8**	6.6	5.9	13.6	1.0
7.	**9**	18.8	21.3	23.8	17.1
8.	**10**	12.7	13.9	16.3	11.4
9.	**11**	>100	87.7	>100	>100
10.	Cisplatin	10.1	N.D.[b]	23.2	6.7

[a]The data represent the mean values from three independent determination. [b]N.D.: not determined.[9]

Scheme 2. Anti-flavivirus Hydrazone Derivatives based on DHEA Scaffold (12a−w)



in 2020.[8] Therefore, it is necessary to critically review the recent update on the synthesis and biological activities of the dehydroepiandrosterone derivatives. Further, we have also highlighted the synthetic reports in the literature on A- and D-ring modification by fusing heterocyclic rings, the transformation of carbonyl to another useful moiety, and modification for the side chain. These mini-reports can also

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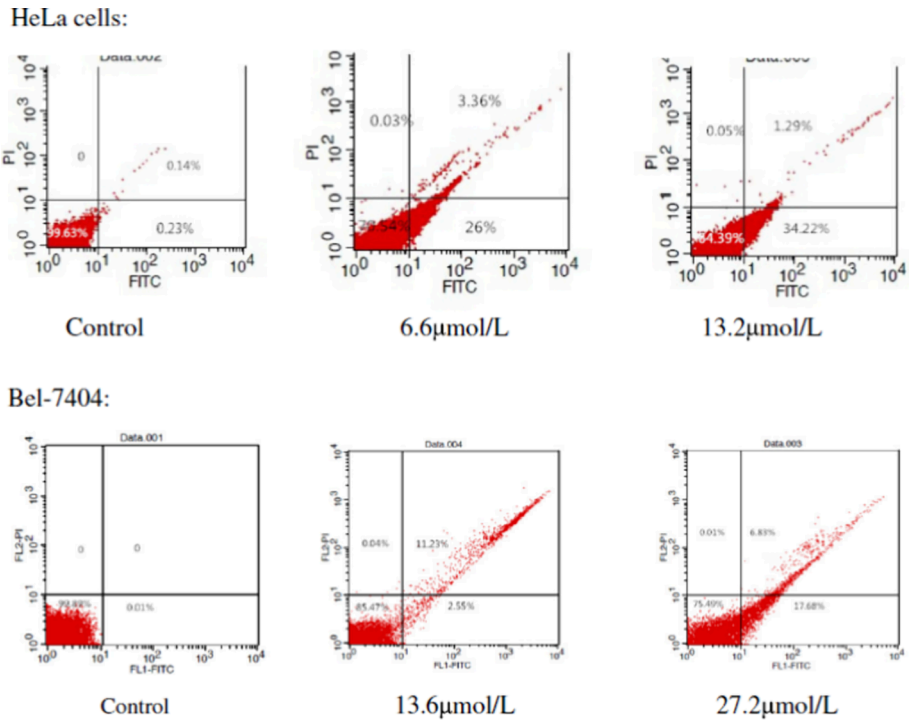
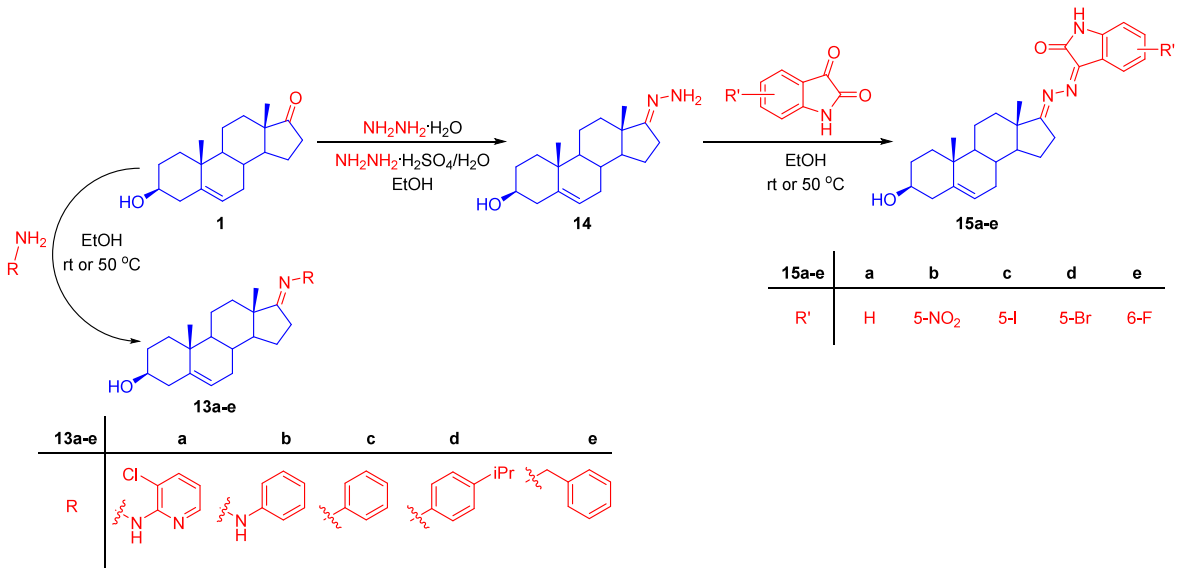


Figure 1. Tumor cells were double-stained with Annexin V/PI and analyzed using flow cytometry. A 24 h treatment with compound **8** induced apoptosis of cells. Reproduced with permission.[9] Copyright 2015, Elsevier.

Scheme 3. Synthesis of Novel DHEA Derivatives (13a−e and 15a−e)



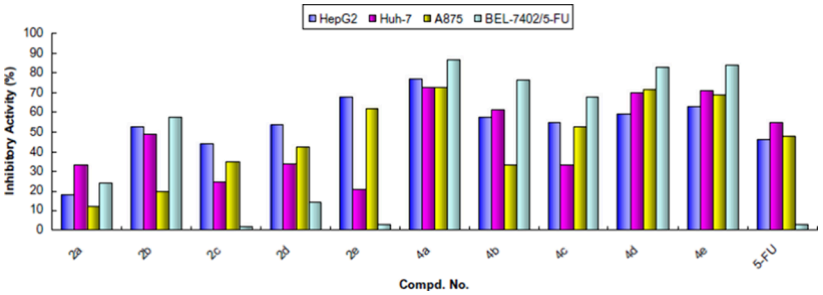


Figure 2. Antitumor activities of compounds **13a−e**[(2a-e)] and **15a−e**[(4a-e)] at 20 μg/mL. Reproduced with permission.[6] Copyright 2015, Elsevier.

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Table 2. IC_{50}[a] (μM) Values of the DHEA Derivatives against Cancer Cell Lines

s. no.	compounds	IC_{50}[a] (μM)			
		HepG2	Huh-7	A875	BEL-7402/5-FU
1.	**13a**	>90	>90	>90	N.T.[b]
2.	**13b**	43.14 ± 8.99	55.35 ± 4.73	>100	29.87 ± 7.53
3.	**13c**	63.45 ± 8.20	>100	82.74 ± 9.22	>100
4.	**13d**	44.31 ± 4.91	>90	61.53 ± 2.07	>90
5.	**13e**	32.60 ± 4.39	>100	42.48 ± 3.71	>100
6.	**15a**	13.33 ± 3.78	28.19 ± 3.59	25.23 ± 1.32	21.79 ± 4.87
7.	**15b**	27.43 ± 4.77	30.55 ± 3.91	69.00 ± 4.18	10.10 ± 3.04
8.	**15c**	22.54 ± 4.45	>70	43.72 ± 7.86	14.11 ± 3.59
9.	**15d**	16.22 ± 4.65	13.90 ± 3.91	14.83 ± 1.47	5.97 ± 2.67
10.	**15e**	19.72 ± 5.97	22.26 ± 6.21	29.59 ± 2.76	12.85 ± 4.88
11.	5-FU[c]	>150	>100	>150	>700

[a]Compounds concentration is required to inhibit tumor cell proliferation by 50%. [b]N.T.: no test.. [c]5-FU: used as a positive control.[6]

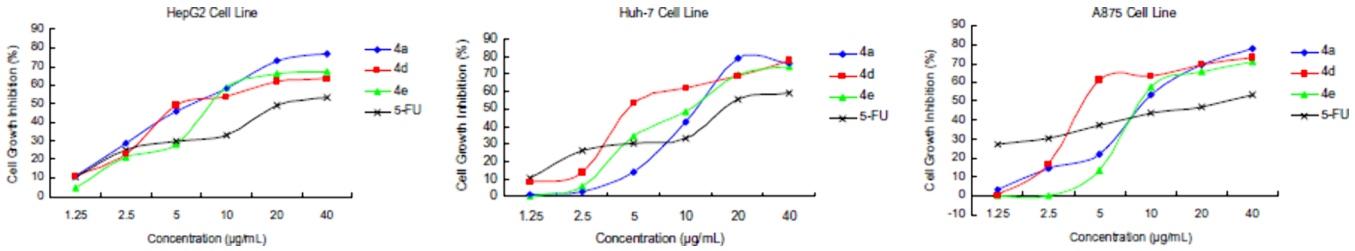

Figure 3. Dose−response analysis of cell growth inhibition activity for representative compounds **15a**$^{(4a)}$, **15d**$^{(4d)}$, **15e**$^{(4e)}$, and 5-FU (positive control) against HepG2, Huh-7, and A875 cell lines. Reproduced with permission.[6] Copyright 2015, Elsevier.

Table 3. IC_{50}[a] (μM) Value of the Compounds against Cancer Cell Lines

s. no.	compounds	IC_{50}[a] (μM)				
		MDA-MB-231	HCC1806	SKOV-3	DU145	HUVEC
1.	**18a**	0.28	2.22	0.61	0.22	0.17
2.	**18b**	0.26	1.71	0.50	0.25	0.17
3.	**18c**	2.43	>60	6.72	2.47	1.47
4.	**18d**	1.03	19.55	2.31	1.22	0.66
5.	**18e**	1.32	2.46	1.58	1.37	0.49
6.	**20b**	0.09	1.80	0.19	0.18	0.12
7.	**20c**	0.0014	1.03	0.0034	0.02	0.0035
8.	**20d**	0.04	1.34	0.15	0.10	0.05
9.	Furoxan **22**	1.62	2.97	2.25	0.65	1.42

[a]The data are the mean of triplicate determinations; IC_{50} is the concentration of sample for 50% cell growth inhibitory rate.[11]

Table 4. VEGF Inhibitory Activities of the Compounds 18a, 20b−d

s. no.	compounds	IC_{50} (μM)[a]	VEGF EC_{50} (μM)[a]	TI (IC_{50}/EC_{50})[b]
1.	**18a**	0.28	0.04	7.00
2.	**20b**	0.09	0.01	9.00
3.	**20c**	0.0014	0.0003	4.67
4.	**20d**	0.04	0.01	4.00
5.	**21c**	6.78	2.71	2.50
6.	Furoxan **22**	1.62	1.47	1.10
7.	2-ME	0.25	0.07	3.57

[a]The data are the mean of triplicate determinations. [b]EC_{50} is the concentration of sample for 50% VEGF inhibitory rate; IC_{50} is the concentration of sample for 50% cell growth inhibitory rate; and TI (therapeutic index) = IC_{50}/EC_{50}.[11]

advance the in vivo and in vitro biological studies carried out on modified DHEA.

2. SYNTHESIS AND PHARMACOLOGICAL PROPERTIES OF DEHYDROEPIANDROSTERONE (DHEA)

2.1. Hydrazone Dehydroepiandrosterone Derivatives.

In 2015, Cui and co-workers reported the series of dehydroepiandrosterone-17-hydrazone derivatives possessing

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Scheme 4. Synthesis of Novel Furoxan-based DHEA Derivatives (18a−e and 20b−d)

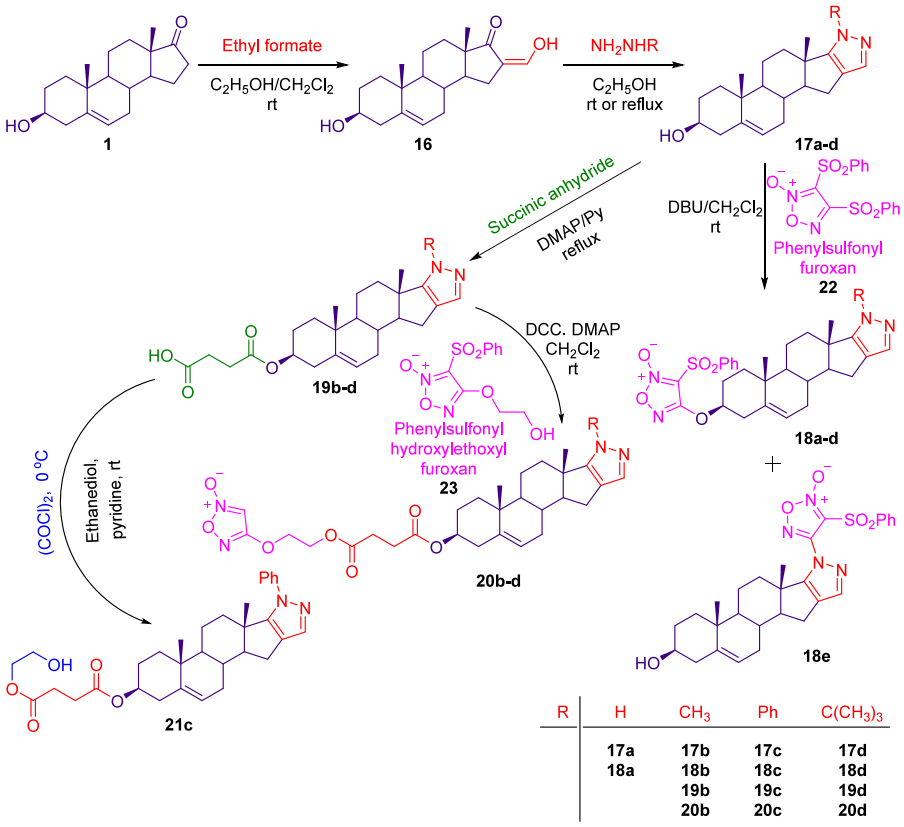

Table 5. In Vitro Antiproliferative Activities (IC_{50}) of Some B-nor-D-Homosteroids against Cancer Cell Lines

s. no.	compounds	IC_{50} (μM)		
		Bel-7404	HeLa	HT-29
1.	**26**	>100	37.6	N.D.[a]
2.	**27**	>100	955	34.5
3.	**28**	15.9	>100	>100
4.	**29**	>100	>100	>100
5.	**30**	>100	71.1	>100
6.	**31**	>100	64.7	15.1
7.	**32**	>100	>100	N.D.[a]
8.	**33**	>100	>100	>100
9.	**34**	>100	>100	>100
10.	**35**	>100	>100	>100
11.	**36**	83.0	>100	16.6
12.	cisplatin	23.2	10.1	N.D.[a]

[a]N.D.: not determined.[5]

Table 6. LC_{50} (μg/mL) of Brine Shrimp Lethality Activity of Some Derivatives[13]

s. no.	compounds	LC_{50}
1.	**38h**	14.62
2.	**38i**	5.34
3.	**38m**	9.81
4.	**38s**	7.81
5.	**41b**	16.22
6.	**41g**	16.89
7.	K_2MnO_4	16.37

various aromatic heterocycles at the C-17-side chain of their steroidal nucleus. These derivatives, prepared from dehydroepiandrosterone **1** (Scheme 1),[9] were evaluated for antiproliferative activity against four cancer cell lines specifically: HeLa (human cervical carcinoma), HT-29 (human colon carcinoma), Bel 7404 (human liver carcinoma), and SGC 7901 (human

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Scheme 5. Synthesis of B-nor-D-Homo-aza-DHEA Derivatives 26−36

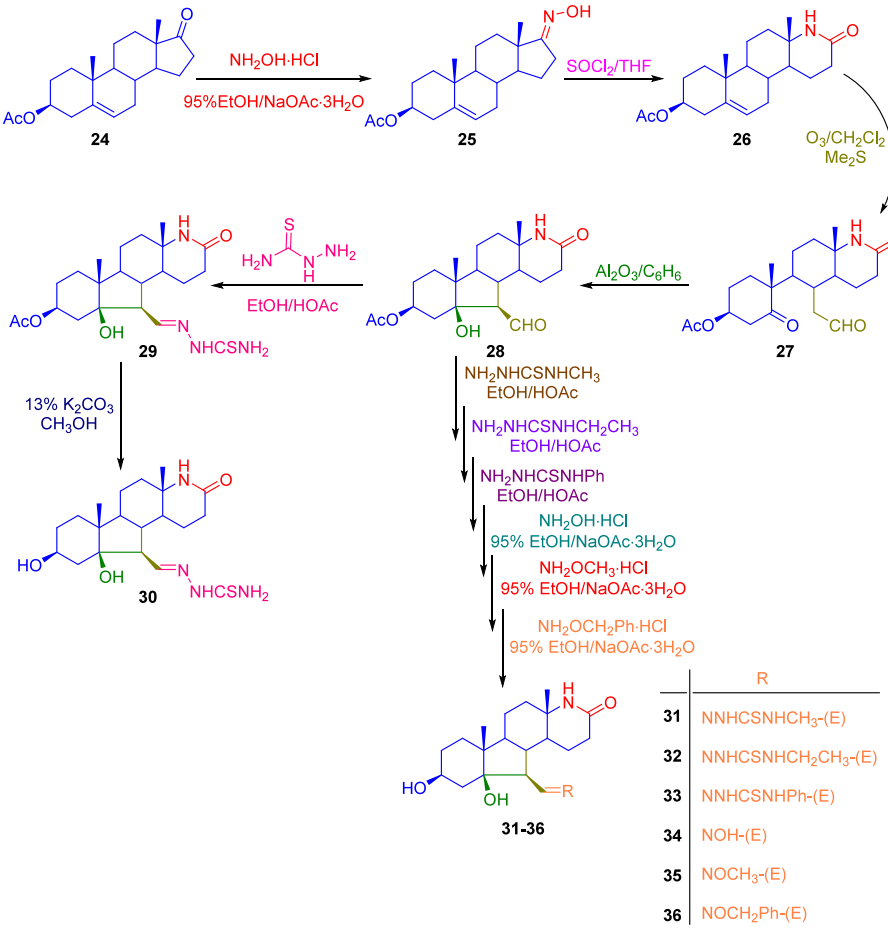

Table 7. IC_{50} (μM) Values of 38s and 41g

s. no.	compounds	IC_{50} (μM)	
		A549	HT29
1.	**38s**	N.D.[a]	9.70
2.	**41g**	8.85	N.D.[a]
3.	paclitaxel	0.003	0.005
4.	DHEA	32.4	28.7
5.	cisplatin[b]	9.6	9.0

[a]N.D.: not determined. [b]Positive control.[13]

Table 8. IC_{50} (μM) Value of the Targeted Compounds 45a−h against Cancer Cell Lines

s. no.	compounds	IC_{50}[a] (μM)			
		A549	SKOV3	MKN-45	MDA-MB-435
1.	**45a**	32.1 ± 3.0	12.5 ± 1.8	70.2 ± 2.2	20.2 ± 1.6
2.	**45b**	22.5 ± 1.6	10.3 ± 1.5	21.2 ± 0.6	18.2 ± 2.2
3.	**45c**	14.3 ± 2.6	9.6 ± 1.3	17.2 ± 1.6	12.6 ± 2.4
4.	**45d**	12.1 ± 1.6	8.9 ± 1.2	15.3 ± 2.2	9.7 ± 2.6
5.	**45e**	11.5 ± 2.0	7.7 ± 0.4	18.4 ± 1.2	8.8 ± 1.5
6.	**45f**	20.2 ± 0.8	10.1 ± 1.0	21.6 ± 2.6	34.6 ± 1.9
7.	**45g**	10.9 ± 1.8	7.5 ± 2.5	16.6 ± 3.2	8.3 ± 2.8
8.	**45h**	20.9 ± 2.8	14.5 ± 1.5	23.6 ± 2.2	32.3 ± 1.6

[a]The results are the average mean of eight replicate determinations ± SD; Used as reference A549: human lung carcinoma, SKOV3: human ovarian carcinoma, MKN-45: human gastric adenocarcinoma, MDA-MB-435: human breast carcinoma.[14]

gastric carcinoma) using MTT assay. The results demonstrated that several dehydroepiandrosterone-17-hydrazone derivatives (**3**−**11**) displayed notable antiproliferative activity by inducing apoptosis in cancer cells. However, compound **11** with a

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Scheme 6. Synthetic Routes of Steroidal Lactams (38a−u and 41a−g)

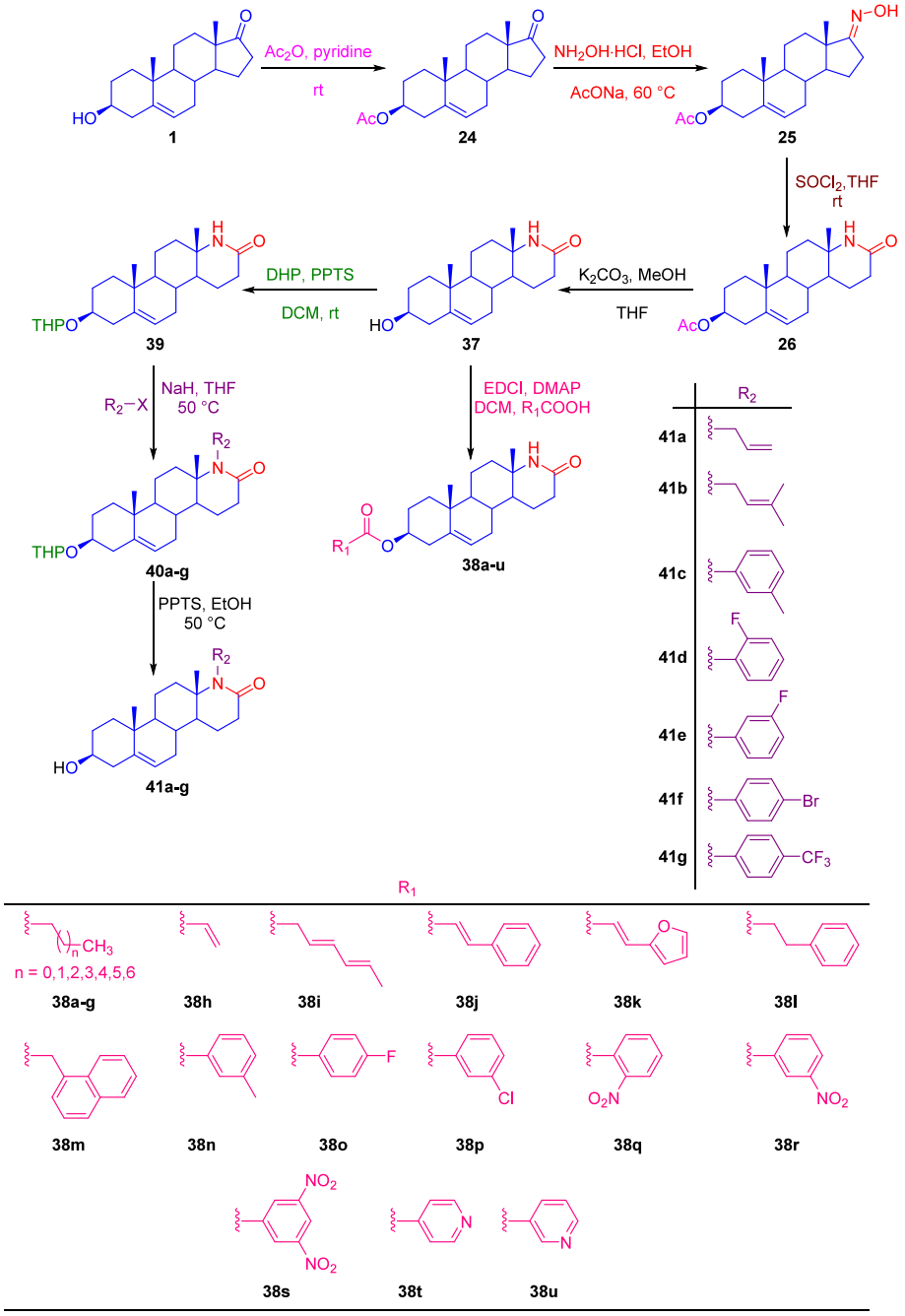

Table 9. In Vitro Activity Data of Synthesized Compounds against 5AR-1 and 5AR-2

s. no.	compounds	activity code	5AR-1% inhibition at 10 μM	5AR-1% inhibition at 2 μM	5AR-2% inhibition at 10 μM	5AR-2% inhibition at 2 μM	IC_{50} of 5AR-2 (nM)
1.	**49**	SAR-43	81.1 ± 2.5	38.7 ± 5.8	100.0 ± 0.0	100.0 ± 0.0	83.8 ± 17.4
2.	**58**	SAR-44	9.6 ± 10.0	N.D.[a]	100.0 ± 0.0	90.6 ± 8.4	220.1 ± 66.2
3.	**59**	SAR-45	6.4 ± 9.0	N.D.[a]	91.2 ± 2.8	84.3 ± 1.6	273.8 ± 45.2
4.	**61**	SAR-46	31.7 ± 0.9	N.D.[a]	100.0 ± 0.0	94.7 ± 7.6	157.0 ± 27.6
5.	finasteride			IC_{50}-453 nM			40 nM

[a]N.D.: not determined; activity of compounds **60** and **62−64** could not be performed due to stability challenges.[15]

structure of propan-2-ylidenehydrazone, was found to be nearly inactive against these cancer cells. The compounds **8−10** with 17-quinoline-4-methanylidenehydrazone **8**, 17-(3-hydroxy)-benzylidenehydrazone **9**, and 17-indol-3-methanylidenehydrazone **10** showed better cytotoxicity. Although, dehydroepiandrosterone quinoline-4-methanylidenehydrazone **8** with a quinoline structure in a 17-side chain exhibits excellent antiproliferative activity against the tested cancer cells SGC

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Scheme 7. Synthesis of Potent Anticancer Active DHEA Derivatives 45a−h

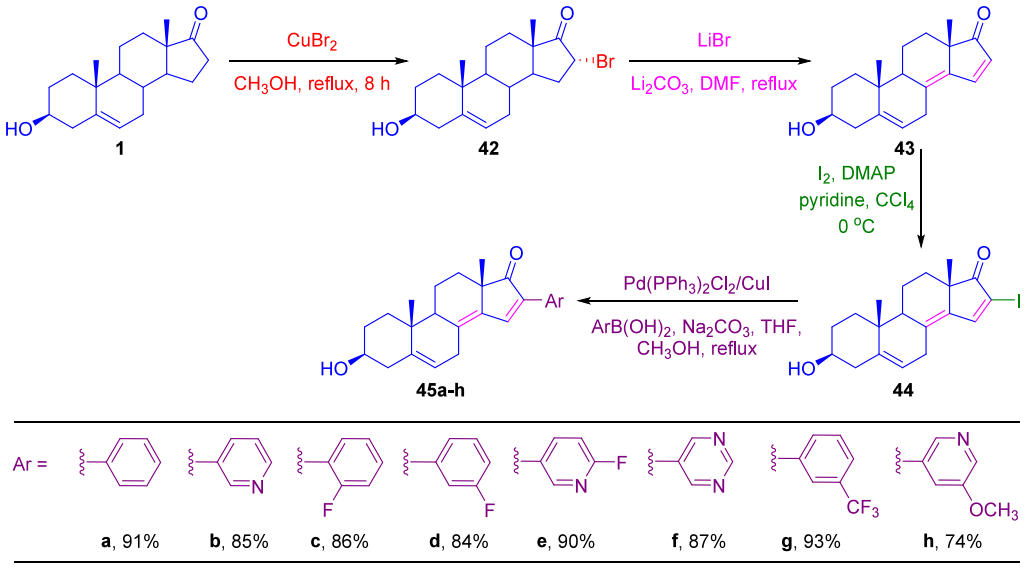

Table 10. In Vitro Activity of Synthesized Compounds against DU-145 Cell Line

s. no.	compounds	activity code	IC_{50} (μg/mL)	IC_{50} (μM)
1.	**49**	SAR-43	N.I.[a]	N.I.[a]
2.	**58**	SAR-44	N.I.[a]	N.I.[a]
3.	**59**	SAR-45	143.20	389.12
4.	**61**	SAR-46	195.10	495.50
5.	finasteride		204.20	548.00

[a]N.I.: no significant inhibition; activity of compounds **60** and **62−64** could not be performed due to stability challenges.[15]

Table 11. Percentage Reduction in Organ Weight Following Treatment with Synthesized Compounds[15]

s. no.	compounds	activity code	ventral prostate	dorsal prostate	vas deferens	epididymis
1.	**49**	SAR-43	35.20	15.25	59.11	36.25
2.	**58**	SAR-44	24.79	37.31	37.09	11.09
3.	**59**	SAR-45	29.94	11.39	11.56	9.03
4.	**61**	SAR-46	33.61	16.94	37.07	9.30
5.	finasteride		44.76	45.32	14.45	14.40

7901 (IC_{50} 1.0 μM) than cisplatin (positive control) (Table 1). Moreover, compound **8** was used to further investigate the antiproliferative mechanism of compounds, and an Annexin V experiment was performed on HeLa and Bel-7404 cells. After 24 h of incubation, treatment of compound **8** with different concentrations resulted in different amounts of PI/Annexin V double-labeled apoptotic cells (control: 0.0%) (the lower right quadrant and the upper right quadrant, which contain early and late apoptotic cells, respectively), indicating compound **8** is a potent apoptotic inducer in these carcinoma cells (Figure 1).

Following the above methodology, Zhang and colleagues also synthesized 23 hydrazone derivatives (**12a−w**) of DHEA (Scheme 2) against flaviviruses in 2021.[10] The transformation was performed at the 17 positions of DHEA, resulting in steroidal derivatives that exhibited significantly stronger antiflavivirus activity compared to DHEA, as reported in the literature.[10]

2.2. Isatin-Dehydroepiandrosterone Derivatives. In 2015, Ke et al. reported the series of isatin-DHEA hybrids (**15a−e**) that were synthesized using a simple condensation technique where DHEA was linked to various isatins via a ═N−N═ bridge (Scheme 3).[6] Also, imine derivatives (**13a−e**) have also been prepared using a similar condensation method, to study the possible structure−activity relationships (SAR). In addition, all newly synthesized compounds (**13a−e** and **15a−e**) were screened for their in vitro cytotoxic effects against HepG2 (hepatocellular liver carcinoma), Huh-7 (hepatoma), A875 (melanoma), and BEL-7402/5-FU (5-fluorouracil-resistant hepatocellular carcinoma) cell lines by the standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay using 5-FU (5-fluorouracil) as a positive control. The preliminary antitumor results of most of the synthesized compounds **13a−e** and **15a−e** exhibited moderate to good antitumor activities against four human cancer cell lines compared with the control, as shown in Figure 2. Especially, the isatin-DHEA conjugates **15a−e** exhibited significantly better inhibitory activities against all tested cell lines at a concentration of 20 μg/mL compared to the positive control 5-FU. However, as compared to the control, the other DHEA derivatives containing imine unit **13a−e** showed moderate to lower activity.

Subsequently, to investigate the high potential activities and the IC_{50} values for all compounds were also evaluated. The compounds **15a**, **15d**, and **15e** exhibited greater cytotoxicity against all tested cancer cell lines compared to the control, 5-FU.

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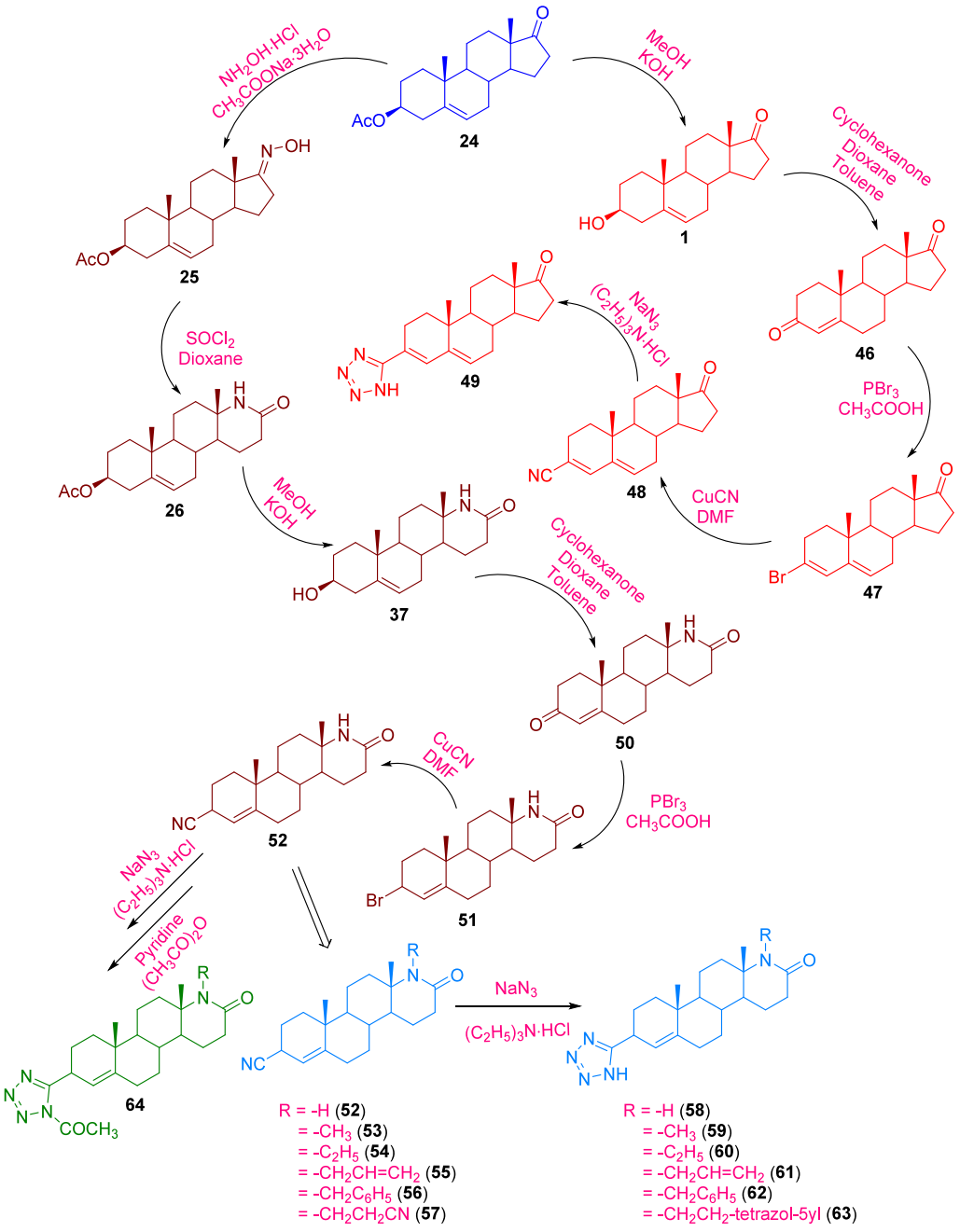
Scheme 8. Synthesis of Tetrazolo-DHEA Derivatives (49, 58-64)



However, compounds **13a−e** indicated poor activities under the same tested conditions. Especially, the highly potential compound **15d** bearing a bromo-group exhibited significant inhibition activities (IC_{50} 5.97 ± 2.67 μM) against BEL-7402/5-FU cell lines that are resistant to 5-FU, which might be developed as a novel main scaffold for anticancer agents. In addition, compound **15b** bearing a nitro group exhibited a selective inhibitory effect on HepG2, Huh-7, and BEL-7402/5-FU cell lines (Table 2).

In addition, the dose−response curve of cell growth inhibition activities for high potential compounds **15a**, **15d**, **15e**, and 5-FU have been outlined in Figure 3, which demonstrated that the targeted compounds had cytotoxic effects on HepG2, Huh-7, and A875 cell lines in a dose-dependent manner. Compound **15d**, in particular, exhibited the most potent growth inhibitory activities against all tested cell lines, with IC_{50} values of 16.22 ± 4.65, 13.90 ± 3.91, and 14.83 ± 1.47 μM (Table 2), respectively, outperforming the control 5-FU.

2.3. Pyrazoannulated Dehydroepiandrosterone Derivatives. A series of eight novel furoxan-based nitric oxides (NO) releasing hybrids (**18a−e** and **20b−d**) of 16,17-pyrazoannulated steroidal derivatives from DHEA **1** via C-16 formylation, condensation with monosubstituted hydrazines, esterification of C-3 hydroxyl group with succinic anhydride, and coupling with phenylsulfonyl-substituted furoxan **23** were synthesized by Huang et al. (2015) (Scheme 4).[11] All newly synthesized compounds (**18a−e** and **20b−d**) were evaluated for their cytotoxic effects against the following five cell lines: MDA-MB-231 (human breast cancer cell line), HCC1806 (tamoxifen-resistant human breast cancer cell line), SKOV-3 (human

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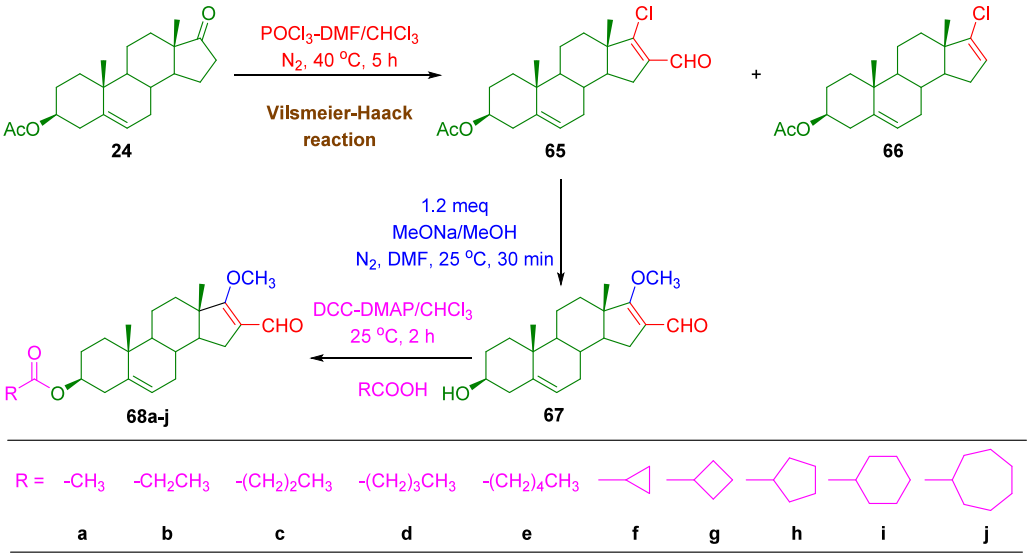
Scheme 9. Route of Synthesis of DHEA Derivatives (68a−j)



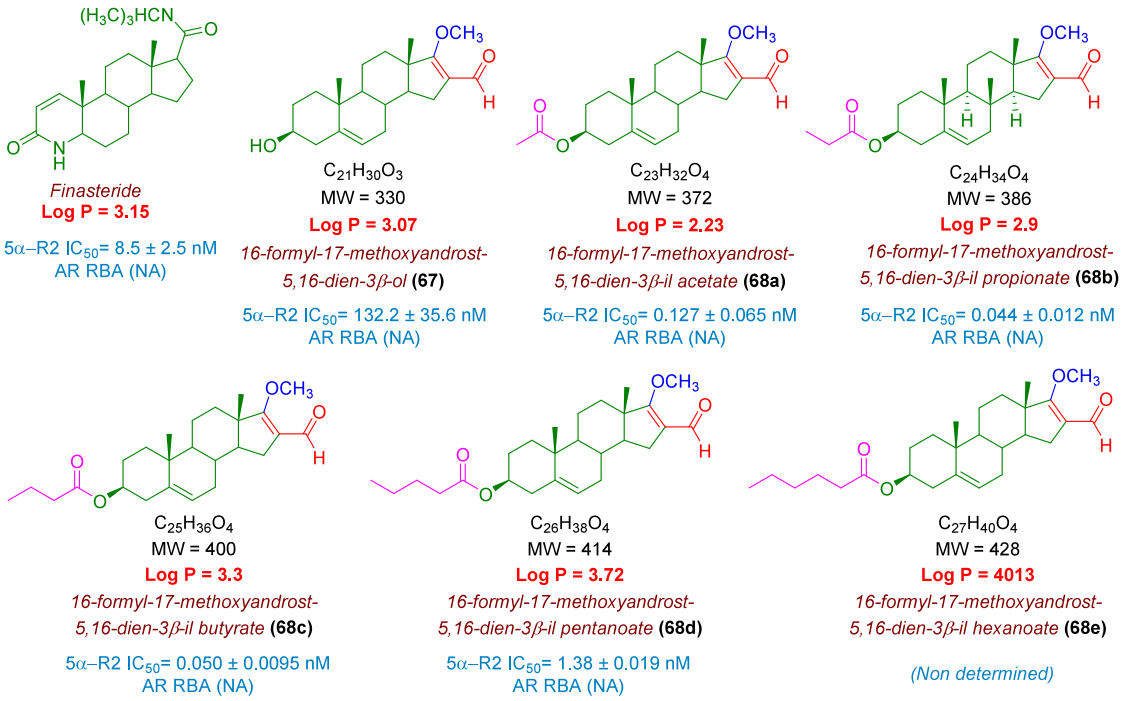


Figure 4. Structures, molecular weight, and biological activities of different DHEA derivatives. All steroids exhibited more potency (lower IC_{50} value) than finasteride to inhibit the 5AR-2 enzyme. IC_{50} values: Concentration of compound required to inhibit 50% of the activity of 5α-reductase type 2 (5AR-2); RBA: Relative binding activity to the androgen receptor (AR).[18]

ovarian cancer cell line), DU145 (human prostate cancer cell line), and HUVEC (umbilical vein endothelium cell line) (Table 3). Among them, compound **20c** exhibited the best activity with IC_{50} values of 20−1.4 nM against four cell lines (MDA-MB-231, SKOV-3, DU145, and HUVEC), and 1.03 μM against the HCC1806 cancer cell line. Moreover, in the preliminary pharmacological study, four compounds **18a** and **20b−d** were selected to screen for VEGF (vascular endothelial growth factor) inhibitory activity. Compounds **18a**, **20b**, and **20c** showed better activity than 2-methoxyestradiol (2-ME) in reducing levels of VEGF secreted by the MDA-MB-231 cell line with the EC_{50} values of 40, 10, and 0.3 nM, respectively, and also exhibited better TI (IC_{50}/EC_{50}) values of 7.00, 9.00, and 4.67 compared with 2-ME (TI = 3.57) and furoxan **22** (TI = 1.10) (Table 4). In order to confirm whether phenylsulfonylfuroxan moiety played an important role in these novel compounds on VEGF inhibitory activity, compound **21c** was produced as a control. It exhibited worse activity, with an EC50 value of 2.71 μM and a TI value of 2.50, compared to the hybrids. These findings revealed that the hybrids of 16,17-pyrazo-annulated steroidal derivatives and furoxan were more effective at inhibiting VEGF secretion than the individual compounds. Furthermore, the preliminary SAR (structure−activity relationship) analysis showed that steroidal scaffolds with a linker at the 3-position enhanced the anticancer activities of the tested compounds. For example, compound **18a** obtained by coupling

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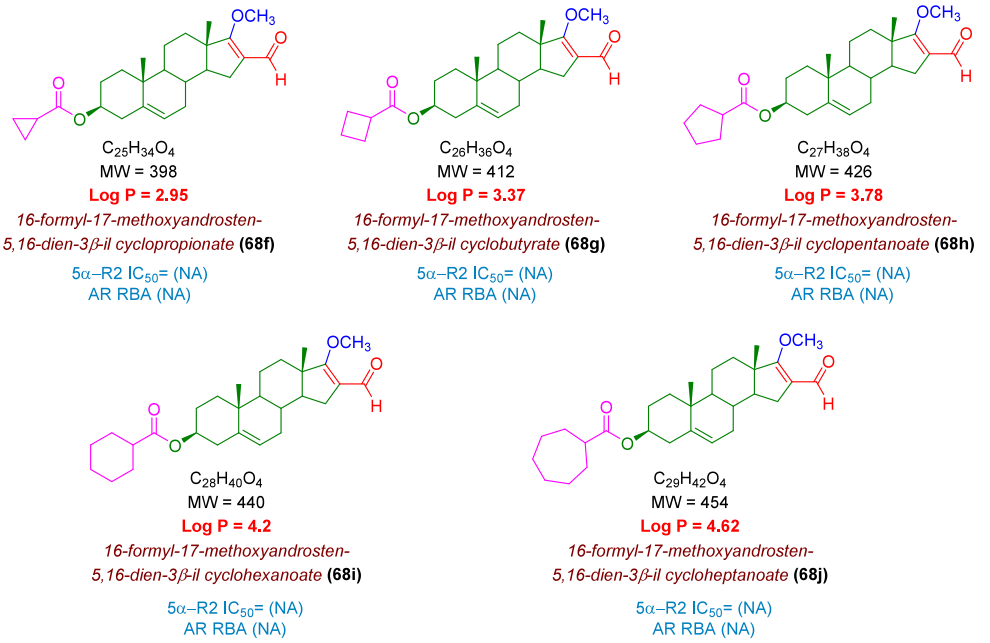
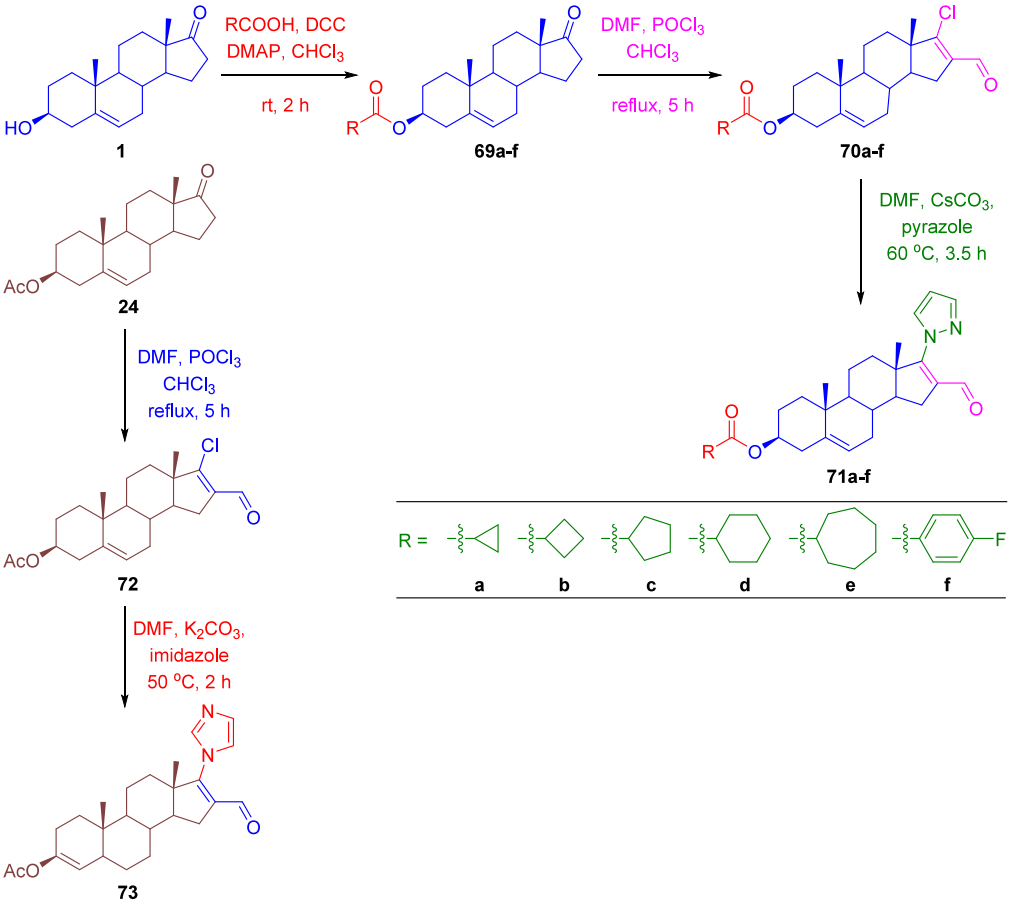


Figure 5. Structures, molecular weights, and biological activities of different DHEA derivatives. None of these compounds was capable of inhibiting the activity of the 5AR-2 enzyme. IC_{50} values: Concentration of compound required to inhibit 50% of the activity of 5α-reductase type 2 (5AR-2); RBA: Relative binding activity to the androgen receptor (AR).[18]

Scheme 10. Synthesis of Pyrazole and Imidazole DHEA Derivatives (71a–f and 73)



furoxan **22**[12] to the 3-OH of the steroidal skeleton, demonstrated greater activity than the byproduct **18e**, which was obtained by coupling furoxan **22** to the 20-position. However, the compounds **20b–d** with a linker in the 3-position showed stronger antiproliferation activities against all four tested cancer cell lines than **18b–d**, respectively. The activities of

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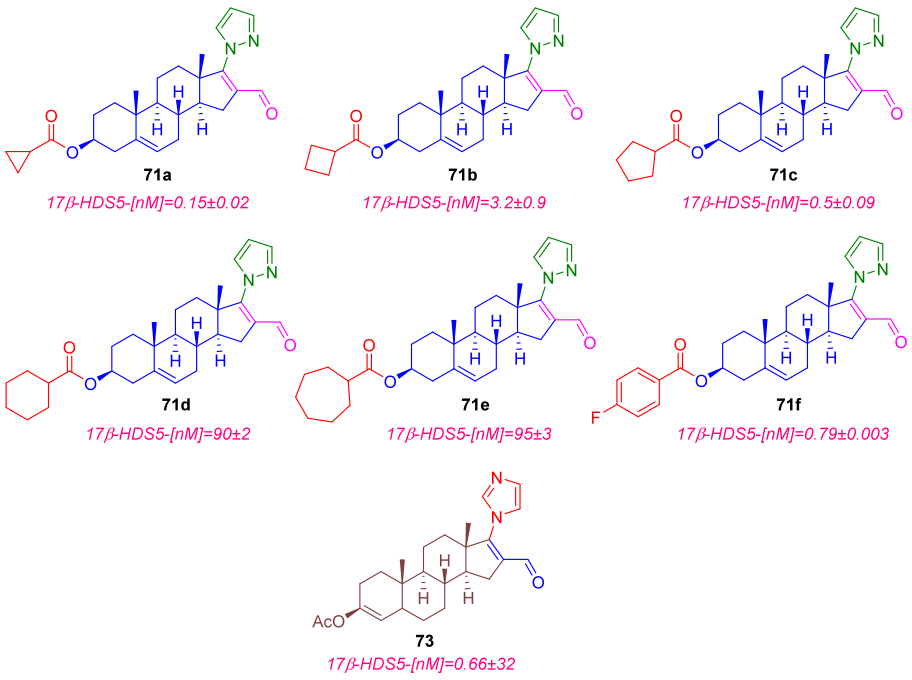
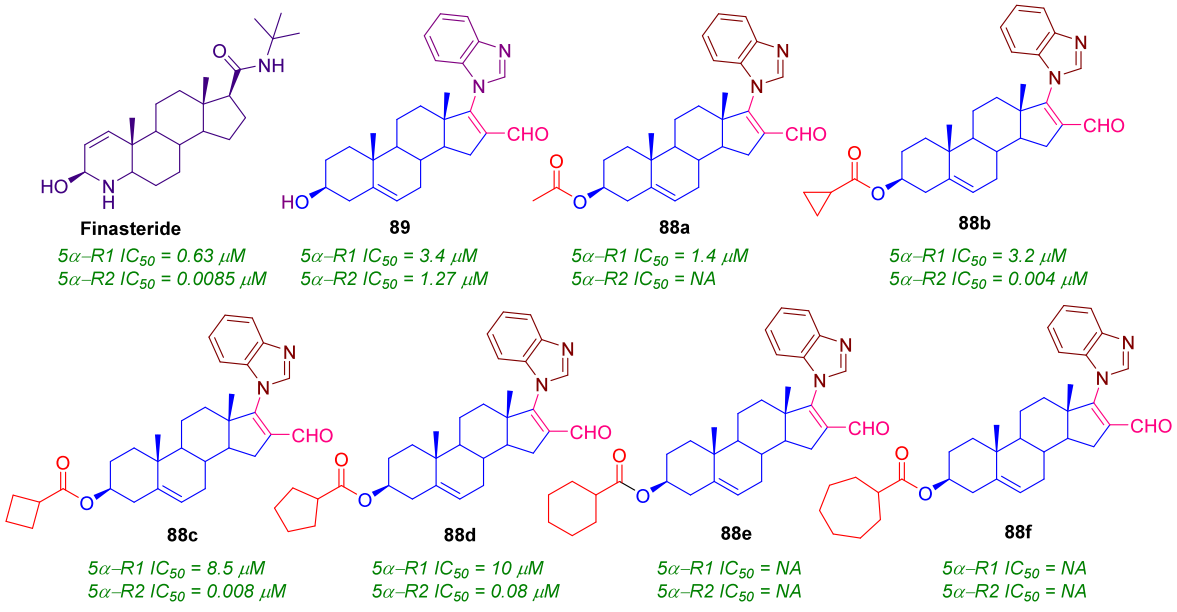


Figure 6. Effect of the novel steroids on the activity of 17β-HSD5 enzyme IC_{50} values shows the concentration for each compound, that inhibited the activity of 17β-HSD5 by 50%.[19]



Note: IC_{50}: Concentration of the compound required to inhibit 50% of the activity of 5α-reductase isoenzymes 1 and 2.

Figure 7. Biological activities of steroidal compounds (**88a−f** and **89**) and finasteride.[21]

compound **20c** bearing the phenyl in the 20-position were enhanced from 60 to 1900 fold than that of **18c** without a linker at the 3-position. However, the structure−activity relationship (SAR) of the substituent group in the 20-position appeared to be ambiguous in those compounds (**18a−d**) without the linker, hydrogen atom, and smaller size group like methyl located at the 20-position were more beneficial to retain the better potent activities, whereas, in the compounds, **20b−d** containing the linker, larger group size like phenyl substituted at 20-position was observed to have the better anticancer activity.

2.4. B-nor-D-Homoaza-dehydroepiandrosterone Derivatives. Cui et al. established a methodology in 2015 to construct a variety of novel nitrogen-containing B-nor-D-homosteroids using oximation, Beckman rearrangement, ozonation, cyclization, and condensation reaction (Scheme 5).[5] Initially, DHEA acetate **24** was transformed into the corresponding hydroxylamine derivative **25** of DHEA by the condensation reaction with hydroxylamine hydrochloride then Beckman rearrangement in $SOCl_2$/THF gave aza-D-homo derivative **26**. Next, the ozonolysis of compound **26** was performed in CH_2Cl_2 at −78 °C. After bubbling O_2 to expel the excess O_3 and adding Me_2S to decompose the resulting ozonide, compound **27** was obtained. The B-nor skeleton of compound **28** bearing the 5β-OH and 6β-formyl was generated through the

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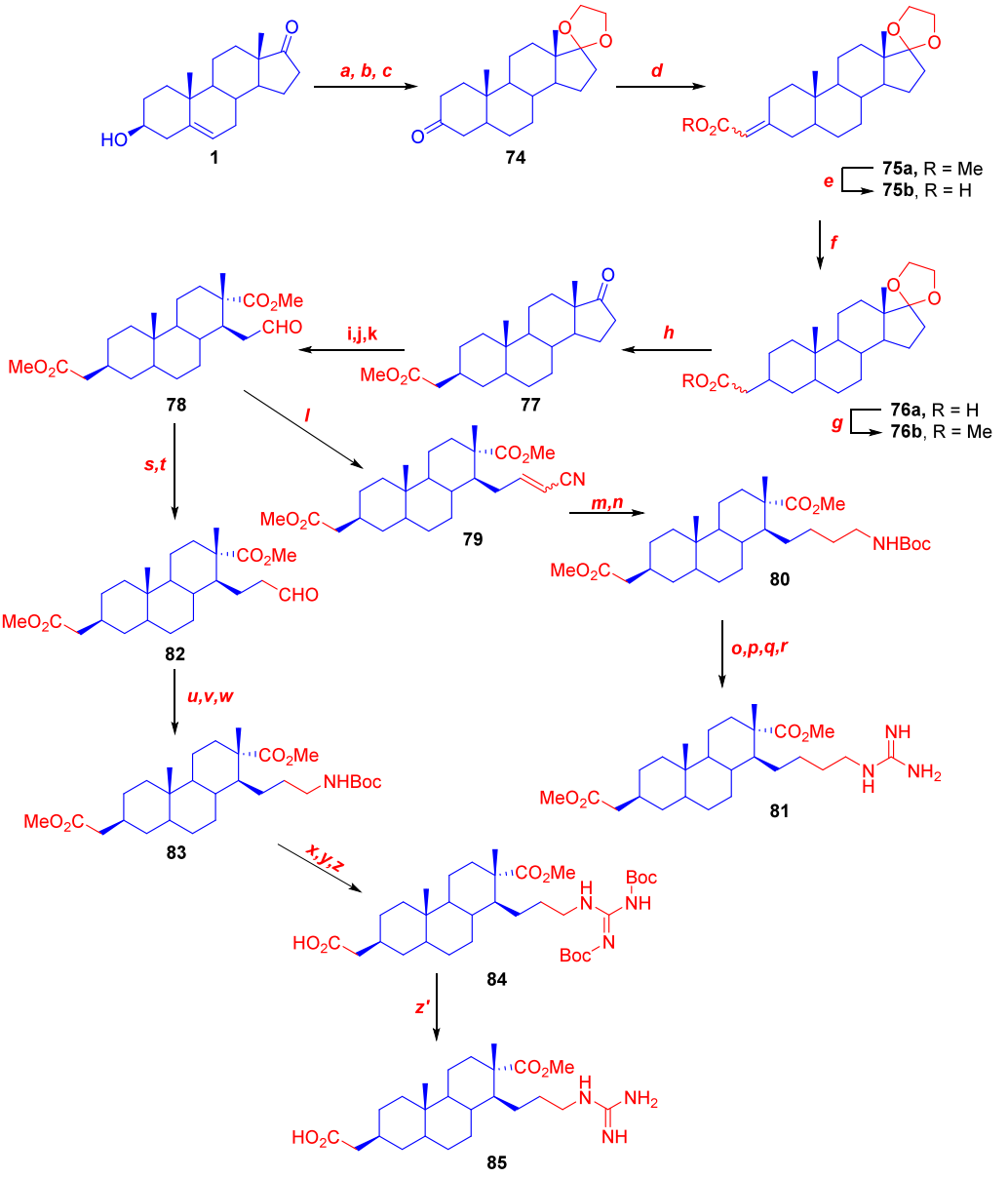
Scheme 11. Synthetic Strategy for RGD Mimics 81 and 85



Reagents and conditions: (a) $(CH_2OH)_2$, CSA, benzene, reflux, 80%; (b) $Al(OPr^i)_3$, cyclohexanone, toluene, reflux, 97%; (c) Li, liq. NH_3, THF, -78 °C, then NH_4Cl, 88%; (d) $(EtO)_2P(O)CH_2CO_2Me$, $KOBu^t$, DMF, 0 °C to rt, 97%; (e) KOH, EtOH/H_2O, 90 °C; (f) Li, liq. NH_3, ^{t}BuOH, THF/dioxane, -78 °C, $\beta:\alpha$ = >10:1; (g) MeI, NaH, DMF, r.t; (h) 1.5 M HCl, THF, r.t, 91% in 4 steps; (i) isoprophenyl acetate, conc. H_2SO_4, reflux, 75%; (j) O_3, CH_2Cl_2/AcOH, then Me_2S, AcOH/H_2O, -78 °C to r.t.; (k) CH_2N_2, Et_2O, 0 °C, 83% in 2 steps; (l) $(EtO)_2P(O)CH_2CN$, $KOBu^t$, DMF, 0 °C to r.t., 96%; (m) PtO_2, H_2, HCl/MeOH/$CHCl_3$, r.t.; (n) Boc_2O, Et_3N, CH_2Cl_2, 0 °C to r.t., 95% in 2 steps; (o) 10% LiOH, aq. Bu_4NHSO_4, MeOH/THF, 0 °C to r.t., quant.; (p) TFA, CH_2Cl_2, 0 °C to r.t; (q) *N,N'*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine, pyridine, 0 °C to r.t. quant. in 2 steps; (r) TFA, CH_2Cl_2, 0 °C to r.t., 87%; (s) $MeOCH_2PPh_3Cl$, LHMDS, THF, -78 °C, 4h, 73%; (t) *p*-TsOH·H_2O, acetonr/H_2O; 0 °C to r.t., 73%; (u) NH_2OH·HCl, Et_3N, EtOH, r.t.; (v) PtO_2, H_2, AcOH, r.t.; (w) Boc_2O, Et_3N, CH_2Cl_2, 0 °C to r.t., 78% in 3 steps; (x) 10% LiOH aq., Bu_4NHSO_4, MeOH/THF, 0 °C to r.t., quant.; (y) TFA, CH_2Cl_2, 0 °C to r.t.; (z) *N,N'*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine, pyridine, 0 °C to r.t. 71% in 2 steps; (z') TFA, CH_2Cl_2, 0 °C to r.t., quant.

intramolecular aldol condensation of **27** with neutral alumina. Finally, **29** and **31**−**36** were obtained by the reaction of **28** with different nitrogen-containing agents. Likewise, the deacetylation of **29** with alcoholic K_2CO_3 gave compound **30** in good yield. Moreover, the antiproliferative activity of all the synthesized compounds was evaluated against HT-29 (colonic carcinoma), HeLa (human cervical carcinoma), and Bel 7404 (human liver carcinoma) cells using an MTT assay. Compound **28**, which has a B-nor and D-homo lactam structure, did not show increased cytotoxicity against HeLa cancer cells compared to its precursor **26**, which has a D-homo lactam structure. However, compound **28** exhibited a significant increase in cytotoxicity against Bel-7404 cells. However, compound **31**, featuring a 6-(4′-methyl)-thiosemicarbazone group, and **36**, with a 6-*O*-benzyloxime

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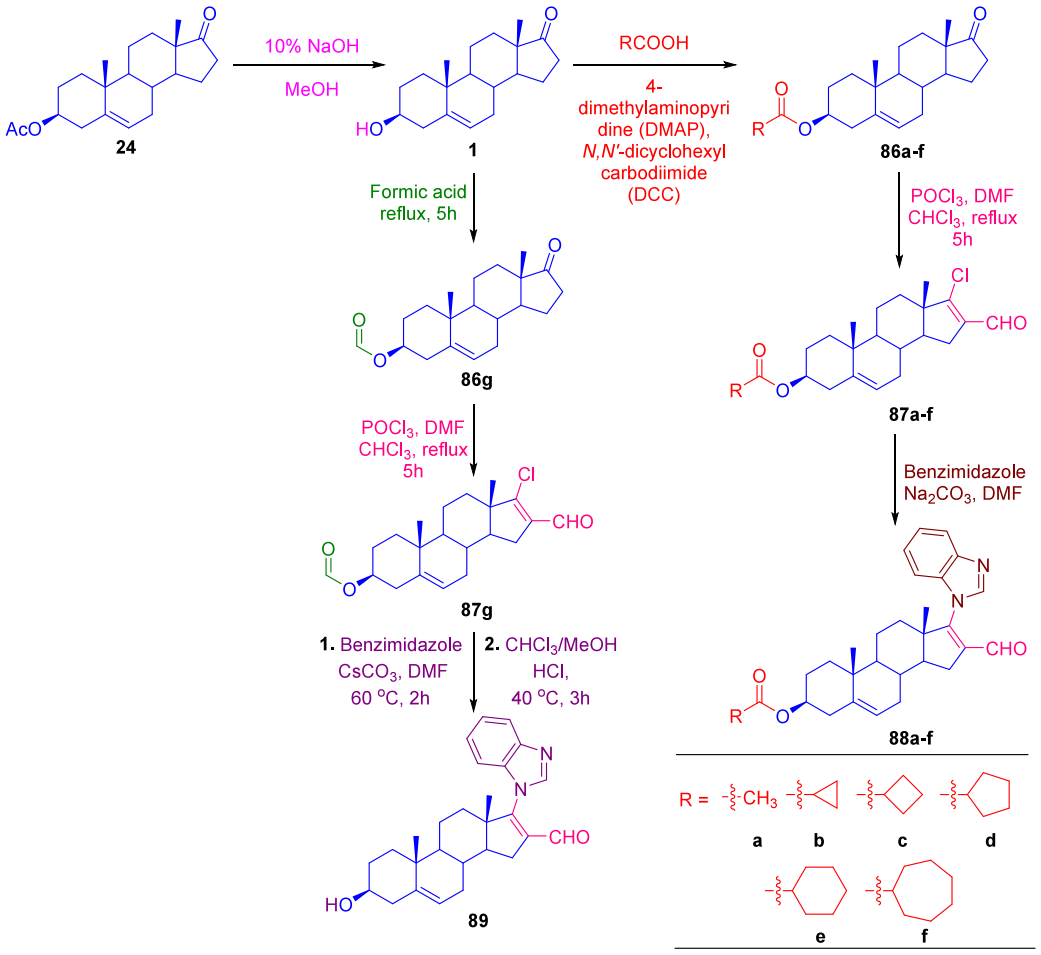
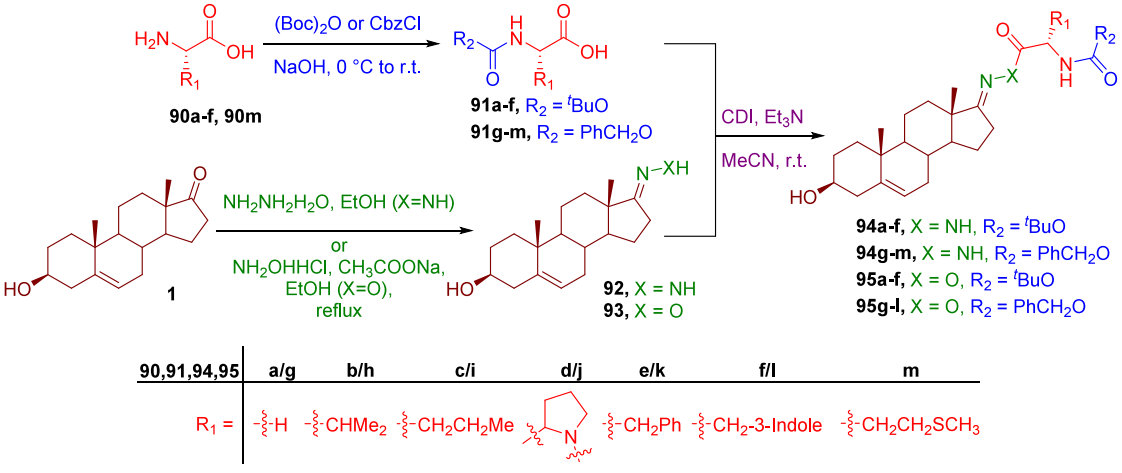
Scheme 12. Synthetic Path for the Synthesis of Seven Novel Steroidal Derivatives (88a−f and 89)



Scheme 13. Synthetic Route of Novel Amino Acid-Conjugates of DHEA Derivatives (94a−m and 95a−l)



group, demonstrated selective antiproliferative activity against HT-29 cells, with IC_{50} values of 15.1 and 16.6 μM, respectively (Table 5).

Similarly, Hong and co-workers also used an oximation reaction, Beckmann rearrangement, hydroxyl protection, *N*-alkylation, and deprotection to synthesize twenty-eight novel 17a-aza-D-homoandroster-17-one derivatives from DHEA **1** (Scheme 6) in 2021.[13] In the beginning, high yields of **38a−u** were obtained by condensation of intermediate **37** with different acids in the presence of DMAP and EDCl. Moreover, compound **39** with the tetrahydropyran protective group may be easily made by reacting compound **37** with DHP in DCM at room temperature under the catalysis of PPTS. The resulting intermediate **39** was then treated with various alkyl halides in dry THF in the presence of NaH to generate the required products **40a−g**. In the end, the treatment of compounds **40a−g** in EtOH in the presence of PPTS at 50 °C to quantitatively obtain the target hybrids, **41a−g**. In addition, all the synthesized

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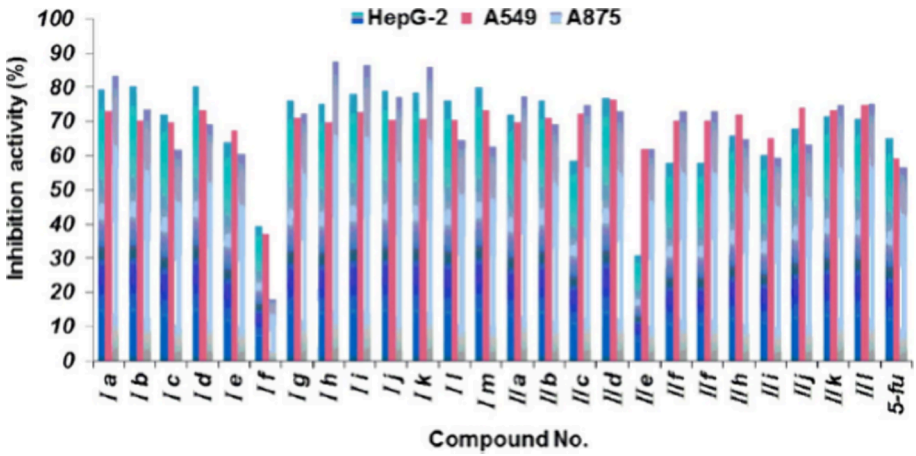

Figure 8. Inhibition activities against cell proliferation for target compounds **94a−m**$^{(Ia\text{-}m)}$ and **95a−l**$^{(IIa\text{-}l)}$ at 40 μg/mL.[22] Copyright 2016, The Author(s). 5-FU: 5-Fluorouracil, used as a positive control.

Table 12. In Vitro Cytotoxic Activity (IC_{50}) of the Compounds against Various Human Cancer Cell Lines

s. no.	compounds	IC_{50}[a] (μM)		
		HepG2	A549	A875
1.	**94a**	20 ± 4[b]	20 ± 3	18 ± 2
2.	**94b**	15 ± 4	17 ± 2	16 ± 6
3.	**94c**	14 ± 4	14 ± 3	14 ± 5
4.	**94d**	12 ± 5	16 ± 3	19 ± 4
5.	**94e**	19 ± 4	22 ± 4	30 ± 8
6.	**94f**	>60	>60	>60
7.	**94g**	35 ± 5	32 ± 7	37 ± 1
8.	**94h**	26 ± 7	24 ± 6	27 ± 3
9.	**94i**	9 ± 3	10 ± 3	14 ± 3
10.	**94j**	24 ± 5	21 ± 2	24 ± 2
11.	**94k**	9 ± 2	6 ± 1	13 ± 1
12.	**94l**	11 ± 4	8 ± 3	15 ± 6
13.	**94m**	10 ± 2	10 ± 4	16 ± 5
14.	**95a**	21 ± 1	27 ± 6	29 ± 4
15.	**95b**	24 ± 7	19 ± 6	24 ± 0
16.	**95c**	29 ± 4	21 ± 5	17 ± 2
17.	**95d**	7 ± 3	13 ± 4	15 ± 4
18.	**95e**	>60	37 ± 5	40 ± 2
19.	**95f**	28 ± 1	21 ± 4	29 ± 9
20.	**95g**	31 ± 6	23 ± 4	20 ± 2
21.	**95h**	23 ± 4	18 ± 2	14 ± 1
22.	**95i**	23 ± 7	23 ± 2	22 ± 5
23.	**95j**	22 ± 2	20 ± 2	18 ± 1
24.	**95k**	16 ± 2	16 ± 3	18 ± 2
25.	**95l**	17 ± 3	15 ± 2	15 ± 2
26.	5-FU[c]	84 ± 25	115 ± 10	100 ± 24

[a]IC_{50} (μM): Compound concentration required to inhibit tumor cell proliferation by 50%; [b]All assays were performed in triplicate on three independent experiments and measurement data were expressed as the mean ± S.D; [c]5-FU: 5-Fluorouracil.[22]

derivatives were also examined for bioactivities, brine shrimp toxicity, and cytotoxicity. The results showed that compounds **38h**, **38i**, **38m**, **38s**, **41b**, and **41g** had excellent toxicity against brine shrimp, with LC_{50} values ranging from 5.34 to 16.89 μg/mL (Table 6). Among them, compounds **38h**, **38i**, **38m**, and **38s** showed more potent activities than compounds **41b** and **41g** with LC_{50} values ranging from 5.34 to 14.62 μg/mL. And these six molecules showed better activities than the positive control, K_2MnO_4 with an LC_{50} value of 16.37 μg/mL. Additionally, the compounds **38s** and **41g** had significant cytotoxicity against HT29 and A549 cells, respectively, with IC_{50} values of 9.70 μM and 8.85 μM (Table 7), which are comparable to the positive control Cisplatin but inferior to Paclitaxel.

2.5. Substituted Aryl-Dehydroepiandrosterone Derivatives. Li et al. (2016) reported the multistep synthesis of potential anticancer agents such as 16-aryl-3β-hydroxyandrosta-5,8(14),15-trien-17-ones **45a−h** (Scheme 7), derived from dehydroepiandrosterone **1**. These compounds were tested against four human cancer cell lines, namely: A549 (lung), SKOV3 (ovarian), MKN-45 (gastric), and MDA-MB-435 (breast). On the basis of IC_{50} values, it was found that compounds **45c−e** and **45g**, which have a fluoro-substituted

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Scheme 14. Synthesis of Phosphocholines Containing DHEA Derivatives

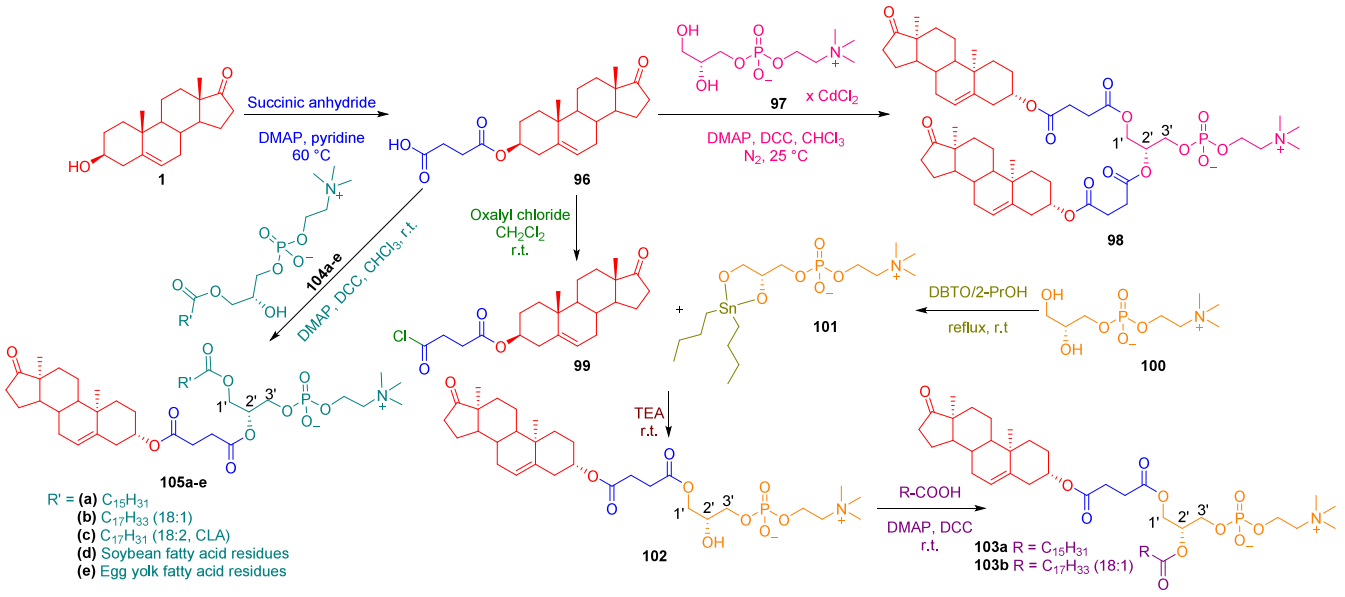

Table 13. IC_{50}[b] Values of the Targeted Compounds against Cancer Cell Lines

s. no.	compounds	IC_{50}[b]			
		Balb/3T3	HL-60	B16	LNCaP
1.	**1**	46.57 ± 4.47	22.23 ± 4.07	30.76 ± 2.28	34.21 ± 4.48
2.	**96**	N.A.[a]	20.88 ± 4.82	37.44 ± 2.85	57.74 ± 2.96
3.	**98**	N.A.[a]	N.A.[a]	73.33 ± 3.27	N.A.[a]
4.	**102**	N.A.[a]	N.A.[a]	N.A.[a]	N.A.[a]
5.	**103a**	N.A.[a]	36.13 ± 4.85	39.43 ± 4.42	N.A.[a]
6.	**103b**	N.A.[a]	31.01 ± 1.29	63.51 ± 6.25	N.A.[a]
7.	**105a**	N.A.[a]	33.29 ± 2.72	55.97 ± 5.78	85.07 ± 10.07
8.	**105b**	N.A.[a]	N.A.[a]	82.86 ± 16.04	N.A.[a]
9.	**105c**	N.A.[a]	30.50 ± 1.83	36.71 ± 1.64	71.11 ± 6.19
10.	**105d**	N.A.[a]	N.A.[a]	62.05 ± 7.88	81.71 ± 13.57
11.	**105e**	N.A.[a]	90.76 ± 12.49	51.75 ± 9.00	N.A.[a]
12.	cisplatin	1.95 ± 0.57	0.28 ± 0.04	0.65 ± 0.27	2.48 ± 0.73

[a]N.A.: not active in the range of concentrations tested (100−0.1 μg/mL). [b]IC_{50} (μg/mL): half-maximal inhibitory concentration.[7]

Table 14. IC_{50} Values of the Compounds 108 and 112 against the Growth of Various Tumor Cell Lines[a]

s. no.	compounds	IC_{50}[b] (μM)								
		T47D	MDA-MB-231	MCF-7	DU145	LNCaP	HCT116	HT29	HL-60	Jurkat
1.	DHEA	2.55	>50	>50	>50	>50	>50	>50	>50	46.5
2.	EPI[c]	1.23	>50	>50	>50	>50	>50	>50	>50	28.8
3.	**108**	1.36	>50	24.1	>50	>50	>50	>50	>50	>50
4.	**112**	0.67	20.1	>50	35.6	48.1	>50	>50	>50	22.7

[a]From SRB assay after 96 h of treatment. [b]IC_{50} data are an average of at least 3 independent experiments, with variation ±10%; [c]EPI (Epiandrosterone) is a reduzate of DHEA.[23]

aromatic ring, exhibited better cytotoxicity against SKOV3, A549, MKN-45, and MDA-MB-435 cell lines than compounds **45a**, **45b**, **45f**, and **45h**. The analogs **45a** with a phenyl ring remarkably decreased their cytotoxic activity against A549, SKOV3, MKN-45, and MDA-MB-435 cells in comparison with the analogs **45b**, **45f**, and **45h**, which have a nitrogen-containing heterocycle. Compound **45e** containing a fluoro-substituted nitrogen containing heterocycle was found to be the best active against SKOV3, A549, MKN-45, and MDA-MB-435 cells tested. In contrast, substitution with a trifluoromethyl group as a strong electron-withdrawing group of the phenyl ring (compound **45g**) showed better anticancer activity (Table 8).[14]

2.6. Tetrazole Dehydroepiandrosterone Derivatives. In 2016, Aggarwal and co-workers developed a series of steroidal tetrazole derivatives, in which the tetrazole moiety was appended at C-3 and 17a-aza locations, and those were synthesized through multiple synthetic steps from dehydroepiandrosterone acetate **24** (Scheme 8).[15] Some of the synthesized compounds were evaluated for their in vitro 5α-reductase (5AR) inhibitory activity at 10 μM and 2 μM concentrations. Compound **49** was found to be a potent dual

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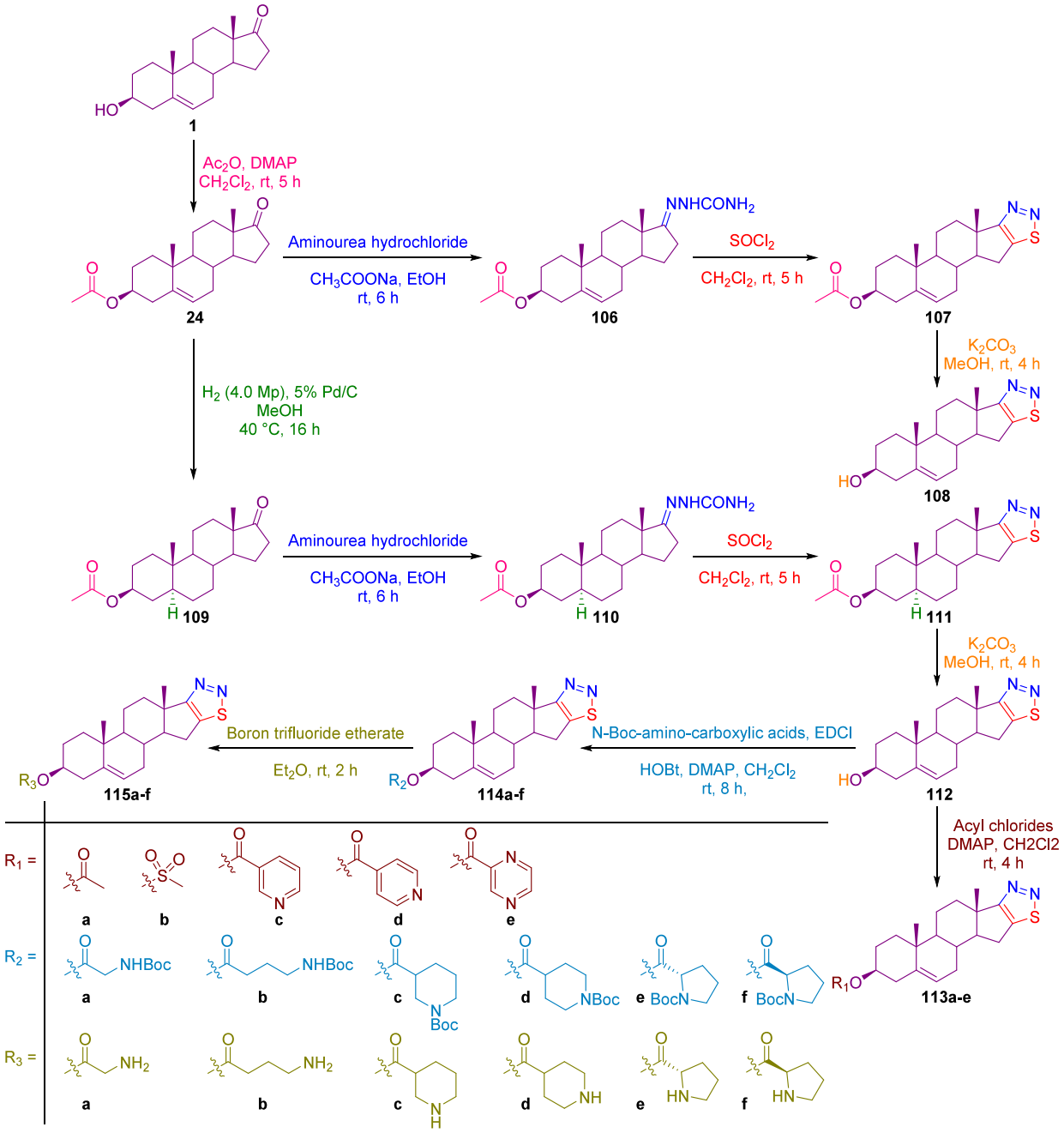
Scheme 15. Synthesis of DHEA Derivatives (108, 112, 113a−e, 114a−f, and 115a−f)



Scheme 16. Synthesis of 16-Arylidene Steroids 116a−d

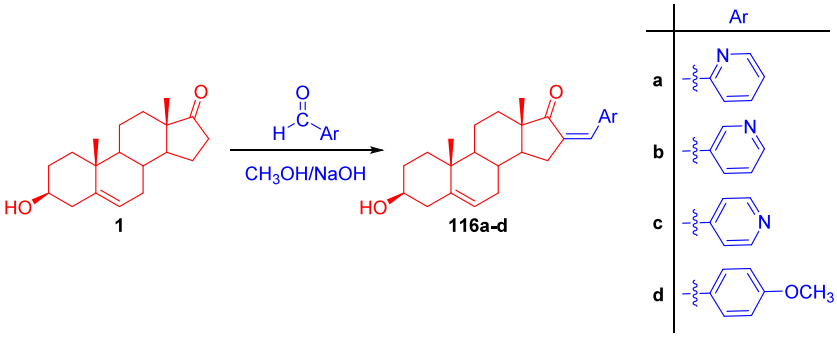


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Table 15. IC_{50} Values of the Compounds against the Growth of T-47D Tumor Cell Line and Normal Human Fibroblast (HAF) Cells[a]

s. no.	compounds	IC_{50} (μM)[b]		SI[c]
		T47D	HAF	
1.	**112**	0.67 ± 0.11[d]	39.05 ± 4.15	32
2.	**111**	2.17 ± 0.09	>50	N.D.[e]
3.	**113a**	2.66 ± 0.85	>50	N.D.[e]
4.	**113b**	2.83 ± 0.49	>50	N.D.[e]
5.	**113c**	2.25 ± 0.30	>50	N.D.[e]
6.	**113d**	3.28 ± 0.51	>50	N.D.[e]
7.	**113e**	3.04 ± 0.99	>50	N.D.[e]
8.	**115a**	0.92 ± 0.05	26.1 ± 0.77	28
9.	**115b**	1.15 ± 0.10	18.0 ± 0.22	16
10.	**115c**	0.042 ± 0.028	2.95 ± 0.28	70
11.	**115d**	0.051 ± 0.010	1.82 ± 0.03	36
12.	**115e**	0.24 ± 0.06	36.0 ± 2.05	150
13.	**115f**	0.058 ± 0.016	21.1 ± 5.06	364
14.	DHEA	2.55 ± 0.01	>50	N.D.[e]
15.	ADM[f]	0.040 ± 0.018	0.068 ± 0.064	1.7

[a]From SRB assay after 96 h of treatment. [b]IC_{50} data are an average of at least 3 independent experiments; [c]The selectivity indexes (SI) were calculated by IC_{50} values in HAF cells divided by IC_{50} values in the T47D cancer cell line; [d]Data from Table 14; [e]ND: not determined; [f]ADM is Adriamycin.[23]

Table 16. IC_{50} Values of the Compounds 115c and 115(e, f) against the Growth of Various Tumor Cell Lines[a]

s. no.	compounds	IC_{50} (μM)[b]								
		T47D[c]	MDA-MB-231	MCF-7	DU145	LNCaP	HCT116	HT29	HL-60	Jurkat
1.	**115c**	0.042	1.24	1.41	0.92	4.28	2.01	2.12	5.33	2.57
2.	**115e**	0.24	7.69	15.0	24.3	36.2	8.52	13.6	31.5	1.41
3.	**115f**	0.058	9.66	27.1	17.7	31.6	11.7	16.5	46.0	2.49

[a]From SRB assay after 96 h of treatment. [b]IC_{50} data are an average of at least 3 independent experiments, with variation ±10%; [c]Data from Table 15.[23]

Table 17. Neuroprotective Effects of 16-Arylidene Steroids 116a−d on Mice Injected with LPS Intraperitoneally

compounds	Morris water maze		elevated plus maze
	escape latency (s)	TSTQ[c] (s)	% ITL[d] (s)
control	41.5 ± 1.9	55 ± 3.4	11.4 ± 2.6
LPS	73.2 ± 1.6[a]	13 ± 2.9[a]	91.4 ± 2.9[a]
celecoxib	52.9 ± 2.4[b]	44 ± 3[b]	24.4 ± 2.2[b]
dexamethasone	56.7 ± 2.5[b]	40 ± 3[b]	31.1 ± 2.4[b]
116a	62.5 ± 1.8[b]	35 ± 4[b]	37.7 ± 2.2[b]
116b	65.2 ± 2[b]	32 ± 3[b]	40 ± 2.7[b]
116c	57.9 ± 2[b]	40 ± 4[b]	30.2 ± 4.4[b]
116d	60.3 ± 2.3[b]	38 ± 3[b]	33.8 ± 2.8[b]

[a]$p < 0.05$ as compared to control. [b]$p < 0.05$ as compared to LPS ($n = 5$). One-way ANOVA followed by Tukey's test was used to determine intergroup variation, with values considered statistically significant ($p < 0.001$). [c]TSTQ: Time spent in the target quadrant. [d]ITL: Initial transfer latency.[24]

inhibitor of 5α-reductase showing 100% inhibition for 5AR-2 isozyme at both 10 μM and 2 μM concentrations with IC_{50} being 83.8 nM. It showed around 81.1% inhibition for 5AR-1 isozyme at 10 μM. Similarly, compound **59** exhibited 91.2% inhibition at 10 μM and around 84.3% inhibition against 5AR-2 at 2 μM concentration with IC_{50} of 273.8 nM. Compound **61** showed 100% inhibition at 10 μM and 95% inhibition at 2 μM concentration against 5AR-2 with IC_{50} being 157 nM. Finasteride was used as the standard drug in the assay. It showed an IC_{50} value of 453.0 nM for the 5AR-1 enzyme and 40 nM for the 5AR-2 enzyme. Compounds **60**, **62**−**64** could not be evaluated because of the stability challenges for these molecules (Table 9).[16] In addition, some of the synthesized compounds were evaluated against DU-145 prostate cancer cell lines, which are androgen-independent. Finasteride, a selective 5AR-2 inhibitor, has been reported to reduce the proliferation rate of the DU-145 prostate cancer cell line in vitro. Compounds **59** and **61** exhibited weak activity against the DU-145 cell line, likely due to their activity as 5AR-2 inhibitors, which inhibit dihydrotestosterone (DHT) synthesis. Consequently, they were unable to demonstrate an effect on androgen-independent cell lines (Table 10).[17] However, in vivo, 5α-reductase inhibitory activity also showed a significant reduction ($p < 0.05$) in rat prostate weight. Compound **49** significantly reduced the weight of the ventral prostate, vas deferens, and epididymis thereby exhibiting strong inhibition of 5α-reductase as demonstrated in

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Scheme 17. Synthesis of Steroidal[17,16-*d*]pyrimidines Derivatives 118a−p

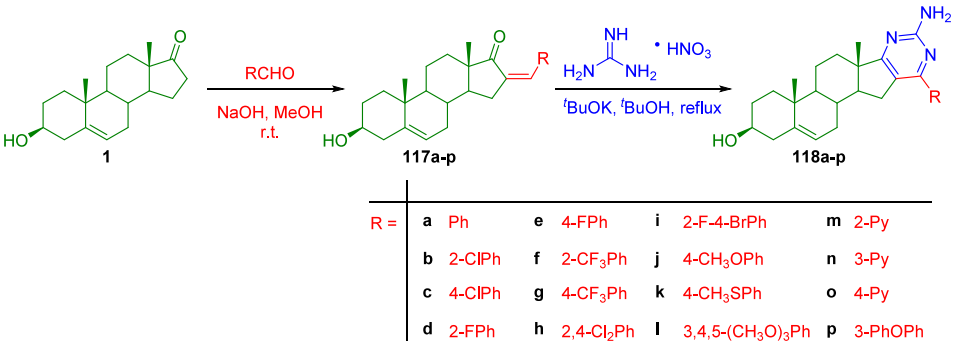

Table 18. Neuroprotective Effects of 16-Arylidene Steroids 116a−d in Rats Injected with LPS Intranigrally

compounds	locomotor activity		elevated plus maze	catatonic response
	ambulation (min)	rearing (min)	% ITL[d] (s)	degree of catatonia (score)
Sham[24]	300 ± 2.5	145 ± 2.0	16.6 ± 3.2	0
LPS + vehicle	56.3 ± 3.8[a]	16.3 ± 3.3[a]	83.3 ± 4.4[a]	1.2 ± 0.02[a]
LPS	55.8 ± 4.6[b]	16.0 ± 2.3[b]	82.9 ± 3.1[b]	1.22 ± 0.03[b]
celecoxib	215 ± 3.8[c]	73.3 ± 2.0[c]	31.3 ± 2.0[c]	0.21 ± 0.04[c]
dexamethasone	200 ± 3.9[c]	63 ± 3.0[c]	37.1 ± 2.2[c]	0.33 ± 0.05[c]
116a	181.5 ± 2.9[c]	53 ± 3.2[c]	48.3 ± 3.0[c]	0.52 ± 0.03[c]
116b	164 ± 4[c]	47 ± 4[c]	51.6 ± 4[c]	0.57 ± 0.06[c]
116c	194.5 ± 4.4[c]	63 ± 3.4[c]	39.7 ± 3.0[c]	0.45 ± 0.05[c]
116d	190 ± 3.5[c]	57 ± 2.8[c]	44.6 ± 3.3[c]	0.48 ± 0.04[c]

[a] $p < 0.05$ as compared to sham. [b] $p < 0.05$ as compared to LPS + vehicle. [c] $p < 0.05$ as compared to LPS ($n = 5$). One-way ANOVA followed by Tukey's test was employed to find out the intergroup variation, and values were considered statistically significant ($p < 0.001$). [d] ITL: Initial transfer latency.[24]

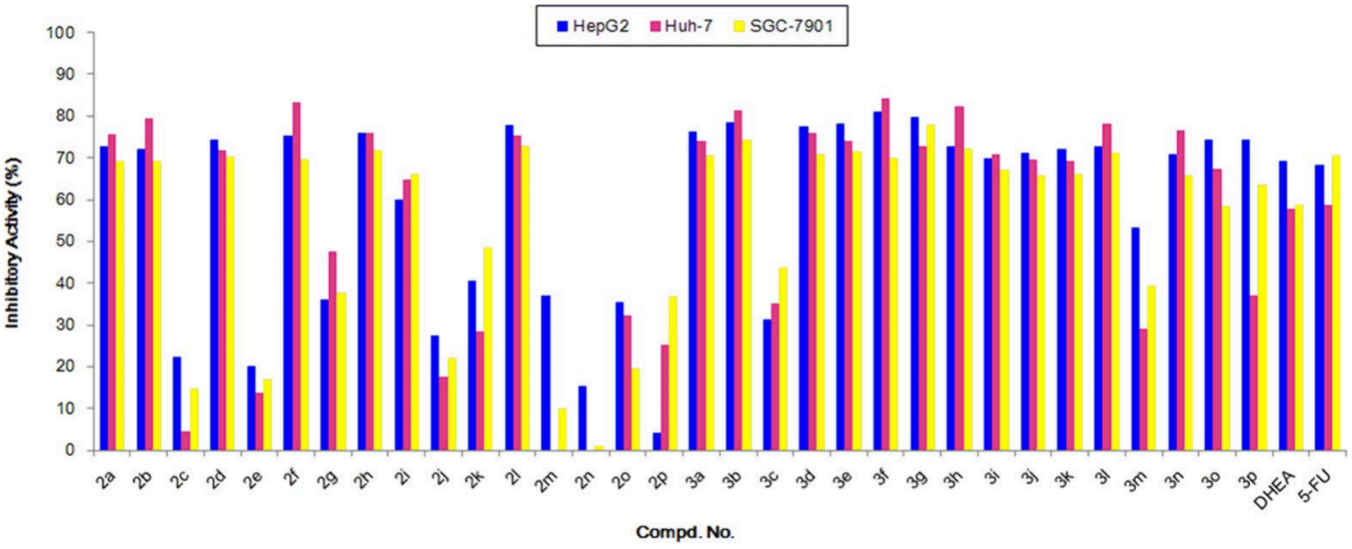

Figure 9. Antitumor activities of compounds **117a−p**$^{(2a\text{-}p)}$ and **118a−p**$^{(3a\text{-}p)}$ at 40 μg/mL.[25] Copyright 2017, The Author(s).

vitro activity. Compound **58**, in its unsubstituted form, led to reduced weights of the ventral prostate, dorsal prostate, and epididymis, with significance observed only in the vas deferens. Upon *N*-alkylation, the resulting methyl **59** and allyl **61** derivatives caused a significant reduction in the weight of the ventral prostate. Additionally, the *N*-allyl derivative also significantly reduced the weight of the vas deferens (Table 11).

2.7. Methoxyformyl Dehydroepiandrosterone Derivatives. Marquez and teammates synthesized 16-formyl-17-methoxy dehydroepiandrosterone derivatives **68a−j** through a series of Vilsmeier−Haack, addition-substitution, and esterification reactions (Scheme 9) in 2016,[18] and the in vitro effect of **68a−j** was investigated on the activity of 5α-reductase type 2 (5AR-2) obtained from the human prostate. Among them, the presence of an aliphatic ester moiety at the C-3 position of these derivatives enhances their in vitro potency as inhibitors of 5AR-2 activity compared to finasteride, recognized as a potent inhibitor of 5AR-2 (Figure 4). In this case, the augmentation of the lipophilicity of these dehydroepiandrosterone derivatives increased their potency as inhibitors of 5AR-2. However, the

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Table 19. In Vitro Cytotoxic Activity (IC_{50}) of the Steroidal Derivatives (117a−p and 118a−p)

s. no.	compounds	IC_{50}[a] (μM)		
		HepG2[b]	Huh-7[b]	SGC-7901[b]
1.	**117a**	31.74 ± 6.11	29.24 ± 5.66	31.66 ± 7.71
2.	**117b**	29.21 ± 2.78	27.67 ± 3.71	27.04 ± 4.58
3.	**117c**	>95	>95	>95
4.	**117d**	35.24 ± 3.96	29.60 ± 7.00	30.04 ± 5.18
5.	**117e**	>100	>100	>100
6.	**117f**	24.09 ± 4.91	18.30 ± 2.14	21.63 ± 3.31
7.	**117g**	>90	>90	>90
8.	**117h**	33.50 ± 3.44	27.17 ± 5.72	25.06 ± 5.07
9.	**117i**	72.21 ± 9.40	50.88 ± 9.64	34.25 ± 6.10
10.	**117j**	>95	>95	>95
11.	**117k**	>90	>90	>90
12.	**117l**	16.68 ± 2.55	15.93 ± 3.28	18.85 ± 3.43
13.	**117m**	>100	>100	>100
14.	**117n**	>100	>100	>100
15.	**117o**	>100	>100	>100
16.	**117p**	>85	>85	>85
17.	**118a**	17.94 ± 2.19	12.98 ± 3.25	20.49 ± 3.76
18.	**118b**	5.41 ± 1.34	5.65 ± 1.02	10.64 ± 1.49
19.	**118c**	>85	>85	>85
20.	**118d**	9.58 ± 3.60	12.53 ± 2.05	17.33 ± 2.61
21.	**118e**	16.80 ± 2.91	21.92 ± 1.96	23.61 ± 3.05
22.	**118f**	11.18 ± 2.46	13.87 ± 2.26	14.88 ± 1.53
23.	**118g**	12.17 ± 3.91	7.43 ± 1.39	11.11 ± 0.83
24.	**118h**	15.34 ± 3.33	6.02 ± 0.25	13.35 ± 3.52
25.	**118i**	14.77 ± 4.52	8.69 ± 1.21	18.88 ± 3.36
26.	**118j**	19.60 ± 5.17	20.57 ± 5.75	25.71 ± 2.07
27.	**118k**	20.42 ± 2.86	22.85 ± 6.42	24.67 ± 4.49
28.	**118l**	6.59 ± 1.78	5.38 ± 1.84	15.71 ± 2.34
29.	**118m**	84.48 ± 6.77	>95	>95
30.	**118n**	22.84 ± 5.09	25.10 ± 6.87	33.97 ± 6.44
31.	**118o**	29.86 ± 7.30	35.34 ± 10.09	60.77 ± 2.67
32.	**118p**	32.33 ± 6.86	>75	40.63 ± 2.23
33.	DHEA	39.04 ± 10.26	>100	>95
34.	5-FU[c]	>100	>95	>100

[a]IC_{50}: Compound concentration required to inhibit tumor cell proliferation by 50%; [b]Abbreviations: HepG2 (Human hepatocellular liver carcinoma cell line), Huh-7 (Human hepatoma cell line), SGC-7901 (Human gastric cancer cell line); [c]5-FU (5-Fluorouracil): used as a positive control.[25]

presence of cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, or cycloheptyl rings as the cycloaliphatic ester moiety at C-3 of the formyl methoxy dehydroepiandrosterone scaffold did not inhibit the activity of this enzyme. This could be attributed to the presence of steric factors arising from the spatial structure of these derivatives interacting with the enzyme (Figure 5).

2.8. Pyrazole and Imidazole Dehydroepiandrosterone Derivatives. Cabeza and co-workers reported the synthesis of various novel DHEA derivatives with pyrazole or imidazole moiety at C-17 and an ester moiety at C-3 (Scheme 10) in 2016,[19] which were tested as inhibitors of enzyme 17β-HSD5 (17β-hydroxysteroid dehydrogenase type 5). Inhibition of 17β-HSD5 by these DHEA derivatives could offer therapeutic potential for treating prostate cancer and benign prostatic hyperplasia. All derivatives inhibited the 17β-HSD5 enzyme. Compound **71a** exhibited the highest potency (IC_{50} = 0.15 ± 0.02 nM) followed by **71c** (IC_{50} = 0.5 ± 0.09 nM), **7** (IC_{50} = 0.66 ± 0.32 nM), **71f** (IC_{50} = 0.79 ± 0.003 nM), **71b** (IC_{50} = 3.2 ± 0.9 nM), **71d** (IC_{50} = 90 ± 0.02 nM), and **71e** (IC_{50} 17β-HSD5 = 95 ± 3 nM) (Figure 6). Therefore, adding a pyrazole or imidazole group at C-17 on the DHEA skeleton, along with an ester moiety at C-3 (compounds **71a−d** and **71f**), enhanced the ability of these steroids to inhibit the 17-βHSD5 enzyme. This ester moiety increased the molecule's lipophilicity, resulting in effective in vitro inhibition of the enzyme. Overall, the incorporation of a pyrazole ring or its isomer, imidazole, at C-17 led to strong 17β-HSD5 inhibition using the human prostate membrane fraction as a source.

2.9. Perhydrophenanthrene Dehydroepiandrosterone Derivatives. Higuchi et al. in 2016, developed a concise method for the synthesis of the Arg-Gly-Asp (RGD) mimics perhydrophenanthrene compounds **81** and **85** from DHEA **1** via intermediate **78,** and their antiplatelets activity was tested.[20] Intermediate **78** was formed by the methylation of carboxylic acid **76b**/deacetalization of **76a** and subsequent ozonolysis of the enol acetate of compound **77** (Scheme 11).[20]

2.10. Benzimidazolyl Dehydroepiandrosterone Derivatives. Likewise, the Marquez and Aggarwal group and Arellano and co-workers reported the synthetic strategy toward seven novel DHEA derivatives with benzimidazole moiety at C-17 (Scheme 12) in 2016 and determined their effect on the activity of 5α-reductase types 1 and 2 (5AR-1 and R-2) isoenzymes.[21]

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Scheme 18. Synthesis of DHEA triazole derivatives 123a−w

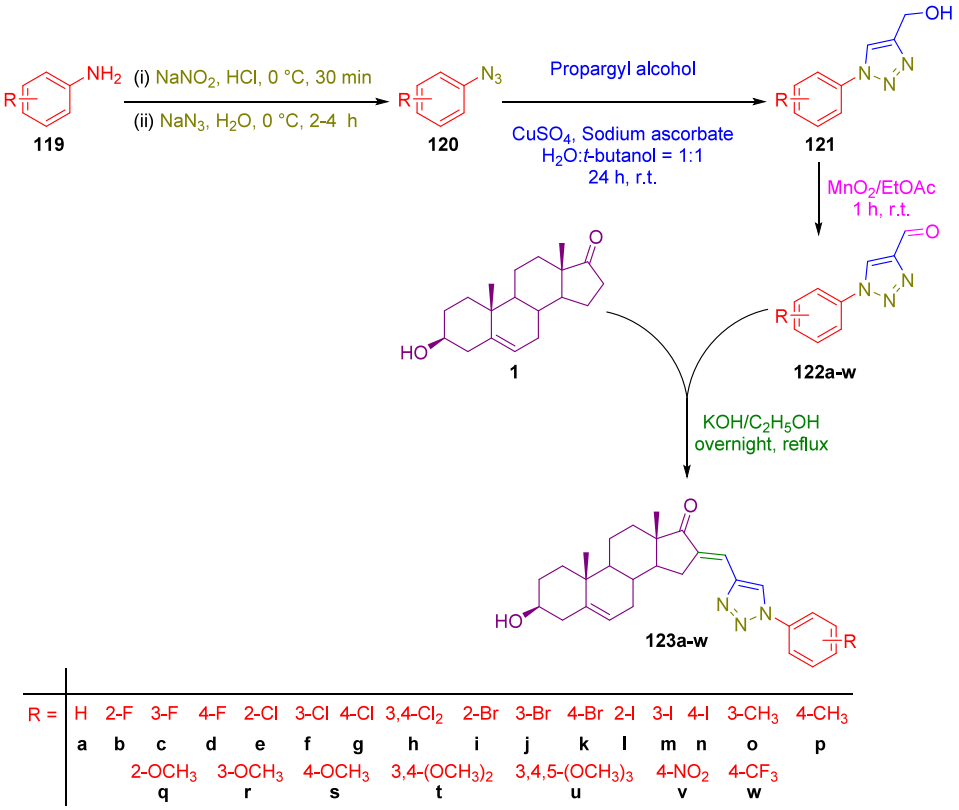

Table 20. Antiproliferative Activity of Compounds 123a−w (% Growth Inhibition at 100 μM)

s. no.	compounds	% growth inhibition (100 μM)						
		A549	Hela	HepG-2	BEL7402	HCT116	MCF-7	L02
1.	DHEA	24.8	15.7	10.9	46.5	47.3	17.1	44.6
2.	**123a**	30.3	N.A.[a]	10.2	12.9	11.1	N.A.[a]	22.3
3.	**123b**	N.A.[a]	N.A.[a]	N.A.[a]	N.A.[a]	N.A.[a]	N.A.[a]	N.A.[a]
4.	**123c**	60.5	55.2	78.8	66.7	78.3	31.2	79.3
5.	**123d**	68.8	78.9	65.6	60.4	84.9	83.0	80.3
6.	**123e**	N.A.[a]	32.8	N.A.[a]	10.5	23.0	37.3	12.4
7.	**123f**	11.9	9.2	51.7	36.5	41.9	41.0	60.2
8.	**123g**	69.8	62.8	32.5	66.9	78.7	39.9	40.9
9.	**123h**	70.7	68.7	48.2	70.1	74.4	80.0	67.9
10.	**123i**	N.A.[a]	17.0	N.A.[a]	N.A.[a]	20.2	N.A.[a]	N.A.[a]
11.	**123j**	N.A.[a]	20.5	10.8	37.6	42.0	42.1	43.8
12.	**123k**	47.8	30.7	66.4	N.A.[a]	N.A.[a]	45.5	11.1
13.	**123l**	N.A.[a]	N.A.[a]	N.A.[a]	N.A.[a]	N.A.[a]	N.A.[a]	N.A.[a]
14.	**123m**	N.A.[a]	N.A.[a]	N.A.[a]	14.1	7.6	N.A.[a]	N.A.[a]
15.	**123n**	46.8	17.3	83.0	N.A.[a]	65.5	81.3	34.2
16.	**123o**	N.A.	12.7	12.8	30.5	40.9	11.2	40.1
17.	**123p**	63.1	64.6	76.0	23.7	76.5	21.7	67.1
18.	**123q**	7.6	N.A.[a]	N.A.[a]	10.0	14.3	34.6	N.A.[a]
19.	**123r**	N.A.[a]	N.A.[a]	N.A.[a]	N.A.[a]	N.A.[a]	N.A.[a]	16.9
20.	**123s**	44.0	14.9	42.3	38.4	70.7	58.1	58.5
21.	**123t**	N.A.[a]	N.A.[a]	N.A.[a]	N.A.[a]	N.A.[a]	N.A.[a]	9.4
22.	**123u**	40.3	60.5	59.1	51.2	59.2	36.3	36.4
23.	**123v**	N.A.[a]	N.A.[a]	12.4	13.2	10.2	N.A.[a]	5.5
24.	**123w**	75.2	44.1	48.3	62.2	75.0	41.3	57.6

[a]N.A.: antiproliferative activity <5%.[2]

The isozymes 5AR-1 and R-2 play an important role in prostate gland development, as they are found within this gland and exhibit distinct locations and biochemical characteristics. The derivatives with an aliphatic ester at C-3 of the dehydroepiandrosterone scaffold **88a** induced specific inhibition of 5AR-1 activity since no effect of **88a** on 5AR-2 activity was detected.

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Table 21. IC_{50} (μM) Values of Some Active Compounds

s. no.	compounds	IC_{50}[a] (μM)						
		A549	HeLa	HepG-2	BEL7402	HCT116	MCF-7	L02
1.	**123c**	51.64	83.52	11.07	68.86	26.32	>100	83.37
2.	**123d**	15.80	13.21	36.19	40.41	33.25	17.75	27.75
3.	**123g**	37.38	57.3	>100	58.22	23.37	>100	>100
4.	**123h**	17.46	15.11	>100	15.96	11.86	14.93	26.36
5.	**123k**	>100	>100	37.11	>100	>100	>100	>100
6.	**123n**	>100	>100	9.10	>100	31.04	9.18	>100
7.	**123p**	56.72	62.42	18.94	>100	44.07	>100	62.23
8.	**123s**	>100	>100	>100	>100	56.91	69.4	81.08
9.	**123w**	72.55	>100	>100	94.03	22.65	>100	93.69
10.	5-FU[b]	23.65	34.61	10.59	21.30	24.80	28.11	19.12

[a]IC_{50}: Concentration that inhibits 50% of cell growth; [b]5-FU: 5-Fluorouracil.[2]

Scheme 19. Synthesis of Abiraterone acetate 127

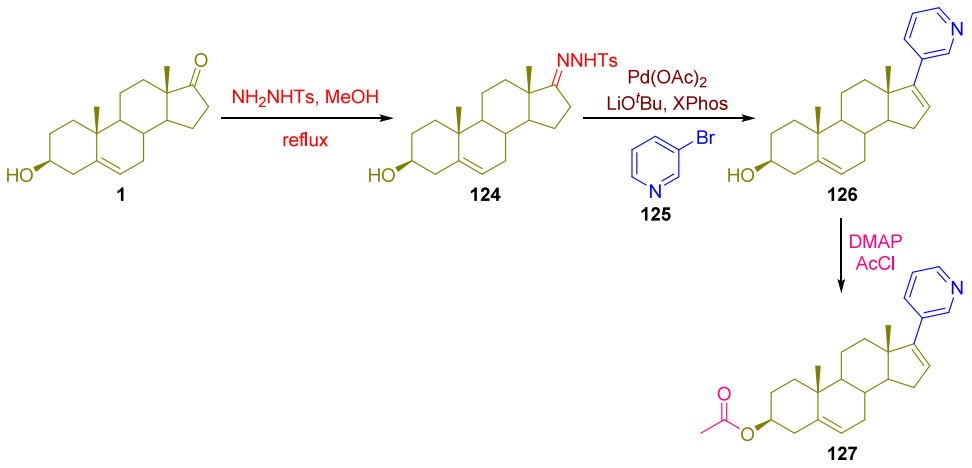

Table 22. IC_{50} (±SD) Values of Compound 139g and Cisplatin[27]

s. no.	compounds	IC_{50}					
		MRC-5	MCF-7	PC-3	A549	HeLa	U2Os
1.	**139g** (μM)	8.9 ± 1.1	6.2 ± 1.0	4.9 ± 1.1	7.9 ± 1.0	4.0 ± 1.0	3.5 ± 1.0
2.	cisplatin (nM)	37.72 ± 1.2	338.5 ± 1.1	902 ± 1.6	369.8 ± 1.2	254.5 ± 1.2	342.8 ± 1.2

Whereas, those with a cycloaliphatic ester (cyclopropyl **88b**, cyclobutyl **88c**, or cyclopentyl ring **88d**) or an alcohol group at C-3 of the dehydroepiandrosterone scaffold **89** inhibited the activity of both isozymes. The potency of these steroids decreased with the increasing size of the cycloaliphatic ring. Derivatives with a cyclohexyl **88e** or cycloheptyl ester **88f** at C-3 showed no inhibitory activity for either isoenzyme. **88b** and **88c** showed higher potency than finasteride, which is considered to be a potent inhibitor of 5AR-2, with IC_{50} values 0.004, 0.008, and 0.0085, respectively, thus specifying that **88b** and **88c** could have therapeutic potency (Figure 7).

2.11. Peptidomimetics Dehydroepiandrosterone Derivatives. Wang et al. (2016) reported a series of novel peptidomimetics bearing DHEA moiety (**94a−m** and **95a−l**) that was designed and synthesized using *N*-protected amino acids **91a−m** and DHEA-17 hydrazone **92**/DHEA-17 oxime **93** through nucleophilic substitution (Scheme 13).[22] Besides, the inhibition activity of the newly prepared compounds was evaluated against HepG2 (human hepatoma carcinoma), A549 (human lung), and A875 (human melanoma) cancer cell lines using the standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay compared with 5-FU (5-Fluorouracil) as a positive control. The majority of the compounds had higher inhibitory actions than 5-FU, as demonstrated in Figure 8. When compared to 5-FU (56.6−65.3%), the compounds **94(a, b, i, j, k, l)**, and **95(d, k, l)** showed strong inhibitory activity against all three cell lines examined, with 70.1−86.4% growth inhibition at 40 μg/mL. It is also worth noting that compound **95e** showed selective cytotoxicity to the A549 and A875 cell lines with 62.1% and 62% growth inhibition respectively, and with 31% inhibitions to HepG2 cell lines. As a result, the IC_{50} values were further analyzed to investigate the potential activities (Table 12). The results also show that under the same conditions, some of the designed peptidomimetics derivatives had stronger inhibitory activity than the control 5-FU. Compound **95d** exhibited the strongest inhibitory effect against HepG2, with an IC_{50} value of 7 μM, and compound **94k** exhibited the strongest inhibitory effect against A549 and A875 with an IC_{50} value of 6 and 13 μM, respectively. Specifically, compounds **94i** (IC_{50} < 14 μM) and **94k** (IC_{50} < 13 μM) exhibited noticeable inhibition activities against all tested cancer cell lines.

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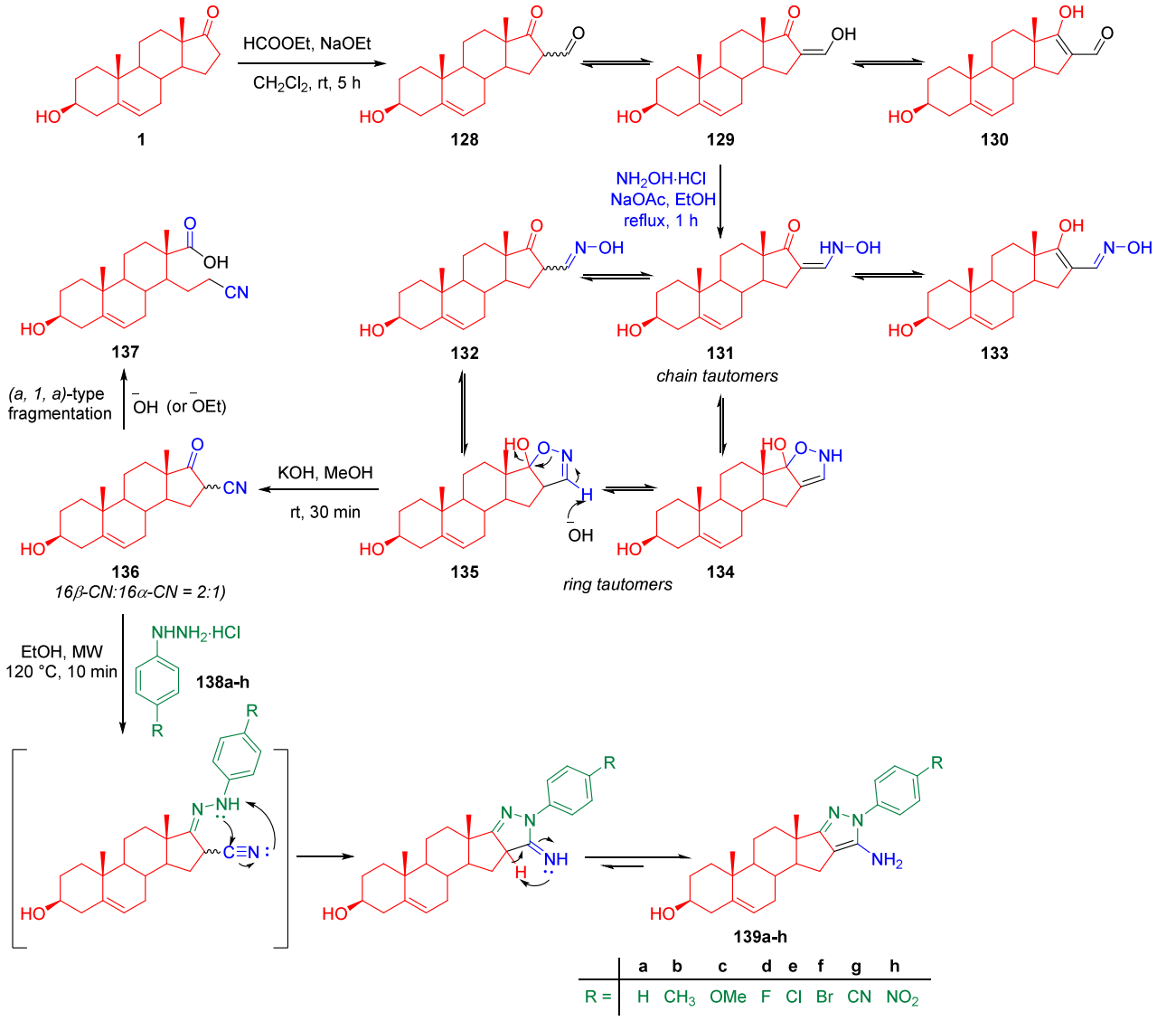
Scheme 20. Synthesis of a Steroidal β-Ketonitrile (136) and D-Ring-Fused Steroidal 5-Amino-1-arylpyrazoles (139a–h)



Scheme 21. Synthesis of Steroidal Benzisoselenazolones (146a–j) by Incorporating Benzisoselenazolone Scaffolds (143a–j)

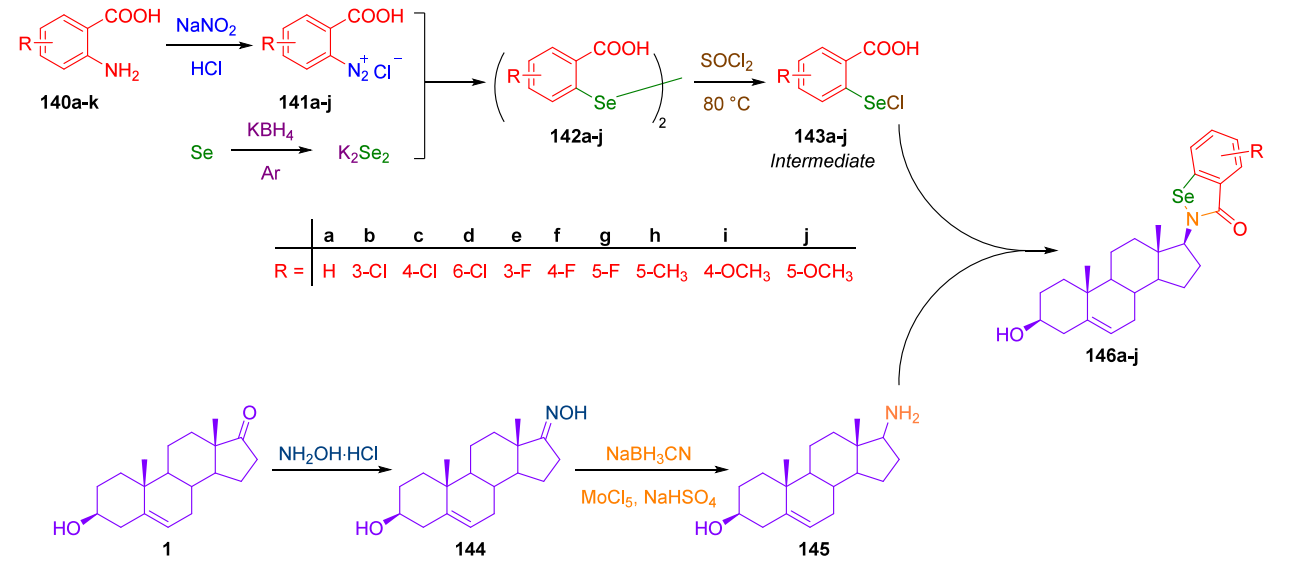


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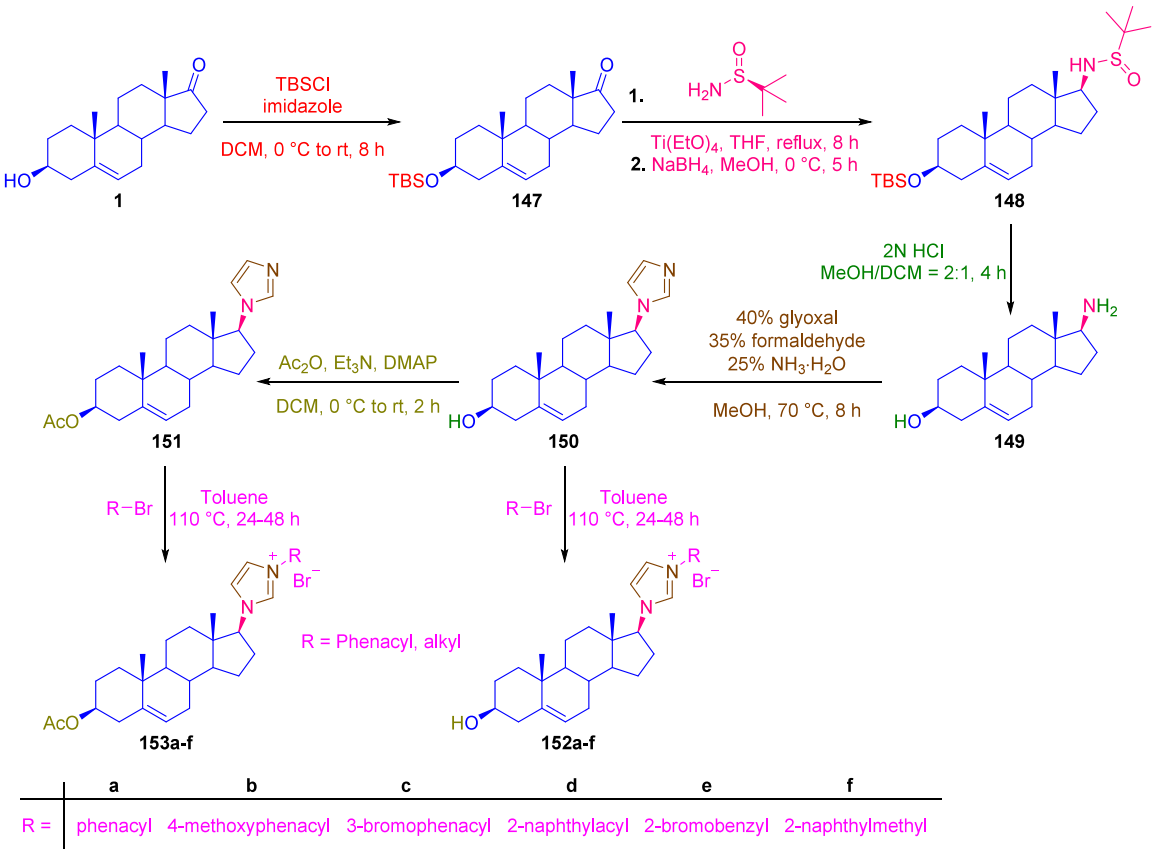
Table 23. In Vitro Antiproliferative Activities of the Compounds 146a−j[3]

s. no.	compounds	IC_{50} (μM)				
		SKOV-3	PC-3	T47D	MCF-7	HEK-293T
1.	**145**	>100	>100	>100	>100	76.58 ± 1.27
2.	**146a**	5.40 ± 3.61	40.75 ± 0.83	44.37 ± 1.18	40.48 ± 0.66	75.16 ± 1.32
3.	**146b**	>100	>100	>100	>100	>100
4.	**146c**	10.41 ± 0.37	16.78 ± 1.04	26.80 ± 1.72	23.39 ± 1.69	70.44 ± 0.16
5.	**146d**	>100	78.60 ± 6.99	84.42 ± 4.27	15.64 ± 1.54	>100
6.	**146e**	>100	>100	>100	>100	>100
7.	**146f**	6.49 ± 0.59	14.26 ± 0.77	35.39 ± 1.35	33.00 ± 2.01	68.19 ± 0.09
8.	**146g**	>100	>100	>100	>100	>100
9.	**146h**	14.13 ± 0.29	30.19 ± 0.48	36.46 ± 2.35	23.27 ± 1.53	60.03 ± 0.28
10.	**146i**	22.95 ± 1.28	29.53 ± 0.19	33.86 ± 0.20	34.24 ± 1.84	51.36 ± 0.60
11.	**146j**	36.52 ± 0.64	35.16 ± 0.12	36.01 ± 0.31	41.58 ± 2.51	74.10 ± 1.31
12.	Abiraterone	51.51 ± 15.98	37.61 ± 0.12	34.66 ± 1.69	44.70 ± 0.67	>100

Scheme 22. Synthesis of DHEA-Imidazolium Salt Derivatives (152a−f and 153a−f)



2.12. Phosphatidylcholines Containing Dehydroepiandrosterone Derivatives. Klobucki et al. in 2016, synthesized a series of new phosphocholines containing DHEA derivatives at *sn*-1 and/or *sn*-2 positions. Initially, a phospholipid with a DHEA moiety at the *sn*-1 and *sn*-2 position **98** was synthesized from a cadmium chloride complex of *sn*-glycero-3-phosphocholine **97** and DHEA hemisuccinate **96** (Scheme 14).[7] Succinic acid was used as a linker between the active drug and *sn*-glycero-3-phosphocholine to the formation of **96**. Next, two phosphocholines containing DHEA hemisuccinate at the *sn*-1 position **103a−b** were synthesized using stannylene acetal **101**, DHEA hemisuccinate chloride **99**, and lysophosphocholine **102**. Finally, the phosphocholines with DHEA hemisuccinate at the *sn*-2 position **105a−e** was synthesized by reacting DHEA hemisuccinate **96** with 1-acyl-2-hydroxy-*sn*-glycero-3-phosphocholines **104a−e**. Additionally, all the synthesized compounds were evaluated in vitro for their antiproliferative activities against Balb/3T3 (mouse embryonic fibroblasts), HL-60 (human promyelocytic leukemia cells), B16 (mouse skin melanoma cells), and LNCaP (human prostate cancer cells) cell lines in comparison with cisplatin as the positive control. The phosphocholines with DHEA at *sn*-1 and/or *sn*-2 positions (**98**, **102**, **103a,b**, and **105a−e**) exhibited no cytotoxic effects on the normal cell line (Balb/3T3). The most active compound **105c** showed a moderate cytotoxic effect against the HL-60 and B16 cell lines. Further, it was found that the compounds with DHEA hemisuccinate at the *sn*-1 position (**103a−b**) were more active than those with DHEA hemi-

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Table 24. In Vitro Cytotoxic Activities of Steroidal Imidazolyl Derivatives and Steroidal Imidazolium Salt Derivatives

s. no.	compounds	IC_{50}[a] (μM)				
		HL-60[b]	SMMC-7721[b]	A-549[b]	MCF-7[b]	SW480[b]
1.	**1**	>20	>20	>20	>20	>20
2.	**154**	>20	>20	>20	>20	>20
3.	**155**	>20	>20	>20	>20	>20
4.	Imidazole	>20	>20	>20	>20	>20
5.	Benzimidazole	>20	>20	>20	>20	>20
6.	5,6-Dimethyl-benzimidazole	>20	>20	>20	>20	>20
7.	**154xa′**	2.69	7.38	7.76	5.50	7.39
8.	**154xb′**	1.25	7.32	7.18	4.49	7.03
9.	**154xc′**	1.28	7.28	7.03	5.21	6.46
10.	**154xd′**	1.03	3.46	7.27	2.26	4.01
11.	**154xe′**	1.24	4.58	6.60	3.86	6.33
12.	**154xf′**	1.22	2.45	4.92	1.95	4.03
13.	**154x′a′**	1.09	7.15	7.30	5.18	8.18
14.	**154x′b′**	1.25	1.51	1.94	1.47	2.59
15.	**154x′c′**	1.25	1.71	1.55	1.43	1.65
16.	**154x′d′**	0.96	1.89	3.34	2.27	3.62
17.	**154x′e′**	0.43	0.95	1.22	1.41	1.66
18.	**154x′f′**	1.01	1.15	1.12	1.09	1.59
19.	**154ya′**	1.19	1.81	2.55	2.49	2.59
20.	**154yb′**	1.22	1.45	2.45	2.11	2.55
21.	**154yc′**	1.36	1.75	1.77	1.53	1.74
22.	**154yd′**	1.10	1.33	1.32	1.67	1.75
23.	**154ye′**	0.34	0.21	4.47	2.14	2.10
24.	**154yf′**	1.02	0.88	1.25	2.31	1.80
25.	**154za′**	0.44	0.90	9.57	2.91	3.22
26.	**154zb′**	0.74	0.44	5.02	2.31	2.01
27.	**154zc′**	0.98	1.39	7.15	2.78	2.22
28.	**154zd′**	0.45	0.57	5.53	3.05	3.10
29.	**154ze′**	0.49	0.44	0.67	0.73	0.79
30.	**154zf′**	0.42	0.59	5.12	1.90	1.60
31.	**154x′′a′**	1.18	2.14	1.86	5.66	7.96
32.	**154x′′b′**	0.94	1.61	1.21	1.96	4.15
33.	**154x′′d′**	1.37	5.49	3.49	5.83	6.55
34.	**154x′′e′**	0.92	2.27	2.99	1.88	5.37
35.	**155xa′**	1.53	1.29	7.28	6.98	6.06
36.	**155xb′**	1.52	1.10	6.68	6.54	7.18
37.	**155xc′**	1.42	1.22	7.84	8.70	8.23
38.	**155xd′**	2.41	1.23	8.53	8.00	6.25
39.	**155xe′**	5.53	1.16	7.65	10.83	10.90
40.	**155xf′**	1.64	1.04	7.67	8.04	7.48
41.	**155x′a′**	6.85	1.49	8.42	8.41	9.03
42.	**155x′b′**	6.00	2.90	13.22	5.25	4.87
43.	**155x′c′**	9.91	1.70	7.22	8.51	6.33
44.	**155x′d′**	10.63	1.88	8.82	>20	10.54
45.	**155x′e′**	1.59	0.94	7.36	6.02	8.32
46.	**155ya′**	0.88	1.65	7.51	7.18	10.18
47.	**155yb′**	4.41	1.45	7.66	9.85	10.10
48.	**155ye′**	4.47	1.56	7.31	8.97	10.60
49.	**155za′**	0.28	1.77	8.11	9.87	10.44
50.	**155ze′**	6.96	1.81	8.44	14.25	13.16
51.	**155x′′a′**	7.73	1.42	8.10	8.84	6.56
52.	**155x′′b′**	7.97	1.52	8.75	8.71	10.66
53.	**155x′′c′**	7.26	2.10	9.65	>20	11.28
54.	**155x′′d′**	2.35	1.44	8.05	7.62	7.75
55.	**155x′′e′**	5.39	1.41	6.95	8.35	9.29
56.	**152a**	>20	>20	>20	>20	>20
57.	**152b**	>20	>20	>20	>20	>20
58.	**152c**	6.42	14.90	>20	7.25	9.78
59.	**152d**	2.00	3.50	9.69	4.38	5.59

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Table 24. continued

s. no.	compounds	IC_{50}[a] (μM) HL-60[b]	SMMC-7721[b]	A-549[b]	MCF-7[b]	SW480[b]
60.	**152e**	6.30	7.63	>20	9.61	>20
61.	**152f**	2.68	4.41	7.87	5.08	6.47
62.	**153a**	6.42	12.03	>20	9.07	11.12
63.	**153b**	2.20	4.31	10.70	3.46	6.06
65.	**153c**	4.35	5.47	7.20	4.88	7.26
65.	**153d**	1.26	1.75	4.39	2.10	4.45
66.	**153e**	1.48	3.83	6.52	4.84	6.87
67.	**153f**	1.22	2.45	4.74	2.53	5.64
68.	cisplatin	2.11	11.27	6.94	17.43	17.05

[a]IC_{50} (μM): Concentration of a compound that reduced by 50% the optical density of treated cells with respect to untreated cells using the MTS assay; [b]Data represents the mean values of three independent determinations.[1]

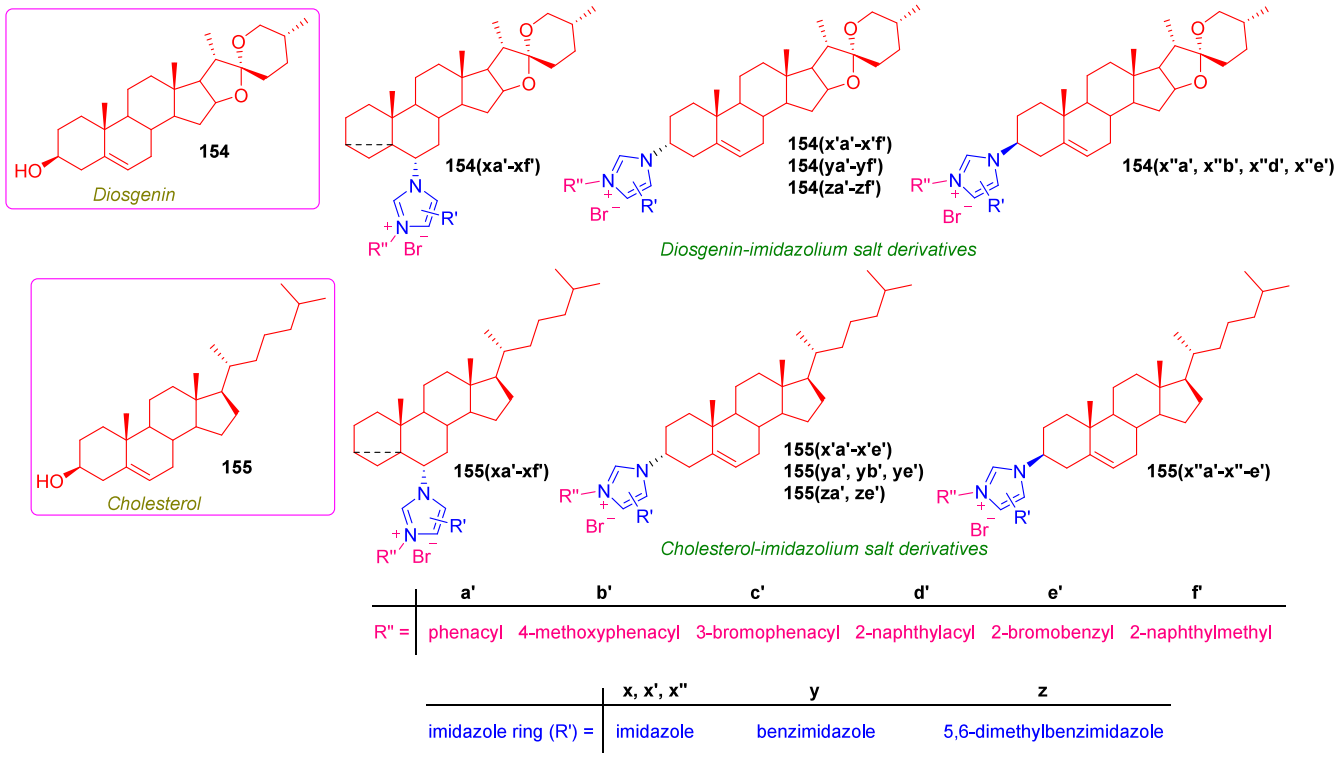

Figure 10. Diosgenin- and cholesterol-imidazolium salt derivatives.[1]

succinate at the *sn*-2 position (**105a−b** and **105d−e**) (Table 13). As a result, phospholipids containing DHEA in the *sn*-1 or *sn*-2 position showed adequate antiproliferative action against cancer cells, suggesting that they might be employed as anticancer drugs without harming noncancer cells.

2.13. Thiadiazole Dehydroepiandrosterone Derivatives. In 2016, Cui and co-workers reported the synthetic methods of D-ring fused 1,2,3-thiadiazole DHEA derivatives (**108**, **113a−e**, **114a-f**, and **115a−f**) (Scheme 15).[23] Compound **106** was prepared by a reaction of **24** with aminourea hydrochloride and sodium acetate in EtOH and compound **108** was obtained by condensing **106** with thionyl chloride, followed by hydrolysis with K_2CO_3 in MeOH. Reduction of the double bond at B-ring of **24** under H_2 and palladium in carbon in autoclave gave compound **109**. Compound **112** was prepared under the same procedure as compound **108**. Also, compounds **113a−e** were obtained by the reaction of compound **112** with corresponding acyl chlorides under DMAP in CH_2Cl_2. Compounds **115a−f** were obtained by condensation of **112** with corresponding *N*-Boc-amino-carboxylic acids under EDCI, HOBt, and DMAP in CH_2Cl_2, then deprotection of the Boc group under boron trifluoride etherate in Et_2O. Additionally, all the synthesized compounds were tested for antiproliferative activity against the growth of various tumor cell lines and one normal cell line including, breast cancer (T47D, MCF7, and MDA-MB-231), promyelocytic leukemia (HL60), prostate cancer (LNCaP and DU145), colon carcinoma (HCT116 and HT29), T lymphocyte (Jurkat), and normal human fibroblast cell (HAF), using the sulforhodamine B (SRB) assay. It is clear from the results that the T47D cell line was much more sensitive to the tested compounds (DHEA, EPI, **108**, and **112**) than other tumor cell lines. However, these compounds had weaker or no inhibitory activity for other cancer cell lines (Table 14). Exclusively, compound **112** shows around 2 and 4 times higher activity than epiandrosterone (EPI) and DHEA, respectively. Further, compound **112** and its derivatives were also tested on the T47D cell line using the SRB assay (Table 15). The results revealed that the alkyl esters (**111**, **113a,b**, and **115a,b**) and

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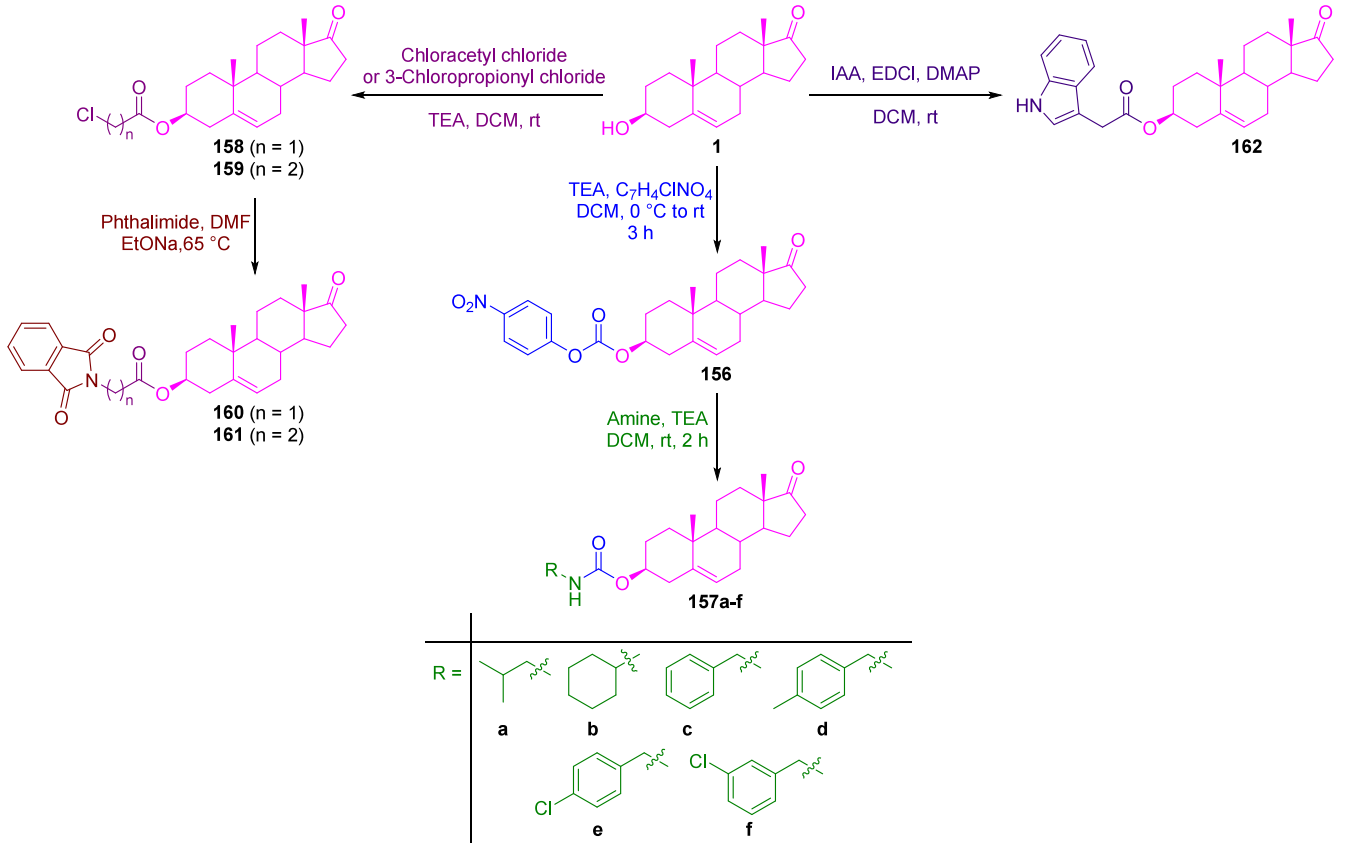
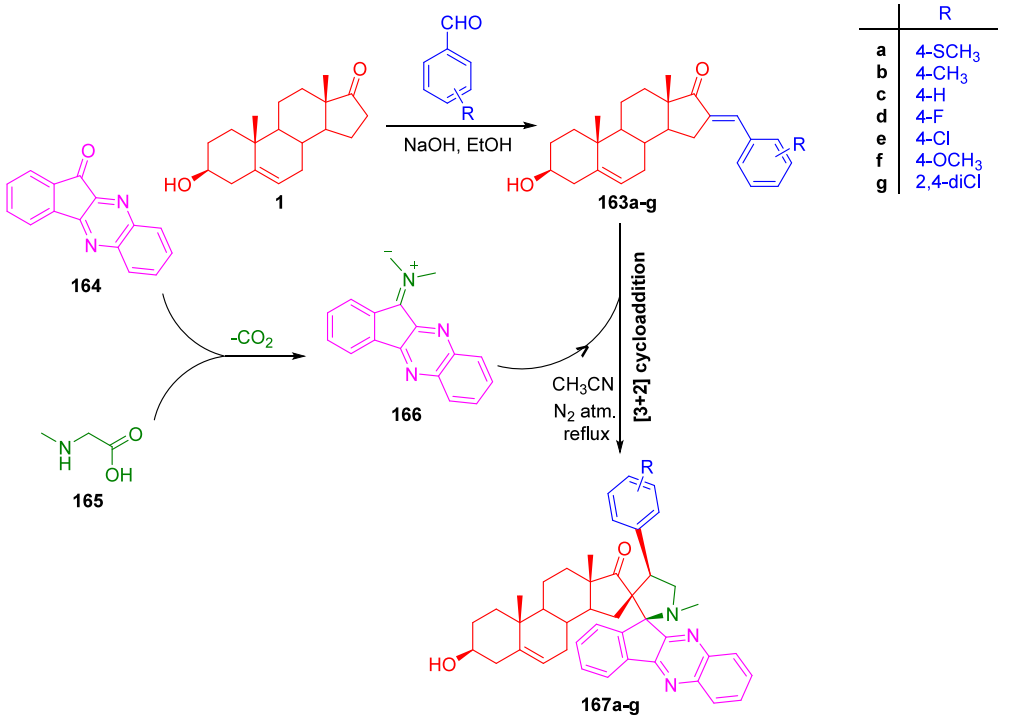
Scheme 23. Synthesis of DHEA-Carbamate (157a−f), -Phthalimide (160 and 161), and -Indoleacetic Acid (162) Derivatives



Scheme 24. Synthesis of DHEA Spiro-Pyrrolidine Derivatives 167a−g



aromatic *N*-heterocyclic esters (**113c−e**) at C-3 position were not favorable substituent for enhancing antitumor activity, leading to a lesser inhibition of cell growth compared to **112**. Steroidal derivatives (**115c−f**) with a saturated *N*-heterocycle in position C-3, had more potent antiproliferative activity than **112**. Also, the unnatural D-proline modified derivative **115f** (IC_{50} = 0.058 μM) had more potent antiproliferative activity than its natural L-proline derivative **115e** (IC_{50} = 0.24 μM) on

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Table 25. Brine Shrimp Bioassay Data of Raw Materials, Products, and Podophyllotoxin

s. no.	compounds	LC_{50} (μg/mL)
1.	**163a**	>200
2.	**163b**	>200
3.	**163c**	117.54
4.	**163d**	>200
5.	**163e**	120.42
6.	**163f**	>200
7.	**163g**	>200
8.	**164**	72.05
9.	**167a**	20.86
10.	**167b**	27.20
11.	**167c**	20.36
12.	**167d**	6.19
13.	**167e**	24.59
14.	**167f**	14.30
15.	**167g**	9.92
16.	sarcosine	>200
17.	DHEA	>200
18.	podophyllotoxin	2.16
19.	DMSO	N.D.[a]

[a]N.D.: not detected.[32]

T47D cells. The most potent saturated *N*-heterocyclic derivatives **115c−e** had an antitumor effect comparable to the positive control ADM (adriamycin) on T47D cells, which was 44−60 times much more potent than DHEA (IC_{50} = 2.55 μM) (Table 16). To advance the research, compound **112** and its derivatives were chosen for the selectivity test on a normal human fibroblast (HAF) cell line using the SRB assay. The selectivity indexes (SI) were calculated by IC_{50} values in HAF cells divided by IC_{50} values in the T47D cell line. The results, presented in Table 15, indicated that most of the tested compounds were less toxic to HAF cells than to tumor cells. In particular, compound **115f** possessed the highest selectivity (SI = 364), which was 214-fold better than the positive control ADM (SI = 1.7).

2.14. Arylidene Dehydroepiandrosterone Derivatives. Singh and co-workers reported neuroprotective steroids **116a−d** in 2017, which were synthesized using base-catalyzed aldol condensation of DHEA with desired aromatic aldehydes (Scheme 16)[24] and found to be effective for the treatment of various neurodegenerative illnesses such as Alzheimer's and Parkinson's disease. As a result, all of the synthesized compounds were tested as neuroprotective agents in lipopolysaccharide (LPS)-treated animal models. Compound **116c** was found to be the most active neuroprotective agent among all the steroidal derivatives **116a−d**, producing effects comparable to standard drug celecoxib at a much lower dose and better than dexamethasone at the same dose, as shown in Tables 17 and 18.[24]

2.15. Pyrimidine Dehydroepiandrosterone Derivatives. In 2017, Ke and co-workers designed a series of 16 heterocyclic steroidal[17,16-*d*]pyrimidine derivatives **118a−p** and prepared them from DHEA **1** by a sequence heterocyclic transformation (Scheme 17),[25] and in vitro anticancer activities for these compounds were tested against three human cancer cell lines like HepG2 (hepatocellular liver carcinoma), Huh-7 (hepatoma carcinoma), and SGC-7901 (gastric carcinoma). Generally, as depicted in Figure 9, the majority of the heterocyclic steroidal compounds **118a−p** had higher cytotoxic activities than the comparable steroidal intermediates **117a−p**. Compounds **118a**, **118b**, **118d−h**, and **118l** displayed admirable inhibitory activities against all three cell lines with 70−82% growth inhibition at the concentration of 40 μg/mL compared to 5-FU (58.6−70.5%) as a positive control. As a result, the compounds **118a**, **118b**, **118d−h**, and **118l** were found to have higher inhibitory activities than the natural compound DHEA (57.9−69.4%), indicating that these heterocyclic steroidal compounds could be used as potential lead compounds in the development of novel anticancer drugs. To evaluate the extreme potential activities, the IC_{50} values for these compounds **118a−p** were investigated further and compared to **117a−p**, DHEA, and 5-FU. Some of the heterocyclic pyrimidine derivatives showed significantly good cytotoxic activities against all tested cell lines compared with 5-FU, especially, compound **118b** exhibited high potential growth inhibitory activities against all tested cell lines with the IC_{50} values of 5.41 ± 1.34, 5.65 ± 1.02, and 10.64 ± 1.49 μM, respectively, as shown in Table 19. In addition, the possible structure−activity relationships (SARs) for these prepared steroidal derivatives can be determined. For the pyrimidine ring system, the compounds with 2-ClPh and 3,4,5-$(MeO)_3$Ph groups have the highest potential activities, however, when the substituents R are 2-Py, 3-Py, 4-Py, and 3-PhOPh, the efficacy is minimal. Also, among the series of pyridine derivatives, the compound **118m** with the pyridin-2-yl substituent had a significantly lower cytotoxic effect. In contrast, for the series of aromatic enone moieties, the compounds containing 3,4,5-$(MeO)_3$Ph and 2-CF_3Ph groups had higher activity than the compounds bearing other substituents. Table 19 also reveals that the ortho-substituted compound is better than the para-substituted compound among the series of halogen derivatives. Especially, the compounds containing the 3,4,5-$(MeO)_3$Ph group (**117l** and **118l**) all show good inhibitory activities, which could be owing to the steric size of the trisubstituted phenyl group being favorable for receptor binding. Thus, these preliminary structure−activity relationships were to categorize the target pyrimidines derivatives **118a−p** that might be used as lead anticancer compounds in drug discovery.

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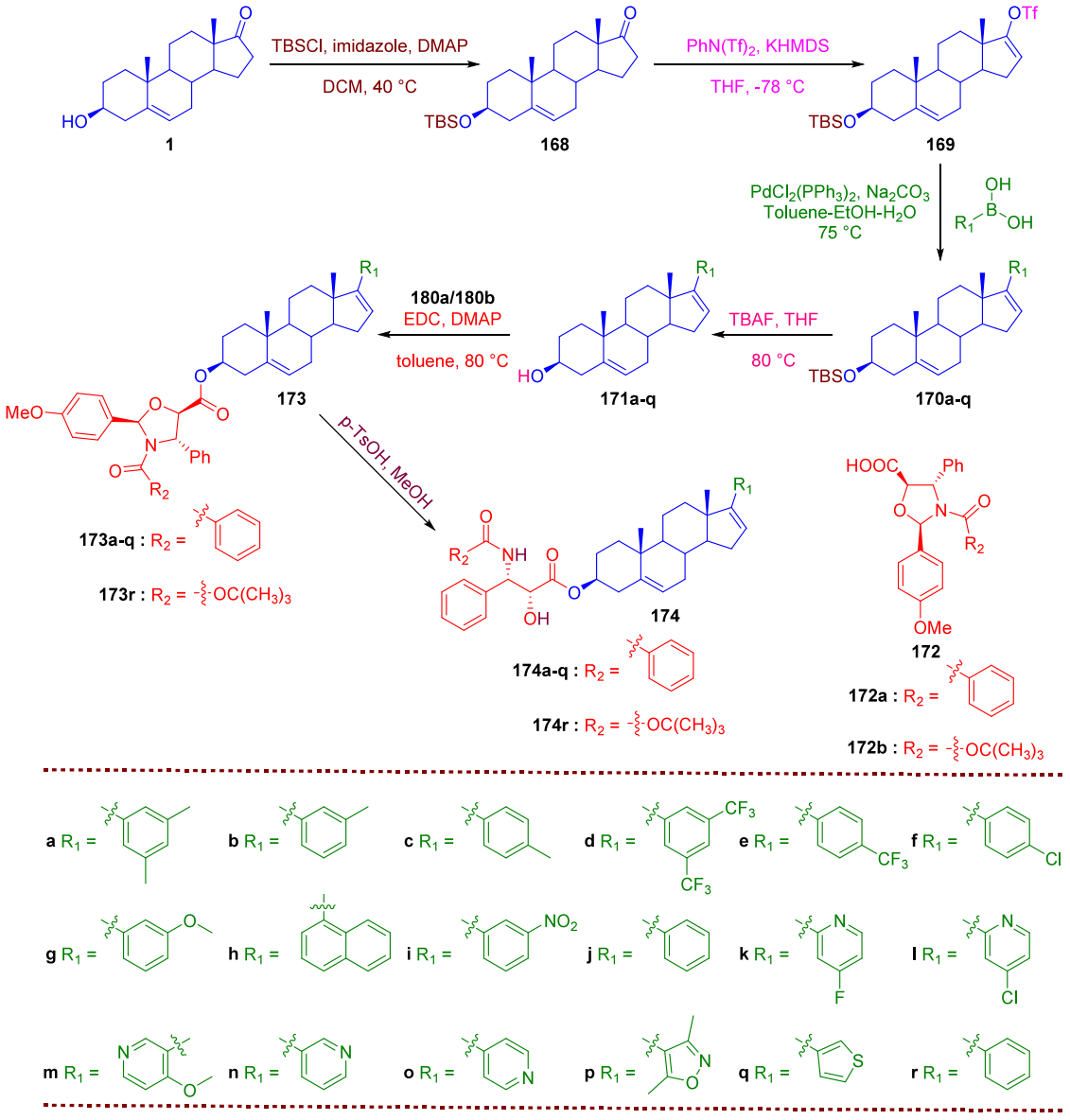
Scheme 25. Synthesis of Designed Paclitaxel-DHEA Derivatives (174a−r)



2.16. Triazole Dehydroepiandrosterone Derivatives. Huang et al. reported the synthesis of a series of novel DHEA derivatives **123a−w** containing triazole at the C_{16} position (Scheme 18) in 2018.[2] Initially, the diazotization of substituted aniline takes place to generate substituted 1-azidobenzene **120**, which then reacts with propargyl alcohol in the presence of $CuSO_4{\cdot}5H_2O$ and sodium ascorbate to produce substituted phenyltriazole **121** in the second step. Finally, the oxidation of **121** by MnO_2 takes place and gave **122a−w**. Subsequently, compounds **123a−w** were obtained with good yield by reacting DHEA with **122a−w** in anhydrous C_2H_5OH under the catalysis of KOH. Further, the antiproliferative activity of all synthesized compounds **123a−w** was also evaluated against six human cancer cell lines, including HeLa (cervical), A549 (lung), HepG-2 (liver), BEL7402 (liver), MCF-7 (breast), and HCT116 (colorectal) as well as one normal cell line, L02 (liver) and compared with DHEA and 5-fluorouracil (5-FU). As a result, Tables 20 and 21 show that several of the targeted compounds, **123n** exhibited significantly high inhibitory activity against HepG-2 (IC_{50} 9.10 μM) and MCF-7 (IC_{50} 9.18 μM) cell lines, while compound **123k** displayed inhibitory activity only against HepG-2, and for other cancer cells, this compound exhibited weaker activity. Compound **123h** displayed activity against all cancer cells except for HepG-2. In general, compounds with electron-withdrawing substituents on the 1,2,3-triazole ring exhibited potent activity against all six cancer cell lines, whereas those with electron-donating substituents on the 1,2,3-triazole ring exhibited no apparent activity. Only the 3-F substitution demonstrated substantial activity among the 3-substituent compounds, while all 2-substituent compounds showed no significant activity. The 3,4-Cl_2 substituent, on the other hand, showed extremely strong activity.

2.17. Pyridine Dehydroepiandrosterone Derivative. In 2018, Ma and co-workers synthesized abiraterone acetate **127** from DHEA **1** by a three-step reaction including the formation of tosylhydrazone, cross-coupling reaction, and acetylation. In the progress of the reaction, the Bamford-Stevens reaction involved using dehydroisoandrosterone-17-*N*-toluenesulfonyl-hydrazone **124** as an intermediate (Scheme 19).[26]

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Table 26. Anticancer Activity of Synthesized Paclitaxel-DHEA Hybrids (174a−r)[33]

s. no.	compounds	IC_{50} (μM) HepG-2
1.	**174a**	81.88
2.	**174b**	35.23
3.	**174c**	56.69
4.	**174d**	73.37
5.	**174e**	>300
6.	**174f**	69.35
7.	**174g**	34.5
8.	**174h**	46.78
9.	**174i**	26.39
10.	**174j**	>300
11.	**174k**	81.61
12.	**174l**	153.66
13.	**174m**	67.72
14.	**174n**	128.86
15.	**174o**	125.63
16.	**174p**	>300
17.	**174q**	>300
18.	**174r**	125.63
19.	taxol	0.78

Scheme 26. Synthesis of A-Homo Lactam D-Homo Lactone Androstane Derivative 179 from DHEA through Previously Reported D-Homo Lactone Androstane 175[35]

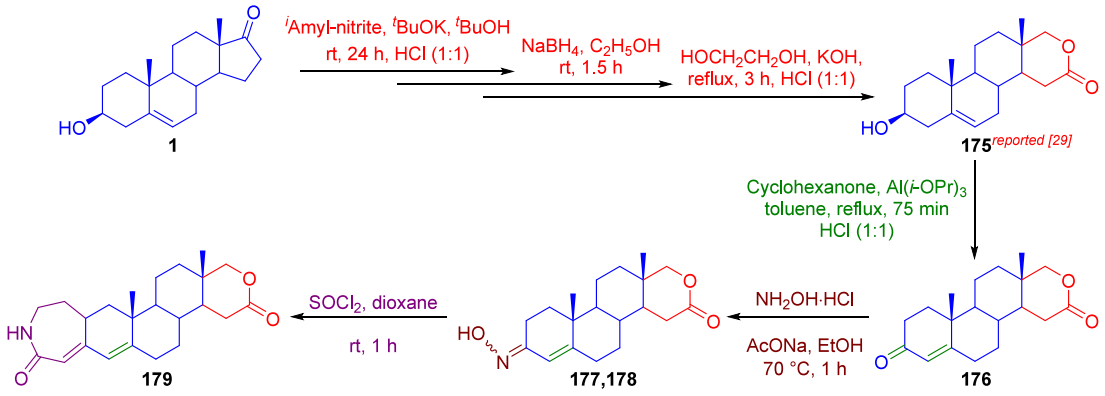

Table 27. In Vitro Cytotoxicity of the Tested Compounds

		IC_{50} (μM)			
s. no.	compounds	MCF-7	MDA-MB-231	PC3	MRC-5
1.	**175**[35]	>100	20.20	89.00	>100
2.	**176**[35]	>100	9.30	>100	>100
3.	**179**	>100	16.61	2.18	>100
4.	Doxorubicin[a]	0.75	0.12	95.61	0.12
5.	Formestane[a]	>100	55.50	48.36	>100

[a]Doxorubicin and Formestane were used as positive controls.[35]

2.18. Aminopyrazole Dehydroepiandrosterone Derivatives. In 2019, Motyan and co-workers developed a multistep reaction sequence from DHEA **1** to produce novel D-ring-condensed 5-amino-1-arylpyrazoles **139a−h**. The condensation reaction of 16-formyl-DHEA with hydroxylamine produced the respective oxime, which exhibited stability in one of its cyclic isoxazoline **134** forms, likely due to potential ring−chain tautomerism. The desired pyrazoles **139a−h** were obtained after base-induced dehydration to a diastereomeric steroidal β-ketonitrile **136**, followed by microwave-assisted heterocyclization with various arylhydrazines **138a−h** (Scheme 20).[27] However, the ^{1}H NMR spectra of compound **136** revealed the presence of two diastereomers in a 2:1 ratio, as evidenced by the appearance of duplicated proton peaks (16-H, 18-CH_3) at distinct chemical shifts and with different integrals. The peak shapes of the 16-H protons allowed us to distinguish the epimers; for the 16β-CN derivative, a triplet at 3.78 ppm (J = 9.3 Hz) was allocated, while a doublet at 4.36 ppm (J = 8.7 Hz) was assigned for the 16α-CN isomer. However, despite the elevated temperature and efficient heat transfer facilitated by the MW conditions, the ring closures were observed to be largely unaffected by the electron demand of the substituent R on the aromatic ring of the reagents. The only exception was the strong electron-withdrawing NO_2-group, where its strong electron-

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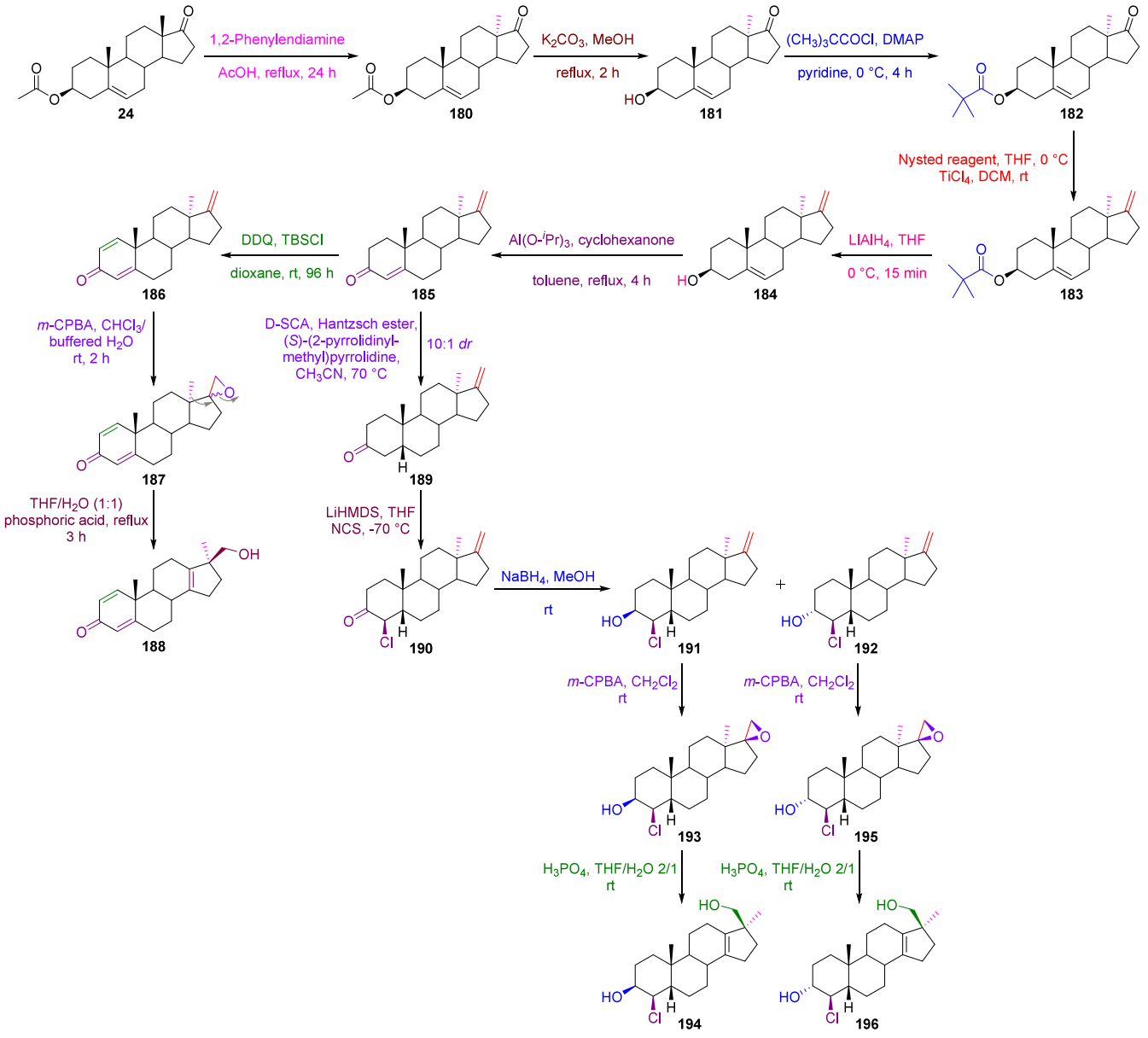
Scheme 27. Synthesis of D-Ring Modifications of Chlorohydrins (188, 194, and 196)



withdrawing effect hindered the ring-closure process, leading to a significant reduction in the yield of the desired product **139h**. Moreover, the antiproliferative effects of the 5-aminopyrazoles **139a−h** were investigated in vitro using human cancer cell lines, namely: HeLa (cervical), U2Os (osteosarcoma), MCF-7 (breast), PC-3 (prostate), A549 (lung), as well as noncancerous fibroblasts cell line (MRC-5). Compound **139g** had the strongest antiproliferative activity for all the tested cancer cells compared to the other compounds, which had less cancer-specific antiproliferative properties hence it was chosen for further studies among all the other compounds (Table 22).

2.19. Benzisoselenazolone Dehydroepiandrosterone Derivatives. Cui and colleagues reported the synthesis of steroidal benzisoselenazolone hybrids **146a−j** by incorporating benzisoselenazolone scaffold into DHEA (Scheme 21) in 2019.[3] Initially, the key intermediates **143a−j** with various substituent groups on the benzene ring were synthesized[28] and reacted with **145** to provide desirable products **146a−j**. The compound **145** was formed by the reduction of compound **144** using sodium cyanoborohydride as a reductant in the presence of molybdenum pentachloride and sodium hydrogen sulfate.[29] Moreover, the antiproliferative activity of all the synthesized compounds was investigated against four types of human hormone-related cancer cell lines, including SKOV3 (ovarian carcinoma), PC-3 (prostate carcinoma), T47D (breast infiltrating duct carcinoma), MCF-7 (breast adenocarcinoma) as well as one normal cell line, HEK293T (kidney epithelial), using MTT assay. Some of the compounds exhibit superior inhibitory activity compared to abiraterone against tumor cell proliferation linked to human growth hormone, while showing lower cytotoxicity toward normal human cells. For compounds **146a−j**, a significant correlation exists between the antiproliferative activity and the substituent on the benzene ring of benzisoselenazolone.. When there is no substituent or there is an electron-withdrawing substituent, such as halogen, present on the 6-position of the benzisoselenazolone group, the compounds show an excellent antitumor activity, such as **146a**, **146c**, and **146f**, have an IC_{50} value of less than 10 μM to SKOV-3 cell.

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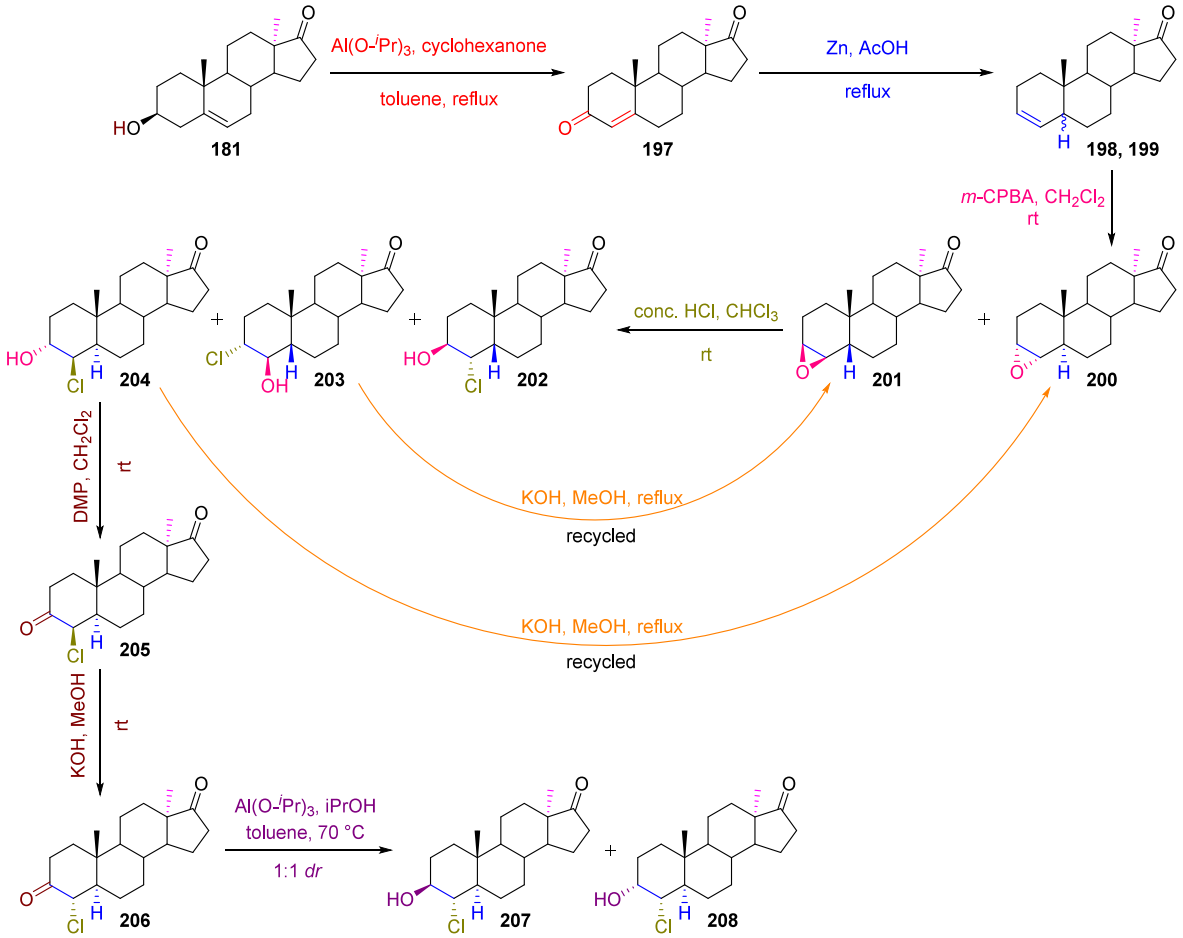
Scheme 28. Synthesis of 17-Keto-chlorohydrins (207, 208)



However, when the substituent is located elsewhere, the compound has little cytotoxicity to the tumor cells tested for the same type of substituent (**146b**, **146d**, **146e**, **146g**). Also, the compounds show moderate cytotoxicity when a substituent is an electron-donating group (**146h**–**j**). Additionally, compounds **146a**–**j** have higher cytotoxicity than that of their precursor compound **145** (Table 23).

2.20. Imidazolium Salt-Dehydroepiandrosterone Derivatives. In 2019, Deng and co-workers synthesized new DHEA-imidazolium salt derivatives (**152a**–**f** and **153a**–**f**) from DHEA **1** (Scheme 22).[1] In the beginning, compound **147** was prepared by the protection of the 3β-OH group of DHEA **1** with *tert*-butyl(dimethyl)silyl, followed by treatment of **147** with (R)-(+)-2-methyl-2-propanesulfinamide in the presence of Ti-(EtO)$_4$ to yield the corresponding imine, which was subsequently reduced to yield the 17β-tertbutylsulfinamido derivative **148**. Moreover, acid-mediated deprotection of the *N*-tert-butanesulfinyl and OH group gave amine **149** in good yield. Following that, an imidazole ring was added to C-17, yielding the key intermediate 17β-imidazolyl derivative **150**.[30] The 3β-OH group was further protected with acetyl to give **151**. Finally, as illustrated in Scheme 22, 12 DHEA-imidazolium salt derivatives **152a**–**f** and **153a**–**f** were produced from the coupling of **150** and **151** with several alkyl and phenacyl bromides, respectively. In addition, the cytotoxic potential of all the synthesized compounds was tested against five human cancer cell lines, including HL-60 (myeloid leukemia), SMMC-7721 (liver carcinoma), A-549 (lung carcinoma), MCF-7 (breast carcinoma), and SW480 (colon carcinoma) using the MTS assay. The results showed that the DHEA derivatives **152a**–**f** and **153a**–**f** were found to have lower cytotoxic activities (Table 24) than cholesterol- and diosgenin-imidazolium salt derivatives (Figure 10).

Furthermore, imidazolium salts with the 3β-AcO group (**153a**–**f**) had greater cytotoxic effects than those with the 3β-OH group (**152a**–**f**) in DHEA derivatives (**152a**–**f** and **153a**–**f**).

2.21. Carbamate, Phthalimide, and Indole-Linked Dehydroepiandrosterone Derivatives. Gan and group reported the synthesis of a series of DHEA derivatives synthesized by linking DHEA with active fragments such as carbamate, phthalimide, and indoleacetic acid (Scheme 23) in 2019.[31] For the synthesis of DHEA-carbamate, DHEA and TEA were dissolved in dry DCM with *p*-nitrophenyl chloroformate at 0 °C to room temperature to give intermediate **156**, which is further treated with the corresponding amine and TEA in DCM to form the desired product **157a**–**f**. Subsequent, for the synthesis of DHEA-phthalimide, acetylation of DHEA using chloroacetyl chloride or 3-chloropropionyl chloride to give intermediate compounds **158** and **159**, which are further treated with phthalimide to afford the final products **160** and **161**, respectively. In addition, the indole derivative **162** was synthesized using IAA and EDCI in dry DCM under argon in the presence of DMAP as a catalyst.

2.22. Spiro-Pyrrolidine Dehydroepiandrosterone Derivatives. In 2021, Tao and colleagues reported the facile

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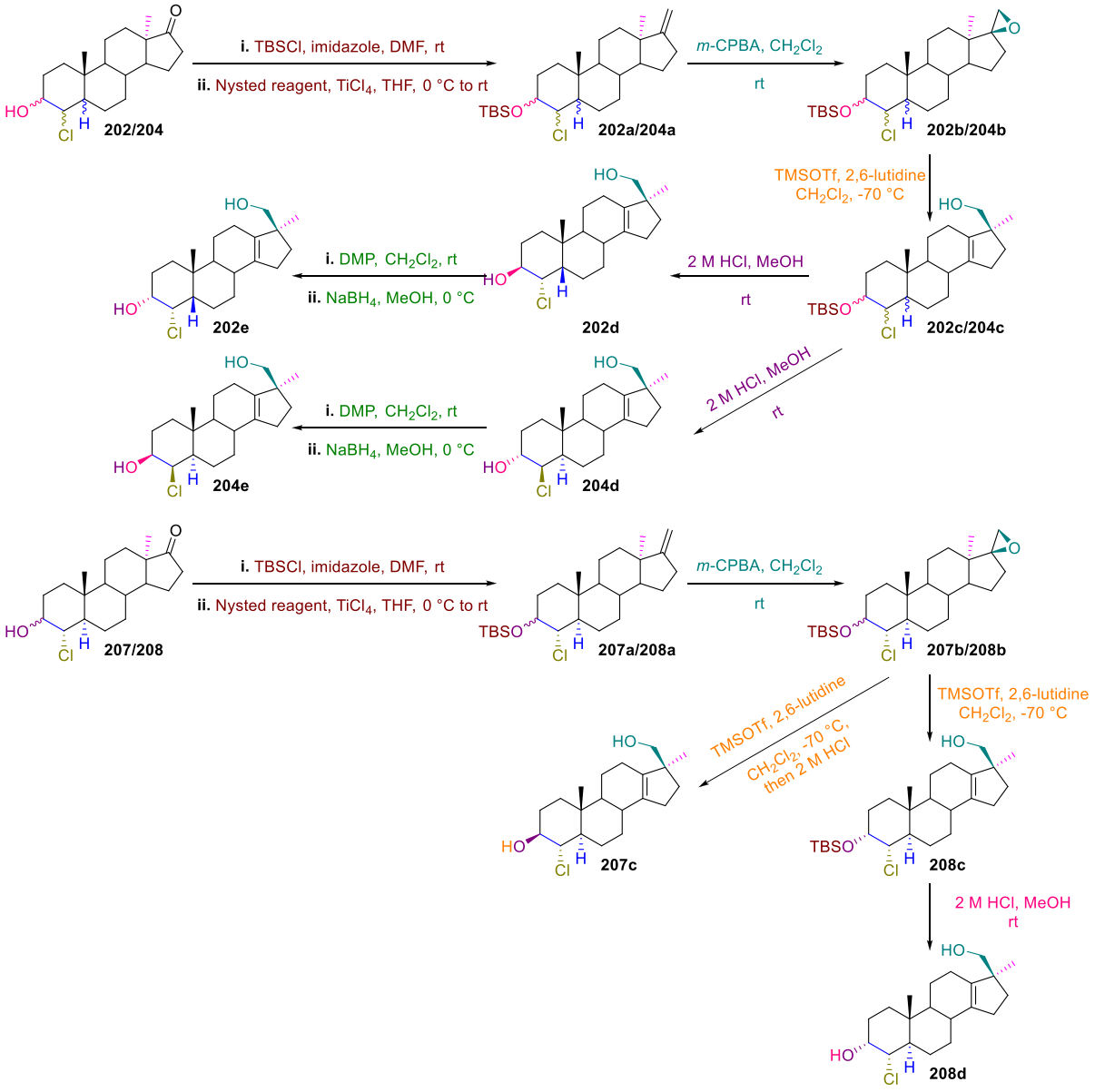
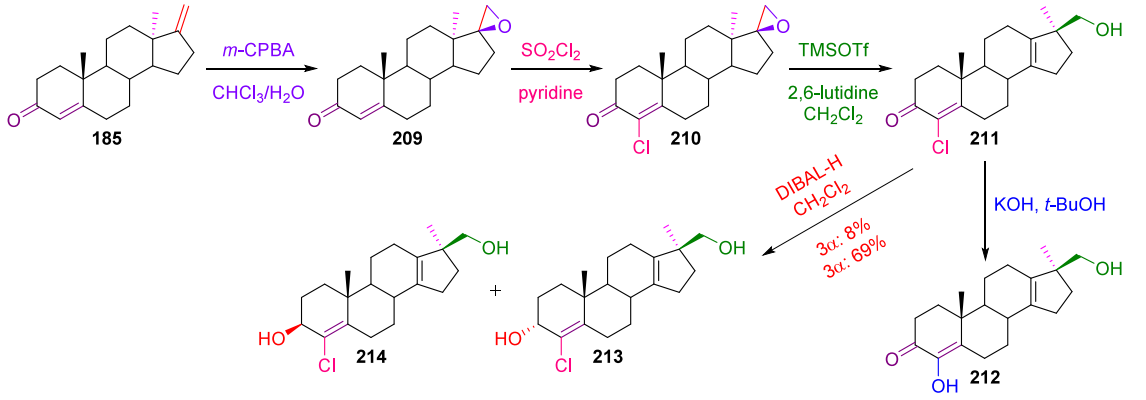
Scheme 29. Synthesis of D-Ring Modifications of Chlorohydrins (202e, 204e, 207c, and 208d)



Scheme 30. Synthetic Route for Long-Term Metabolites (212−214)



synthesis of DHEA-C-16 spiro-pyrrolidine derivatives **167a−g**. These derivatives were prepared by the [3 + 2] cycloaddition reaction involving the 16-arylidene DHEA **163a−g** and the azomethine ylide **166**. The azomethine ylide was generated in situ from 11*H*-indeno[1,2-*b*]quinoxalin-11-one **164** and sarcosine **165** (Scheme 24).[32] In addition, the cytotoxicity of

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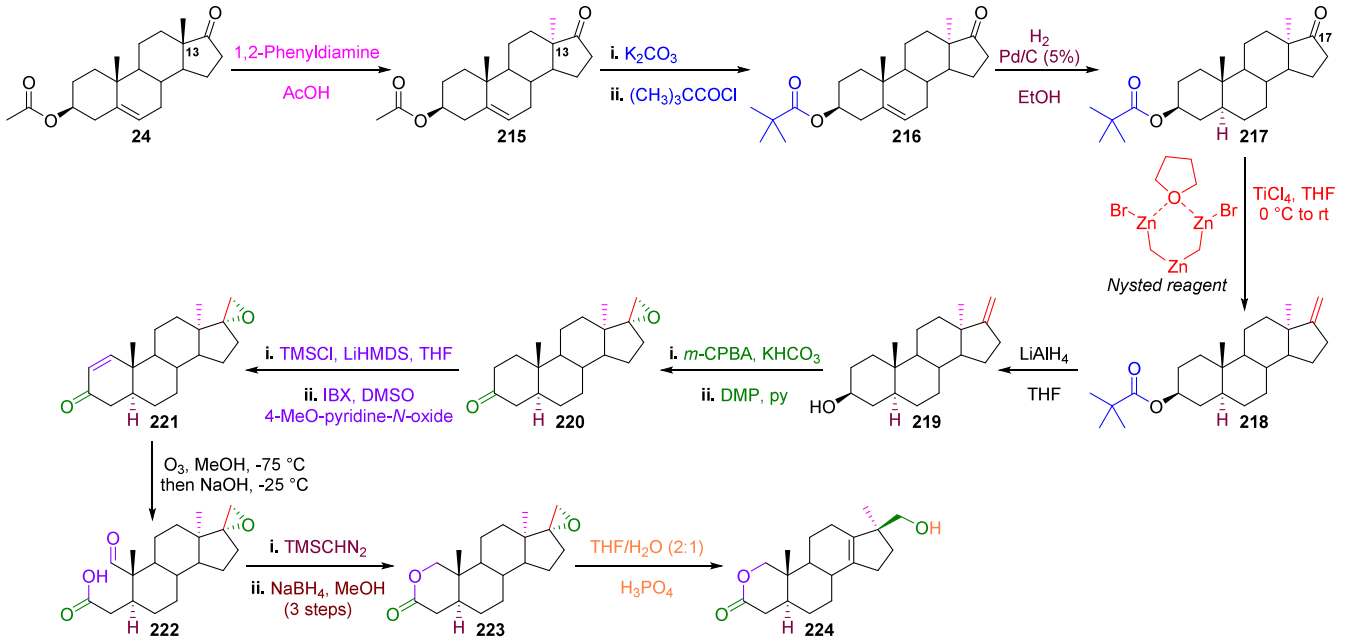
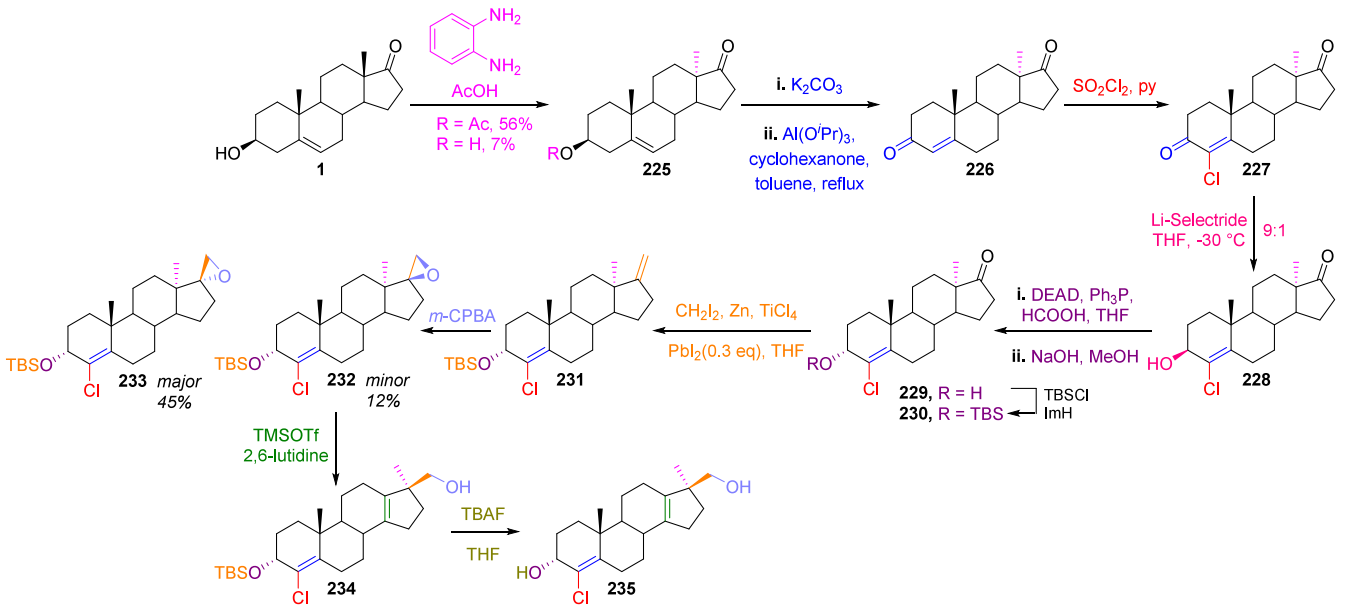
Scheme 31. Synthetic Route for D-Ring Modified Long-Term Metabolites (224)



Scheme 32. Synthetic Route for D-Ring Modified Long-Term Metabolites 235 (4-Chloro-17β-(hydroxymethyl)-17α-methyl-18-norandrosta-4,13-diene-3α-ol)



the compounds was evaluated on brine shrimp assay, and the bioactivities represented by LC_{50} values were found in the range of 6.19−27.2 μg/mL. Among them, **167d** and **167g** had the most significant cytotoxic bioactivities with LC_{50} values less than 10 μg/mL reaching the same level as the positive control (podophyllotoxin) (Table 25). The dehydroepiandrosterone, 16-arylidene dehydroepiandrosterone, and sarcosine did not show cytotoxicity with LC_{50} < 100 μg/mL. The products **167a−g** exhibited much higher cytotoxic activity than the 16-arylidene steroids **163a−g**, which indicated that the introduction of spiro-pyrrolidine moiety significantly increased the cytotoxic activity of steroids.

2.23. Paclitaxel-Dehydroepiandrosterone Derivatives. Lou and co-workers designed 18 new paclitaxel-DHEA derivatives in 2020, which they synthesized using Pd(II)-catalyzed Suzuki-Miyaura cross-coupling of 17-trifluoromethanesulfonic enolate-DHEA **169** with various aryl boronic acids (Scheme 25).[33] The in vitro anticancer activity of the hybrids against a human liver cancer cell line (HepG-2) was assessed through an MTT assay. The results showed that most of these derivatives had moderate antiproliferative activity against the HepG-2 cancer cell line, compared to the positive control Taxol. Three of these derivatives (**174b**, **174g**, and **174i**) had ortho-substituents in the phenyl group of the D-ring of DHEA analogs and had moderate anticancer activity, which was better than the others. Notably, compound **174i** demonstrated the most potent anticancer activity against the HepG-2 cell line, with an IC_{50} value of 26.39 μM (Table 26).

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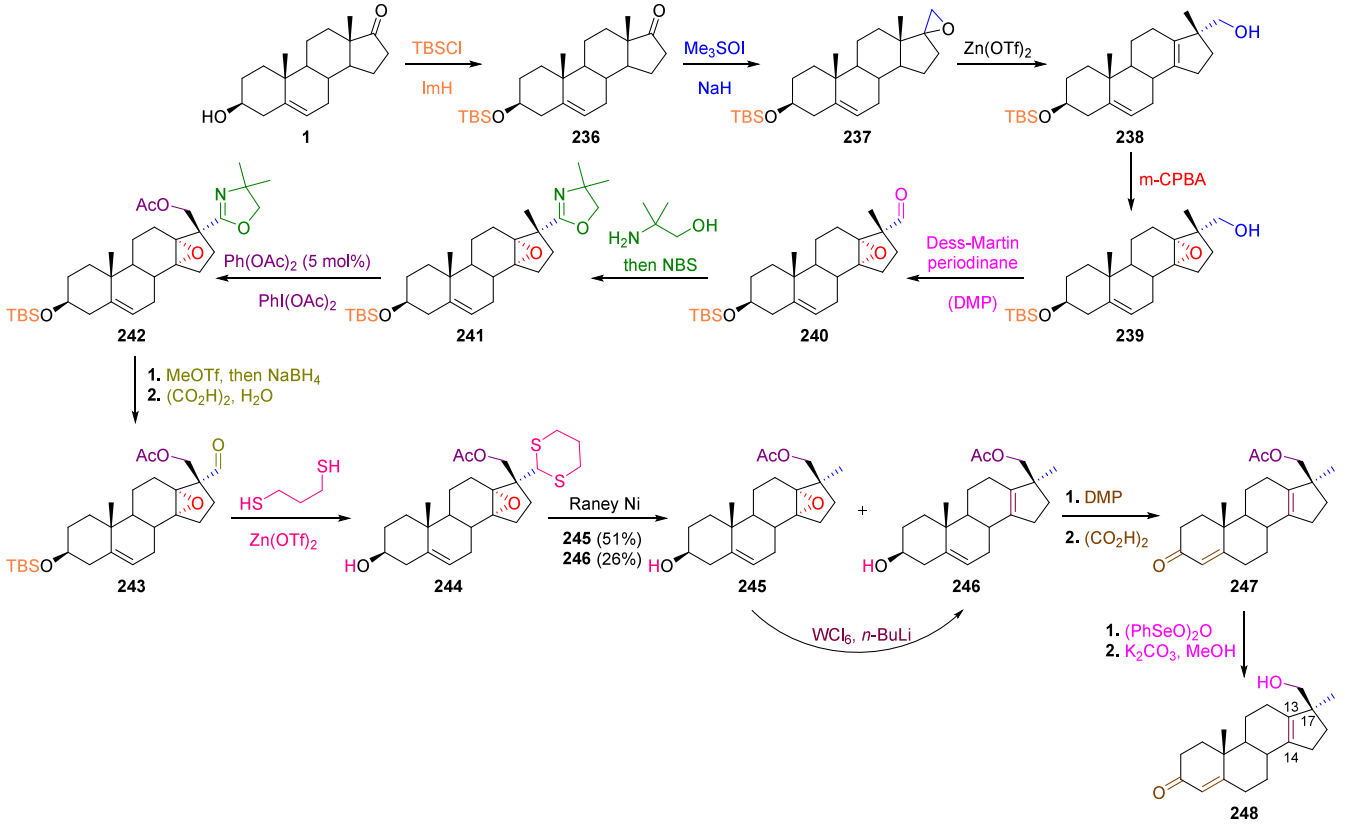
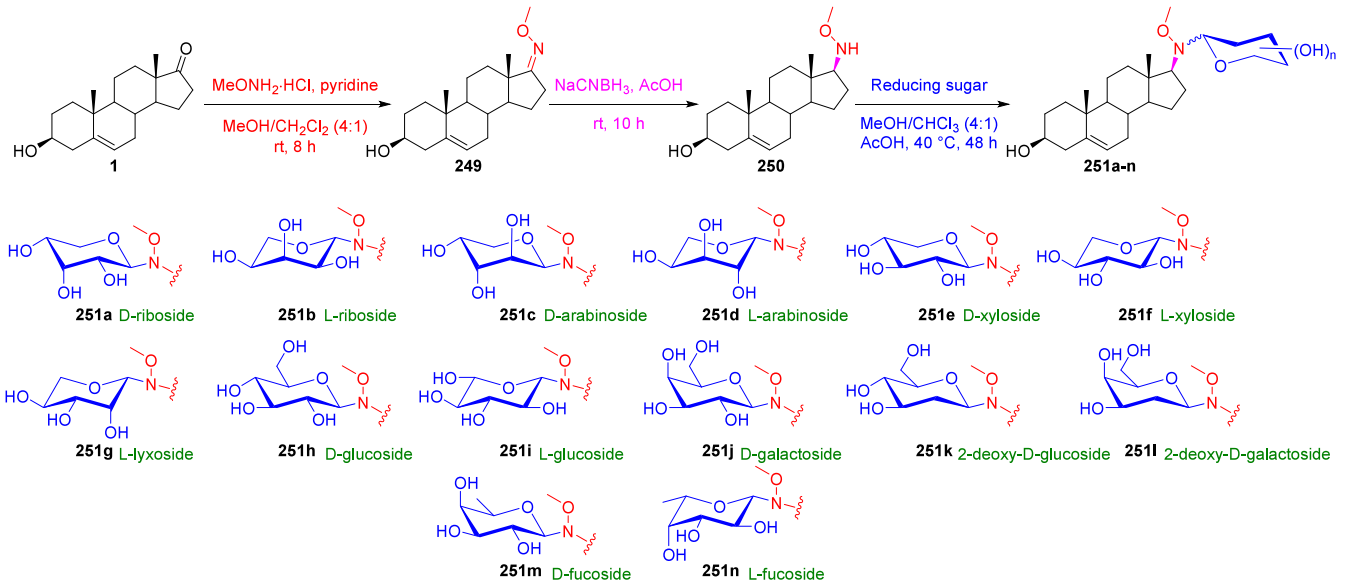
Scheme 33. Pd-Catalyzed D-Ring Modified Long-Term Metabolites 248



Scheme 34. Synthesis of MeON-DHEA 250 and MeON-neoglycosides-DHEA (251a−n)



2.24. Lactam Lactone Dehydroepiandrosterone Derivatives. Savic and colleagues reported the synthesis of a new A-homo lactam D-homo lactone androstane derivative from DHEA **1** (Scheme 26) in 2020,[34] as well as observed the effect of nitrogen addition in the parental scaffold on biological activity. Several human cancer cell lines including breast adenocarcinoma (MDA-MB-231, MCF-7), prostate carcinoma (PC3), and nontumor MRC-5 (lung fibroblasts) cell line, were used to screen the new compound as well as starting compounds for cytotoxic, antiangiogenic, and anti-inflammatory activities. All tested compounds were nontoxic against nontumor (MRC-5) cell line, but Doxorubicin was extremely toxic to MRC-5 normal cell. Compound **179** was found to be inactive against MCF-7 cell. In contrast to Formestane, all of the tested compounds had cytotoxic activity against MDA-MB-231. Compound **176** displayed high activity (IC_{50} 9.30 μM), while compound **179** with A-homo lactam ring showed moderate activity (IC_{50} 16.61 μM). However, compound **175** shows significant cytotoxic activity (IC_{50} 20.20 μM). With regard to PC3 cells, A-homo lactam D-homo lactone derivative **179** had a high cytotoxic

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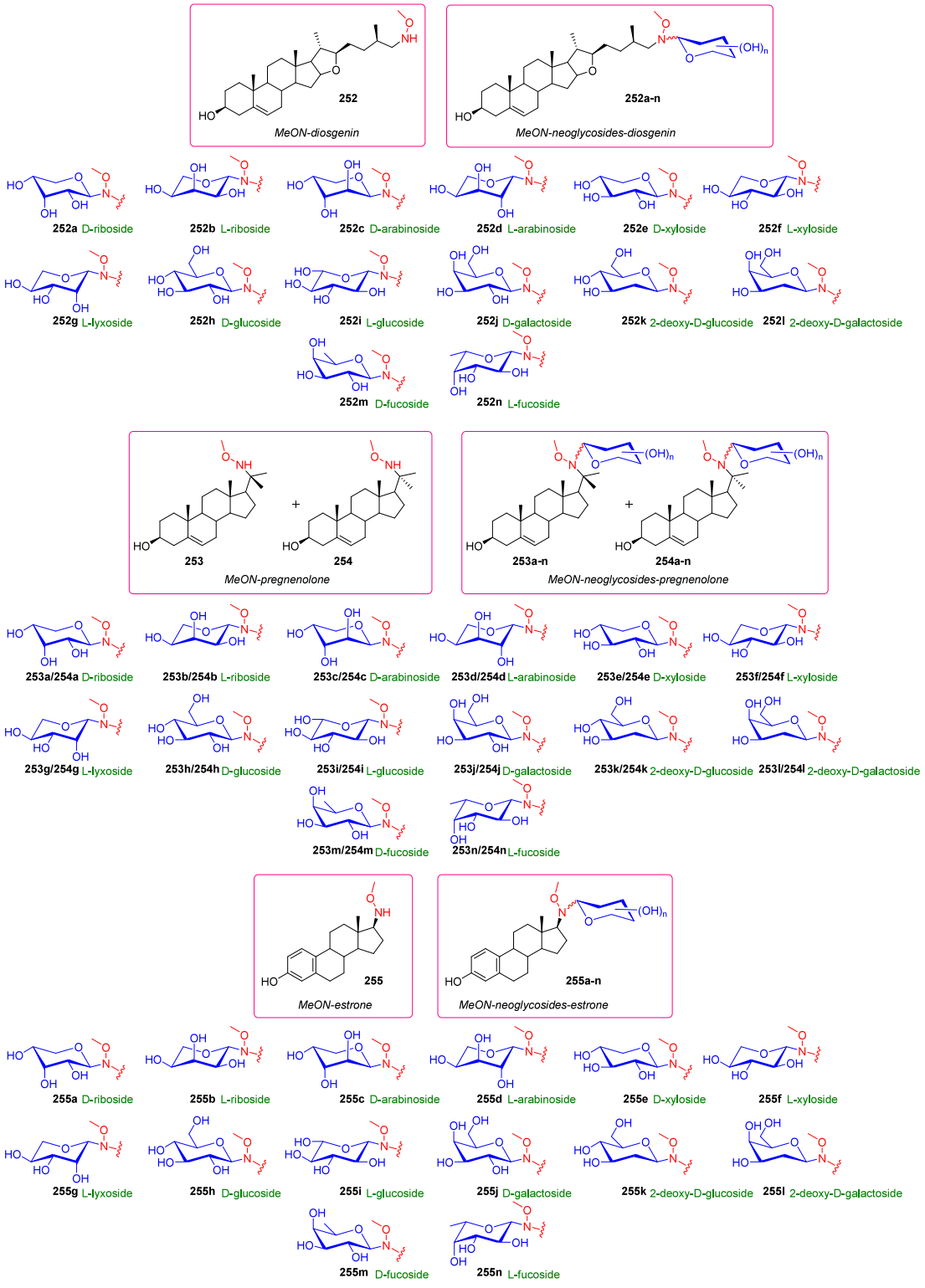

Figure 11. MeON-neoglycosides of diosgenin (**252a−n**), pregnenolone (**253a-n**/**254a-n**), and estrone (**255a−n**).[41]

activity (IC_{50} 2.18 μM), whereas starting compounds **175** and **176** were almost inactive (89.00 and >100 μM, respectively), implying that the A-homo lactam ring is important in D-homo lactone androstane derivatives (Table 27). Consequently, compound **179** showed significantly stronger cytotoxic activity when compared with Doxorubicin and Formestane.

2.25. Human Long-Term Metabolites Dehydroepiandrosterone Derivatives. Kratena and co-workers reported a paper on the synthesis of the long-term metabolites (**212−214**)[4] and chlorohydrins (**194**, **196**, **207**, **208**, **202e**, **204e**, **207c**, and **208d**) (Schemes 27, 28, and 29)[36] in 2020 and 2018 (see Scheme 30), which were synthesized according to a known procedure (2016)[37] that began with dehydroepiandrosterone acetate (Scheme 27). The synthesis started with the epimerization of the angular methyl group on C-13. Only if the methyl group is in the α-position it should give the correct

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Table 28. IC_{50} (μM)[b] Value of Aglycons and Neoglycosides of Diosgenin, Pregnenolone, DHEA, and Estrone against Five Human Cancer Lines[a]

s. no.	compounds	sugar	A375	A549	HCT116	HepG2	MCF-7
1.	**250**	none	>50	>50	>50	>50	>50
2.	**252**	none	>50	36.9 ± 2.6	>50	29.5 ± 3.3	35.9 ± 2.3
3.	**252a**	D-ribose	37.6 ± 2.1	35.7 ± 0.7	34.0 ± 0.5	14.9 ± 1.6	21.5 ± 0.8
4.	**252b**	L-ribose	42.7 ± 3.0	32.1 ± 2.5	34.7 ± 0.6	22.5 ± 0.7	16.6 ± 2.8
5.	**252c**	D-arabinose	17.3 ± 1.5	28.9 ± 1.3	41.2 ± 0.2	17.6 ± 0.6	2.5 ± 0.4
6.	**252d**	L-arabinose	40.2 ± 1.7	21.8 ± 0.3	31.5 ± 0.3	10.5 ± 1.8	3.7 ± 0.6
7.	**252e**	D-xylose	>50	>50	>50	38.0 ± 2.4	27.1 ± 2.8
8.	**252f**	L-xylose	>50	>50	>50	>50	>50
9.	**252g**	L-lyxose	>50	48.4 ± 1.2	>50	20.0 ± 0.9	12.9 ± 1.6
10.	**252h**	D-glucose	23.0 ± 0.7 3	>50	>50	>50	10.2 ± 0.3
11.	**252i**	L-glucose	>50	>50	>50	>50	>50
12.	**252j**	D-galactose	18.2 ± 1.1	>50	34.7 ± 0.1	28.5 ± 1.2	10.4 ± 1.1
13.	**252k**	2-deoxy-D-glucose	27.9 ± 1.8	1.7 ± 0.1	23.5 ± 0.8	1.5 ± 0.3	3.0 ± 0.4
14.	**252l**	2-deoxy-D-galactose	38.8 ± 1.5	13.6 ± 0.3	16.9 ± 0.3	5.9 ± 0.8	1.9 ± 0.3
15.	**252m**	D-fucose	>50	>50	>50	34.6 ± 2.0	>50
16.	**252n**	L-fucose	49.1 ± 0.7	>50	>50	>50	7.9 ± 1.0
17.	**253**	none	32.7 ± 2.1	32.9 ± 0.5	30.5 ± 1.6	37.7 ± 1.2	24.2 ± 2.1
18.	**253a**	D-ribose	>50	>50	>50	>50	>50
19.	**253b**	L-ribose	36.6 ± 0.9	33.0 ± 1.0	43.2 ± 3.1	>50	>50
20.	**253c**	D-arabinose	34.9 ± 1.5	35.7 ± 2.4	38.7 ± 1.1	>50	28.7 ± 2.0
21.	**253d**	L-arabinose	33.7 ± 0.8	34.2 ± 1.0	36.5 ± 0.9	38.1 ± 2.2	23.9 ± 0.7
22.	**253e**	D-xylose	25.9 ± 2.2	27.4 ± 1.8	30.2 ± 0.6	39.6 ± 1.7	22.7 ± 1.6
23.	**253f**	L-xylose	36.7 ± 1.3	37.1 ± 1.2	>50	47.5 ± 1.5	38.2 ± 0.8
24.	**253g**	L-lyxose	22.3 ± 2.6	32.4 ± 0.6	31.8 ± 2.2	42.3 ± 1.9	>50
25.	**253h**	D-glucose	>50	>50	>50	>50	>50
26.	**253i**	L-glucose	>50	>50	>50	>50	>50
27.	**253j**	D-galactose	>50	>50	>50	>50	>50
28.	**253k**	2-deoxy-D-glucose	24.8 ± 0.9	13.8 ± 0.3	32.6 ± 1.5	29.7 ± 0.8	17.2 ± 2.3
29.	**253l**	2-deoxy-D-galactose	27.7 ± 1.6	32.3 ± 1.5	35.2 ± 1.0	35.4 ± 1.4	22.8 ± 1.2
30.	**253m**	D-fucose	38.2 ± 1.8	14.5 ± 0.7	26.8 ± 2.8	>50	39.6 ± 0.9
31.	**253n**	L-fucose	>50	36.7 ± 2.0	32.4 ± 1.3	>50	32.1 ± 1.8
32.	**254**	none	35.0 ± 2.8	39.7 ± 0.7	>50	40.5 ± 0.6	22.7 ± 1.2
33.	**255**	none	18.2 ± 0.4	20.8 ± 0.9	>50	23.3 ± 0.6	>50
34.	**255a**	D-ribose	>50	>50	>50	>50	>50
35.	**255b**	L-ribose	>50	>50	>50	>50	>50
36.	**255c**	D-arabinose	>50	24.2 ± 2.6	>50	25.8 ± 1.0	23.1 ± 1.5
37.	**255d**	L-arabinose	>50	>50	>50	>50	>50
38.	**255e**	D-xylose	>50	15.9 ± 0.4	>50	31.2 ± 1.5	30.7 ± 1.4
39.	**255f**	L-xylose	>50	>50	>50	>50	>50
40.	**255g**	L-lyxose	21.7 ± 0.8	31.9 ± 1.1	>50	22.6 ± 2.8	28.2 ± 2.3
41.	**255h**	D-glucose	>50	>50	>50	>50	>50
42.	**255i**	L-glucose	>50	>50	>50	>50	>50
43.	**255j**	D-galactose	>50	22.4 ± 0.7	>50	31.9 ± 4.7	26.5 ± 1.3
44.	**255k**	2-deoxy-D -glucose	9.7 ± 0.7	4.1 ± 0.1	28.6 ± 1.2	6.1 ± 0.8	4.0 ± 0.4
45.	**255l**	2-deoxy-D -galactose	8.8 ± 1.0	5.7 ± 0.3	27.9 ± 1.3	10.4 ± 0.5	5.4 ± 0.1
46.	**255m**	D-fucose	44.2 ± 1.6	46.6 ± 2.4	>50	>50	>50
47.	**255n**	L-fucose	41.1 ± 3.0	33.0 ± 0.9	>50	23.5 ± 2.4	43.3 ± 0.1
48.	Sorafenib[c]		5.7 ± 0.4	4.8 ± 0.7	2.9 ± 0.1	4.8 ± 0.1	10.5 ± 0.6

[a]Compounds **251a−n** and **254a−n** had IC_{50} > 50 μM against the tested cells (data not shown). [b]Each value was determined in triplicate. The cells were continuously treated with compounds for 72 h. [c]Positive control.[41]

stereochemistry after the rearrangement **180**. Further, the selective epoxidation at C-17 of the exocyclic electron-rich double bond **186** was reached using *m*-CPBA in $CHCl_3/H_2O$ (buffered). Moreover, a spiro-epoxide **187** on C-17 can form a carbocation in the presence of phosphoric acid and the C-13 methyl group migrates from C-13 to adjacent C-17 to furnish the rearranged product **188**. The epoxide ring-opening/rearrangement reaction was first tested under anhydrous conditions using $BF_3{\cdot}Et_2O$ etherate in THF. This gave only small amounts of product, but switching to an aqueous acidic system to phosphoric acid in THF/H_2O yields greatly increased. Next, introducing the chloride through enolate chemistry and it was found that treating the lithium enolate of **189** with NCS yields the 4β-chloro product **190** as a single diastereomer in good yield. Further, the epoxidation of olefins (**191**, **192**) was obtained by the reduction of **190** with $NaBH_4$ to give 17β-epoxy (**193**, **195**)

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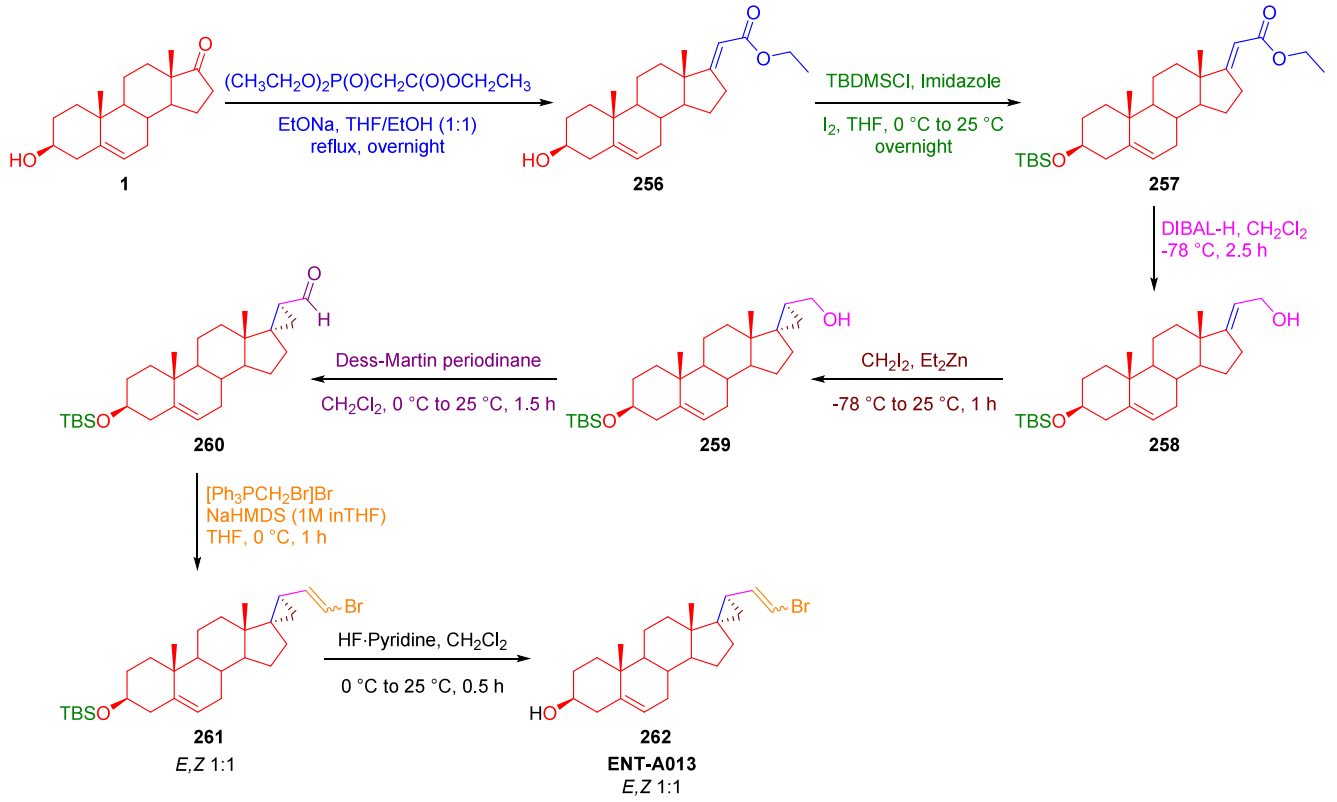
Scheme 35. Synthesis of ENT-A013 (Mixture of *E, Z* isomers 1:1)



when treated with *m*-CPBA. Next, the epoxy ring was opened accordingly in the presence of phosphoric acid and got the rearranged products (**194**, **196**), respectively (Scheme 27).

Additionally, compound **181** undergoes Oppenauer oxidation to yield diketone **197** in a good yield (Scheme 28). Treating this diketone **197** with excess zinc in acetic acid promptly reduces the A-ring enone to an olefin, resulting in the formation of a mixture of new stereocenters at C-5 (**198**, **199**). Further, the epoxidation with *m*-CPBA, the compounds **198** and **199** give two diastereomers **200** and **201**. Subsequently, the epoxides were then treated with concentrated HCl, resulting in a mixture of isomeric chlorohydrins **202**, **203**, and **204**. The configuration and stereochemistry of diastereomer **202** were proved by single-crystal X-ray determination.[36] The undesired chlorohydrin **203** and desired chlorohydrin **204** were used to obtain a pure analytical sample of **201** and **200**, respectively, with a strong base. The last two compounds (**207** and **208**) of the series were obtained by the oxidation of **204** with DMP (Dess-Martin periodinane) and C-4 epimerization of the resulting intermediate **205** with KOH to give **206**, and then finally Meerwein-Ponndorf-Verley reduction of C-3 ketone **206** results in a 1:1 mixture of C-3 epimeric alcohols **207** and **208** (Scheme 28). The synthesis from this point forward is the same for all four chlorohydrins (**202**, **204**, **207**, and **208**) and we get subsequently D-ring modified respective compounds (**202e**, **204e**, **207c**, and **208d**) (Scheme 29).

Additionally, Kratena et al. (2017) also published one more research paper (Scheme 31)[38] on the synthesis of D-ring modified long-term metabolites starting from the dehydroepiandrosterone acetate **24** according to the same procedure that was published in 2016.[37] An extension of Kratena's work, Shostko et al. (2020) also synthesized D-ring-modified long-term metabolites of oral turinabol (4-chloro-17β-(hydroxymethyl)-17α-methyl-18-norandrosta-4,13-diene-3α-ol) **235** starting from DHEA **1** (Scheme 32).[39]

Similarly, in 2017, Hurski and co-workers reported the Pd-catalyzed D-ring modified long-term metabolites of C−H acetoxylation of 17 β-methylated androgenic anabolic steroids (AAS) metandienone **248** (17β-Hydroxymethyl-17α-methyl-18-norandrost-13-ene) (Scheme 33).[40] The Pd-catalyzed C−H acetoxylation of the 17β-methyl group was employed to invert the configuration at a quaternary stereocenter of the epimeric synthetic intermediate. The proposed technique was utilized to manufacture metandienone metabolite **248**, which is employed as a reference drug in antidoping testing, to regulate the abuse of this androgenic anabolic steroid.

2.26. MeON-Neoglycosides Dehydroepiandrosterone Derivatives. In 2021, Du et al. developed a series of MeON-neoglycosides of DHEA **251a−n** and synthesized them according to the neoglycosylation approach (Scheme 34).[41] The compound's cytotoxicity was evaluated on five human cancer cell lines including melanoma (A375), lung (A549), colon (HCT116), liver (HepG2), and breast (MCF-7), using a cell counting Kit-8 (CCK-8) assay. All of the MeON-neoglycosides of DHEA **251a−n** showed no effects against the five cancer cell lines at the concentration of 50 μM as compared to the other MeON-neoglycosides of diosgenin **252a−n**, pregnenolone **253a−n**/**254a−n**, and estrone **255a−n** which are reported in the literature (Figure 11) (Table 28).[41]

2.27. ENT-A013 (Novel Nerve Growth Factor Mimetic) Dehydroepiandrosterone Derivatives. In 2022, Rogdakis and co-workers reported the synthesis of ENT-A013, a nerve growth factor (NGF) that selectively activates TrkA and exerts neuroprotective, antiamyloid actions (Scheme 35).[42] At first, DHEA was treated with triethyl phosphonoacetate in the presence of EtONa to generate **256** in high yield, which was

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subsequently reacted with TBDMSCl to yield the TBDMS-protected alcohol **257**. The allylic alcohol **258** was obtained in quantifiable yield after selective reduction of the ester group in **257** with DIBAL-H, which was then subjected to a Simmons–Smith cyclopropanation reaction in the presence of CH_2I_2 and Et_2Zn to produce cyclopropyl derivative **259**. Compound **259** was then oxidized in DCM with Dess–Martin periodinane to give the corresponding aldehyde **260**. In the presence of NaHMDS solution (1 M in THF), a Wittig reaction of aldehyde **260** with $[Ph_3PCH_2Br]Br$ yielded the vinyl-bromide derivative **261** (1:1 mixture of E, Z isomers) in high yield. Finally, deprotection of the C3 alcohol in **261** using HF·Pyridine complex in dry CH_2Cl_2 yielded ENT-A013 **262** (mixture of E, Z isomers 1:1) in quantitative yield.

3. CONCLUSIONS AND FUTURE PERSPECTIVES

The review presents a wide range of novel approaches to the synthesis of DHEA derivatives, including side-chain modifications, heteroatom substitutions, and heterocycles fused with both D- and A-rings. Every technique is described in great detail, highlighting how easy and effective it is to use. Additionally, a comprehensive summary of the biological activities of the synthetic derivatives, both in vivo and in vitro is provided in chronological order. The scientific community is piqued by this chronological approach, which offers a clear trajectory of advancements over the last decades. In addition, structure–activity relation (SAR) studies were also well studied for many derivatives of DHEA which are also listed in this review. These studies shed important light on the connection between biological function and chemical structure, which is important for designing and refining DHEA derivatives for a range of therapeutic uses.

In summary, this systematic review is an invaluable resource for scholars, especially those who are just starting, and who want to learn more about DHEA and its derivatives. This review serves to both inform and inspire future research endeavors in the field of DHEA-derived therapeutics by encapsulating the latest advancements in synthesis methodologies, biological activities, and SAR studies.

■ ASSOCIATED CONTENT

Data Availability Statement

Data sharing is not applicable to this article since no new data were generated or analyzed in this study.

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Notes

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■ ABBREVIATIONS USED

DHEA	Dehydroepiandrosterone
$(Boc)_2O$	Di*tert*-butyl dicarbonate
CDI	*N*,*N*′-Carbonyldiimidazole
TEA (Et_3N)	Triethylamine
DCC	*N*,*N*′-Dicyclohexylcarbodiimide
CbzCl	Benzyl chloroformate
DBTO	Dibutyltin oxide
DMAP	*N*,*N*′-Dimethylaminopyridine
NMM	4-Methylmorpholine
$CHCl_3$	Chloroform
CLA	Conjugated linoleic acid
EPI	Epiandrosterone
EDCI	1-Ethyl-3-(3-(dimethylamino)propyl) carbodiimide
HOBt	1-Hydroxybenzotriazole
XPhos	Phosphine ligand
TBSCl or TBDMSCl	Tert-butyldimethylsilyl chloride
DCM	Dichloromethane
DMF	Dimethylformamide
IAA	Indole-3-acetic acid
$C_7H_4ClNO_4$	*p*-Nitrophenyl chloroformate
$C_6H_{12}Br_2OZn_3$	Nysted reagent
NCS	*N*-Chlorosuccinimide
LiHMDS	Lithium bis(trimethylsilyl)amide
DMP	Dess–Martin periodinane
MPO	4-Methoxypyridine N-oxide
IBX	*o*-Iodoxybenzoic Acid
$TMSCHN_2$	Trimethylsilyldiazomethane $(CH_3)_3SiCHN_2$
TBSCl, ImH	TBS ether formation
PI/Annexin V	Propidium iodide/Annexin V
LPS	Lipopolysaccharide
$Ti(EtO)_4$	Tetraethyl titanate
Me_3SOI	Trimethylsulfonium iodide
DHP	3,4-Dihydropyran
PPTS	Pyridinium *p*-toluenesulfonate
Trk	Tyrosine kinase
DIBAL-H	Diisobutylaluminum hydride
NaHMDS	Sodium bis(trimethylsilyl)amide solution
$[Ph_3PCH_2Br]Br$	(Bromomethyl)triphenylphosphonium bromide

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