Full Paper

Synthesis and Anti-inflammatory Activities of New Cyanopyrane Derivatives Fused with Steroidal Nuclei

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Thirteen new heterocyclic derivatives containing a cyanopyrane ring fused to a steroidal moiety were conveniently synthesized and screened for their anti-inflammatory potencies comparable to that of the glucocorticoid prednisolone. Four compounds **5a**, **5b**, **6b**, and **8** exhibited superior anti-inflammatory indices (in rats, protection against carrageenan induced edema and inhibition of plasma PGE). All the candidates were less toxic than the reference drug concerning LD_{50} values. Synthetic steroidal structures fused to a substituted cyanopyrane ring seem to be a promising approach in search for novel leads for potent anti-inflammatory agents.

Keywords: Cyanopyranes / Steroidal derivatives / Anti-inflammatory Agents

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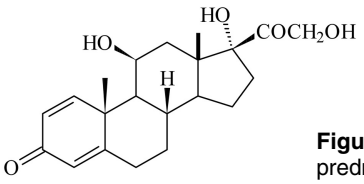
Introduction

Natural steroids and their synthetic congeners were extensively studied during the last decades [1, 2]. Sex hormones, cardiacs, diuretics, antibiotics, neuromusculars are some representative examples. In particular, the steroidal antiphlogistic activity, namely the anti-inflammatory characteristics are still viewed as an active area of the bioorganic medicinal chemistry literature. Regarding their salt retention and gastric and peptic ulceration the search for more potent synthetic agents represents a challenging problem [2, 3].

In a previous work, we reported the synthesis of some substituted pyrane and pyridine derivatives as analgesic, anticonvulsant, anti-Parkinsonian, and antimicrobial agents [4 – 8]. The androgenic, anabolic, and anti-inflammatory activities of many heterocyclic steroidal derivatives were similarly reported [9]. On the other hand, cyanopyridones have promising antimicrobial [10, 11] and anticancer [12 – 14] activities. Recently, we reported the synthesis of some new chiral heterocyclic compounds as anticancer and anti-inflammatory agents [15, 16].



prednisolone (I)

Figure 1. Chemical structure of prednisolone (I).

In view of these reports and in continuation of our previous works in heterocyclic chemistry, we suggested and newly synthesized thirteen cyanopyrane heterocyclics fused with steroidal nuclei. The evaluation of the anti-inflammatory potency of the synthesized compounds was realized comparable to the synthetic corticosteroid prednisolone (I, protection against carragenan induced edema and inhibition of plasma prostaglandin E_2). For the structure of prednisolone see Figure 1.

Results and discussions

Chemistry

Arylmethylene of epiandrosterones **1a**, **b** and the dehydroepiandrosterone **2** were prepared according to the lit-

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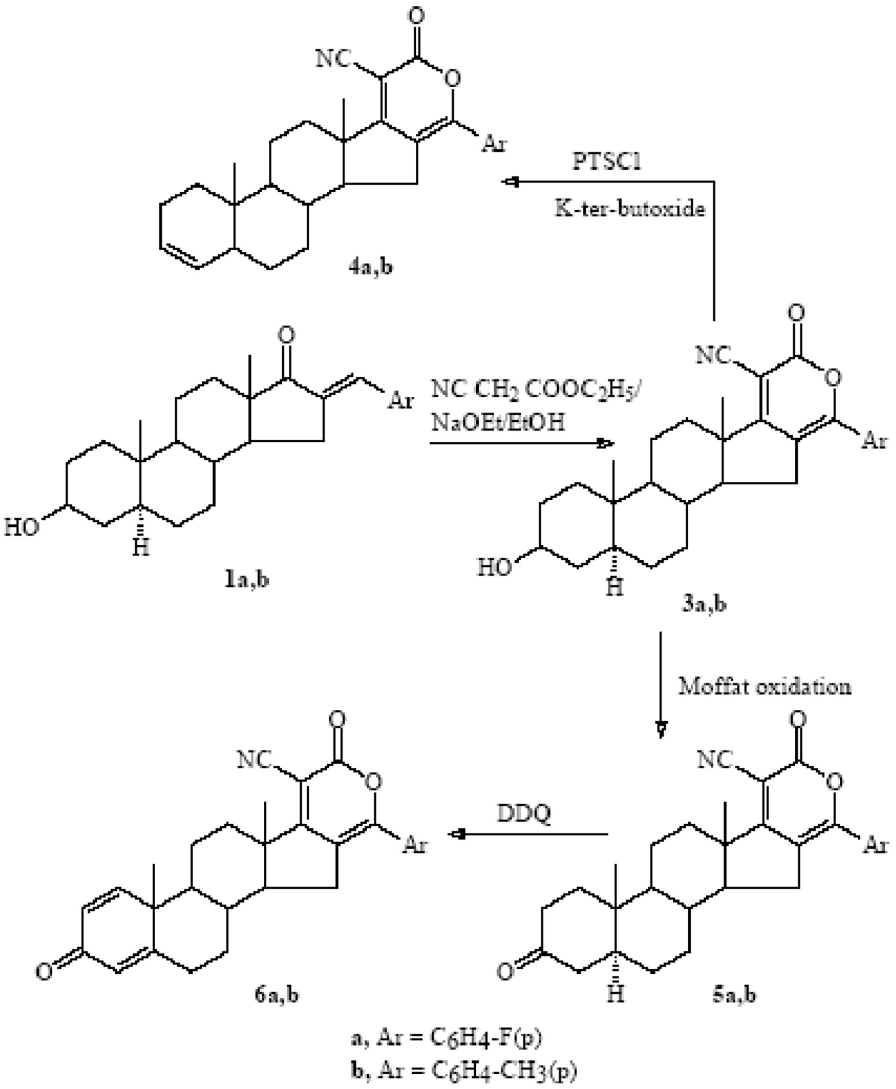


Scheme 1. Synthetic routes for compounds **3**–**6**.

erature [4, 17]. Condensation of **1a**, **b** with ethyl cyanoacetate in the presence of sodium ethoxide gave the corresponding cyanopyranes **3a**, **b**, which were then reacted with *p*-toluene sulfonyl chloride (PTSCl) to afford the cyclohexene (Ring A) derivatives **4a**, **b**, respectively. Oxidation of compounds **3a**, **b** with Moffat oxidizing agents afforded the corresponding 3-oxo-analogue derivatives **5a**, **b**. Compounds **5a**, **b** were oxidized with dichlorodicyanoquinone (DDQ) [18] to the corresponding 3-oxo-diene derivatives, **6a**, **b** (Scheme 1).

The dehydroepiandrosterone **2** was similarly reacted with ethyl cyanoacetate in the presence of sodium ethoxide to yield the corresponding cyanopyrane **7**, which then was reacted with *p*-toluene sulfonylchloride to afford the corresponding cyclohexene derivative **8**. Moffat oxidation of compound **7** gave the 3-oxo analogue **9** without affecting (Δ^5-ene. Oppenauer oxidation of **7** with aluminum isopropoxide resulted in the 3-oxo-analogue with delocalization of (Δ^5-ene into (Δ^4-ene derivative **10**. Modified Oppenauer oxidation [19] of **7** afforded the corresponding ($\Delta^{4,6}$-3-oxo-analogue **11** (Scheme 2).

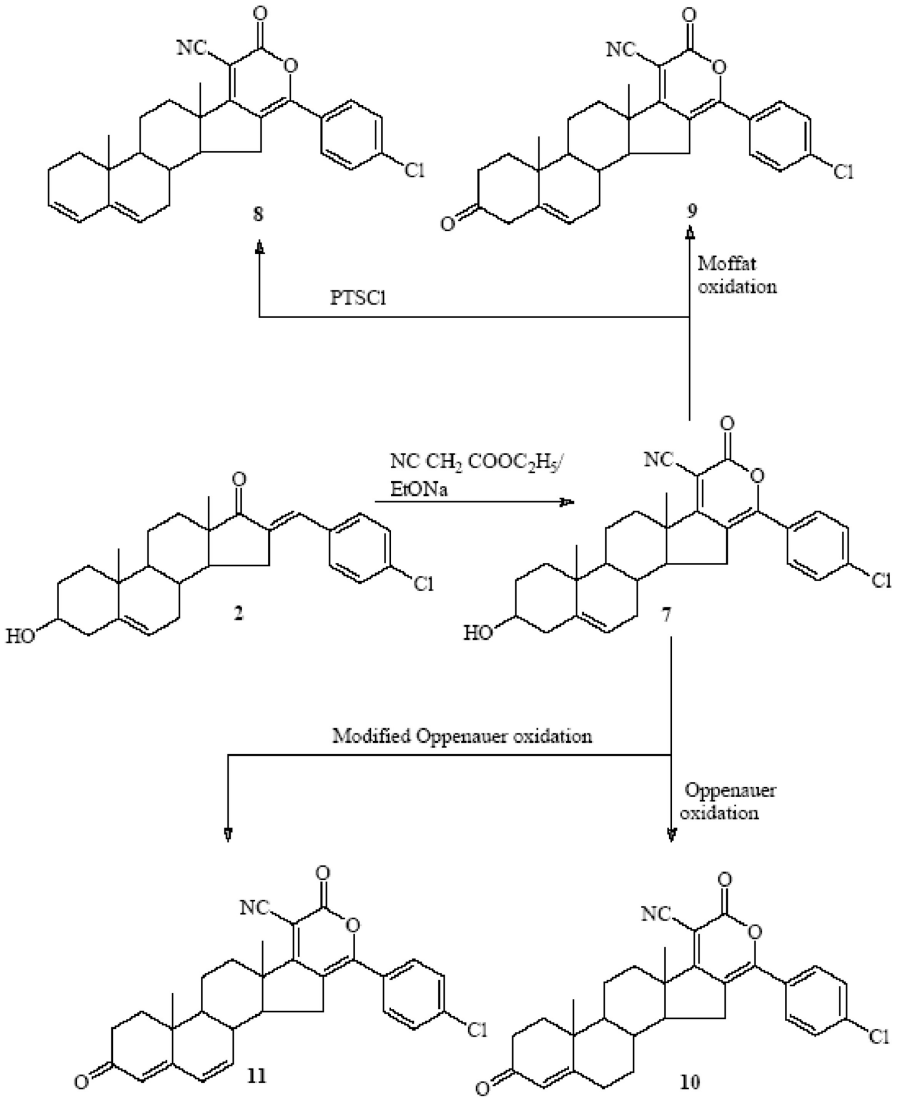
Pharmacological Screening

Anti-inflammatory potency

Initially the acute toxicity of the compounds was assayed determining their LD_{50}. Interestingly, all the compounds, except for **8**, were less toxic than the reference drug (Table 1). Then the newly synthesized compounds were pharmacologically screened for their anti-inflammatory potency using male albino rats (Tables 2 and 3).

Purpose and rational

For the determination of the antiphlogistic potency of the synthesized compounds, two standard tests were rea-



Scheme 2. Synthetic routes for compounds **7**–**11**.

Table 1. Acute toxicity (LD_{50}) of the synthesized compounds.

Compound N°	LD_{50} [mg/kg]
9	4.186
3a	3.813
5a	3.111
11b	3.111
12b	3.068
10	2.868
6b	2.751
6a	2.684
5b	2.681
3b	2.514
11	2.280
10	2.191
4b	1.880
4a	1.711
Prednisolone	1.618
8	1.252

lized at 25 and 50 mg/kg body weight of the rats, namely the protection against carragenan-induced edema according to Winter et al. [20] and the inhibition of plasma PGE2. The later is known as a good confirming indicator for the carragenan-induced rat paw edema [21].

Regarding the protection against carragenan-induced edema, four compounds, namely **5a**, **6b**, **8a**, and **5b**, were found more potent (~120%) than prednisolone in a descending order (Figure 2). For these compounds, a similar but not identical activity profile was realized for the inhibition of plasma PGE2, however, with an inversed order for **8** and **5b**.

It is noteworthy that, regardless of the substitution on the cyanopyrane ring, Moffat oxidation of the synthesized structures was associated with a notable increase in the anti-inflammatory activity. This was exemplified by

Table 2. Anti-inflammatory potencies of the synthesized compounds (protection against carragenan-induced edema).

Compound N°	Dose [mg/kg]	Protection against carragenan-induced edema [%]
4a	25	–
	50	34.19
3a	25	–
	50	39.19
4b	25	–
	50	39.38
10	25	–
	50	54.12
3b	25	–
	50	56.28
6a	25	47.50
	50	51.60
11	25	58.90
	50	61.80
7	25	65.80
	50	88.16
9	25	77.18
	50	88.14
Prednisolone	25	81.00
	50	93.00
5b	25	85.62
	50	98.12
8	25	86.55
	50	98.14
6b	25	98.88
	50	99.41
5a	25	98.91
	50	99.11

Table 3. Anti-inflammatory potencies of the synthesized compounds (inhibition of plasma PGE2).

Compound N°	Dose [mg/kg]	Inhibition of plasma PGE2 [%]
4b	25	–
	50	26.88
4a	25	–
	50	28.55
3a	25	–
	50	31.66
3b	25	–
	50	42.18
10	25	–
	50	49.78
6a	25	40.90
	50	45.00
11	25	44.60
	50	58.60
7	25	48.16
	50	79.77
9	25	71.18
	50	83.65
Prednisolone	25	77.00
	50	91.00
8	25	81.16
	50	93.18
5b	25	83.63
	50	95.65
6b	25	95.32
	50	97.18
5a	25	95.85
	50	96.18

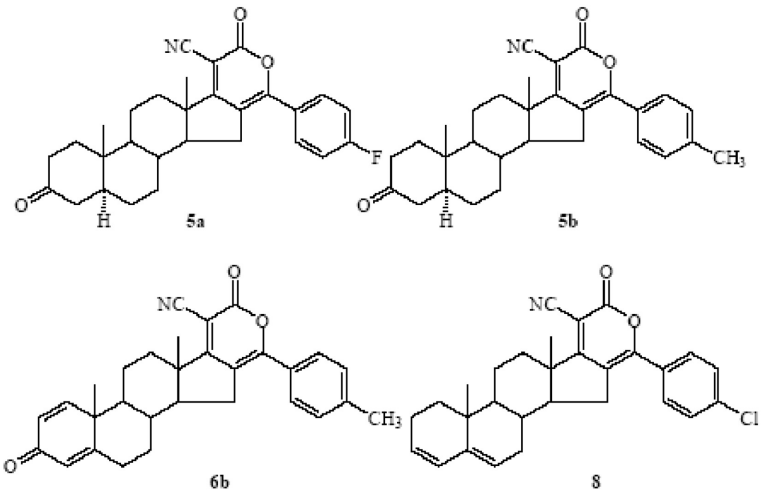


Figure 2. Structures of compounds with potent anti-inflammatory activities.

compound **7** oxidized to yield **9** and more pronounced in the case of compounds **3a**, **b** upon oxidation to **5a**, **b**, respectively. In the latter case, the almost inactive (regarding anti-inflammatory) compounds **3** afforded the significantly potent candidates **5**, comparable to the reference drug prednisolone (Figure 3). Further oxidation (DDQ) of **5b** to **6b** was also accompanied with an enhanced activity.

Figure 3. Anti-inflammatory activities of compounds tested.

Experimental

Chemistry

Melting points were determined on open glass capillaries using an Electrothermal IA 9000 SERIES digital melting point apparatus (Electrothermal, Essex, U.K.) and are uncorrected. Elemental analyses were performed with all final compounds on Elementar, Vario EL, Microanalytical Unit, National Research Centre, Cairo Egypt and were found within ±0.4% of the theoretical values. Analytical data were obtained from the Microanalytical Unit, Cairo University, Egypt. The IR spectra (KBr) were recorded on a FT IR-8201 PC spectrophotometer (Shimadzu, Tokyo, Japan). The ^{1}H-NMR spectra were measured with Jeol FTGNM-EX 270, 270 MHz instrument (Jeol, Tokyo, Japan) in DMSO-d_6 and the chemical shifts were recorded in (δ, ppm) relative to TMS. The Mass spectra were run at 70 eV with a Finnigan SSQ 7000 spectrometer (Thermo electron corporation, Madison, WI, USA) using EI and the values of m/z are indicated in Dalton. TLC (Silica gel, aluminum sheets 60 F_{254}, Merck, Darmstadt, Germany) followed the reactions. The starting materials **1a**, **b** and **2** were prepared according to the published procedure [4, 17].

*Synthesis of 2-oxo-3-cyano-substituted-androsteno[17,16-c]pyran-3β-ol **3a** and **3b***

A mixture of **1** (10 mmol) and ethyl cyanoacetate (1.27 mL, 12 mmol) in 25 mL sodium ethoxide (920 mg sodium metal in 25 mL absolute ethanol) was refluxed for 7 h. The reaction mixture was poured into ice water, the obtained solid was filtered off, washed with water, dried, and crystallized from the proper solvent to give the corresponding pyrano-steroidal derivatives **3a**, **b**, respectively (Table 4).

*2-Oxo-3-cyano-(4-fluorophenyl)androsteno[17,16-c]-pyran-3β-ol **3a***

IR (KBr, cm^{-1}): 3418 (OH), 2238 (CN), 1738 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 0.81 (s, 3H, CH_3, C-19), 0.83 (s, 3H, CH_3, C-18), 0.95 – 1.00 (m, 1H, CH), 1.24 – 1.28 (m, 4H, $2CH_2$), 1.40 – 1.60 (m, 6H, $3CH_2$), 1.65-1.85 (m, 4H, $2CH_2$), 1.98 (m, 1H, CH), 2.28 – 2.45 (m, 2H, CH_2), 2.50 (m, 1H, CH), 3.00 (m, 1H, 5α-CH), 3.55 (m, 1H, 3α – CH), 7.23 – 7.50 (m, 4H, Ar-H), 10.55 (s, 1H, OH, exchangeable with D_2O). MS m/z (%): 461 (M^+, 35), corresponding to the molecular formula $C_{29}H_{32}FNO_3$ and at 323 (100, base peak).

*2-Oxo-3-cyano-(4-methylphenyl)androsteno[17,16-c]-pyran-3β-ol **3b***

IR (KBr, cm^{-1}): 3433 (OH), 2231 (CN), 1741 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 0.78 (s, 3H, CH_3, C-19), 0.82 (s, 3H, CH_3, C-18), 0.90 – 0.95 (m, 1H, CH), 1.20 – 1.25 (m, 4H, $2CH_2$), 1.38 – 1.55 (m, 6H, $3CH_2$), 1.60 – 1.80 (m, 4H, $2CH_2$), 1.95 (m, 1H, CH), 2.20 – 2.40 (m, 2H, CH_2), 2.46 (s, 3H, Ph-CH_3), 2.52 (m, 1H, CH), 3.10 (m, 1H, 5α-CH), 3.56 (m, 1H, 3α – CH), 7.28 – 7.41 (m, 4H, Ar-H), 10.45 (s, 1H, OH, exchangeable with D_2O). MS m/z (%): 457 (M^+, 100), corresponding to the molecular formula $C_{30}H_{35}NO_3$ and also as base peak.

*Synthesis of 2-oxo-3-cyano-6-substituted-androst-3-ene[17,16-c]pyrane **4a** and **4b***

A mixture of compounds **3a**, **b** (5 mmol), *p*-toluene sulfonyl chloride (0.4g, 5 mmol), and triethyl amine (1 mL) in dry benzene (15 mL) was refluxed for 2 h. The solvent was evaporated under reduced pressure. The obtained residue was solidified with water and the solid formed was filtered off, washed with water, and dried, then it was dissolved in dry benzene (15 mL) and potassium *tert*-butoxide (25 mL, 0.5N, in DMSO) was added. The reaction mixture was heated at 50°C for 5 h, The formed solid was filtered off and crystallized from the proper solvent to give the corresponding oxidized cyanopyrane derivatives **4a**, **b**, respectively (Table 4).

*2-Oxo-3-cyano-6-(4-fluorophenyl)androst-3-ene-[17,16-c]pyrane **4a***

IR (KBr, cm^{-1}): 2235 (CN), 1735 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 0.77 (s, 3H, CH_3, C-19), 0.83 (s, 3H, CH_3, C-18), 0.88 – 0.95 (m, 1H, CH), 1.15 – 1.25 (m, 4H, $2CH_2$), 1.40 – 1.54 (m, 4H, $2CH_2$), 1.58 – 1.75 (m, 4H, $2CH_2$), 1.90 (m, 1H, CH), 2.25 – 2.40 (m, 2H, CH_2), 2.56 (m, 1H, CH), 3.25 (m, 1H, CH, C-5), 5.30 (d, 1H, CH-olefinic, C-3), 5.45 (d, 1H, CH-olefinic, C-4), 7.25-7.31 (m, 4H, Ar-H). MS m/z (%): 443 (M^+, 45), corresponding to the molecular formula $C_{29}H_{30}FNO_2$ and at 280 (100, base peak).

*2-Oxo-3-cyano-6-(4-methylphenyl)androst-3-ene-[17,16-c]pyrane **4b***

IR (KBr, cm^{-1}): 2226 (CN), 1732 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 0.76 (s, 3H, CH_3, C-19), 0.81 (s, 3H, CH_3, C-18), 0.90 – 0.95 (m, 1H, CH), 1.10 – 1.25 (m, 4H, $2CH_2$), 1.35 – 1.55 (m, 4H, $2CH_2$), 1.60 – 1.80 (m, 4H, $2CH_2$), 1.85 (m, 1H, CH), 2.20 – 2.42 (m, 2H, CH_2), 2.48 (s, 3H, Ph-CH_3), 2.58 (m, 1H, CH), 3.30 (m, 1H, CH, C-5), 5.42 (d, 1H, CH-olefinic, C-3), 5.48 (d, 1H, CH-olefinic, C-4), 7.18 – 7.23 (m, 4H, Ar-H). MS m/z (%): 439 (M^+, 25), corresponding to the molecular formula $C_{30}H_{33}NO_2$ and at 280 (100, base peak).

*Synthesis of 2-oxo-3-cyano-6-(4-fluorophenyl)-androstano[17,16-c]pyran-3-one **5a** band **5b** (Moffat Oxidation)*

The appropriate compounds **3a**, **b** (2 mmol) were dissolved in a mixture of benzene (3 mL), dimethylsulphoxide (3 mL), pyridine (0.16 mL), and trifluoroacetic acid (TFA, 0.08 mL). Dicyclohexylcarbodiimide (1.24 g, 6 mmol) was then added and the reaction mixture was kept overnight at room temperature. Ether (50 mL) was added followed by oxalic acid (0.54 g, 6 mmol) in methanol (50 mL). After 30 minutes, water (50 mL) was added and the obtained dicyclohexyl urea was removed by filtration. The filtrate was extracted with ether, washed with 5% sodium bicarbonate, then with water. The ethereal solution was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The formed residue was finally crystallized from the proper solvent to give the corresponding oxo-compounds **5a**, **b**, respectively (Table 4).

Table 4. Physico-chemical data of newly synthesized compounds.

Compound N°	Ar	Yield [%]	Mp. [°C]	$[\alpha]_D^{25}$ (c 1, MeOH)	Color and Solvent for crystallization	Mol. Formula[a] (Mol. wt)
3a	C_6H_4-*p*-F	72	271	+ 88	Yellow MeOH	$C_{29}H_{32}FNO_3$ (461.57)
3b	C_6H_4-*p*-CH_3	65	283	+ 76	Pale yellow AcOEt	$C_{30}H_{35}NO_3$ (457.61)
4a	C_6H_4-*p*-F	55	>320	+ 98	White EtOH	$C_{29}H_{30}FNO_2$ (443.56)
4b	C_6H_4-*p*-CH_3	70	310	+ 84	Orange MeOH	$C_{30}H_{33}NO_2$ (439.59)
5a	C_6H_4-*p*-F	72	239	+ 88	Yellow MeOH	$C_{29}H_{30}FNO_3$ (459.56)
5b	C_6H_4-*p*-CH_3	75	166	+ 96	Brown MeOH	$C_{30}H_{33}NO_3$ (455.59)
6a	C_6H_4-*p*-F	58	234	+ 83	White MeOH	$C_{29}H_{26}FNO_3$ (455.52)
6b	C_6H_4-*p*-CH_3	77	209	+ 66	Pale yellow MeOH	$C_{30}H_{29}NO_3$ (451.56)
7	–	72	189	+ 96	Orange MeOH-AcOEt (2 : 1)	$C_{29}H_{30}ClNO_3$ (476.01)
8	–	62	119	+ 69	White MeOH	$C_{29}H_{28}ClNO_2$ (457.99)
9	–	56	261	+ 88	Yellow MeOH	$C_{29}H_{28}ClNO_3$ (473.99)
10	–	68	130	+ 95	Brown MeOH	$C_{29}H_{28}ClNO_3$ (473.99)
11	–	82	243	+ 69	Yellow MeOH-AcOEt (2 : 1)	$C_{29}H_{26}ClNO_3$ (471.98)

[a] Confirmed by elemental analysis showing values within (0.4% of the theoretical values unless otherwise stated.

2-Oxo-3-cyano-6-(4-fluorophenyl)androstano[17,16-c]pyran-3-one ***5a***

IR (KBr, cm^{-1}): 2248 (CN), 1735 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 0.77 (s, 3H, CH_3, C-19), 0.88 (s, 3H, CH_3, C-18), 1.05 – 1.15 (m, 1H, CH), 1.22 – 1.28 (m, 4H, $2CH_2$), 1.40 – 1.61 (m, 6H, $3CH_2$), 1.64 – 1.90 (m, 4H, $2CH_2$), 1.98 (m, 1H, CH), 2.25 – 2.38 (m, 2H, CH_2), 2.52 (m, 1H, CH), 3.05 (m, 1H, 5α-CH), 7.28-7.39 (m, 4H, Ar-H). MS m/z (%): 459 (M^+, 100, base peak), corresponding to the molecular formula $C_{29}H_{30}FNO_3$.

2-Oxo-3-cyano-6-(4-methylphenyl)androstano[17,16-c]pyran-3-one ***5b***

IR (KBr, cm^{-1}): 2231 (CN), 1738 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 0.78 (s, 3H, CH_3, C-19), 0.88 (s, 3H, CH_3, C-18), 1.05 – 1.10 (m, 1H, CH), 1.24 – 1.30 (m, 4H, $2CH_2$), 1.42 – 1.60 (m, 6H, $3CH_2$), 1.62 – 1.92 (m, 4H, $2CH_2$), 2.10 (m, 1H, CH), 2.34 (s, 3H, Ph-CH_3), 2.38 – 2.40 (m, 2H, CH_2), 2.50 (m, 1H, CH), 3.15 (m, 1H, 5α-CH), 7.21 – 7.38 (m, 4H, Ar-H). MS m/z (%): 455 (M^+, 16), corresponding to the molecular formula $C_{30}H_{33}NO_3$ and at 296 (100, base peak).

Synthesis of 2-oxo-3-cyano-6-substituted-androst-4,6-diene[17,16-c]pyran-3-one ***6a*** *and* ***6b***

Anhydrous hydrogen chloride gas was bubbled into a solution of compound **5a**, **b** (6 mmol) and dichlorodicyanoquinone (DDQ) (1.8 g, mmol) in dioxane (40 mL). The mixture was kept for 30 minutes at room temperature. The precipitated hydroquinone was then removed by filtration. The filtrate was extracted with ether. The ethereal solution was washed with 1% sodium hydroxide solution, then water and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure; the residue formed was crystallized from methanol to give the corresponding cyclohexyldiene derivatives **6a**, **b**, respectively (Table 4).

2-Oxo-3-cyano-6-(4-fluorophenyl)androst-4,6-diene[17,16-c]pyran-3-one ***6a***

IR (KBr, cm^{-1}): 2235 (CN), 1775 – 1765 (C=O, enone), 1723 (C=O, ketone). ^{1}H-NMR (DMSO-d_6) δ: 0.79 (s, 3H, CH_3, C-19), 0.84 (s, 3H, CH_3, C-18), 1.10 – 1.15 (m, 1H, CH), 1.25 – 1.30 (m, 4H, $2CH_2$), 1.40 – 1.60 (m, 2H, CH_2), 1.65 – 1.70 (m, 2H, CH_2), 1.75 (m, 1H, CH), 1.85 – 2.00 (m, 2H, CH_2), 2.30 – 2.45 (m, 1H, CH), 5.75 (d, 1H, CH-olefinic, C-2), 5.87 (s, 1H, CH-olefinic, C-4), 6.91 (d, 1H, CH-olefinic, C-1), 7.18 – 7.29 (m, 4H, Ar-H). MS m/z (%): 455 (M^+, 45), corresponding to the molecular formula $C_{29}H_{26}FNO_3$ and at 292 (100, base peak).

2-Oxo-3-cyano-6-(4-methylphenyl)androst-4,6-diene[17,16-c]pyran-3-one ***6b***

IR (KBr, cm^{-1}): 2241 (CN), 1778 – 1767 (C=O, enone), 1728 (C=O, ketone). ^{1}H-NMR (DMSO-d_6) δ: 0.77 (s, 3H, CH_3, C-19), 0.81 (s, 3H, CH_3, C-18), 1.15 – 1.20 (m, 1H, CH), 1.24 – 1.32 (m, 4H, $2CH_2$), 1.36 – 1.58 (m, 2H, CH_2), 1.60 – 1.70 (m, 2H, CH_2), 1.76 (m, 1H, CH), 1.90 – 2.05 (m, 2H, CH_2), 2.32 (s, 3H, Ph-CH_3), 2.35 – 2.45 (m, 1H, CH), 5.71 (d, 1H, CH-olefinic, C-2), 5.91 (s, 1H, CH-olefinic, C-4), 6.89 (d, 1H, CH-olefinic, C-1), 7.21 – 7.31 (m, 4H, Ar-H). MS m/z (%): 451 (M^+, 100, base peak), corresponding to the molecular formula $C_{30}H_{29}NO_3$.

Synthesis of 2-oxo-3-cyano-6-(4-chlorophenyl)androst-5-ene[17,16-c]-pyran-3β-ol ***7***

The compound was prepared by the method given for compounds **3**, using compound **2** as starting material (Table 4). IR (KBr, cm^{-1}): 3518 – 3416 (OH), 2248 (CN), 1723 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 0.76 (s, 3H, CH_3, C-19), 0.81 (s, 3H, CH_3, C-18), 0.90 – 1.00 (m, 1H, CH), 1.25 – 1.30 (m, 4H, $2CH_2$), 1.45 – 1.60 (m, 6H, $3CH_2$), 1.66 – 1.80 (m, 2H, CH_2), 1.95 (m, 1H, CH), 2.30 – 2.50 (m, 2H, CH_2), 2.60 (m, 1H, CH), 3.65 (m, 1H, 3α – CH), 5.70 (m, 1H, CH-olefinic, C-6), 7.33 – 7.41 (m, 4H, Ar-H), 10.45 (s, 1H, OH, exchangeable with D_2O). MS m/z (%): 476 (M^+, 10), corresponding to the molecular formula $C_{29}H_{30}ClNO_3$, at 478 (M^++2, 3) and at 279 (100, base peak).

Synthesis of 2-oxo-3-cyano-6-(4-chlorophenyl)androst-3,5-diene[17,16-c]-pyran ***8***

The compound was prepared by the method given for compounds **4** using compound **7** as starting material (Table 4). IR (KBr, cm^{-1}): 2257 (CN), 1723 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 0.74 (s, 3H, CH_3, C-19), 0.79 (s, 3H, CH_3, C-18), 0.88 – 0.95 (m, 1H, CH),

1.15 – 1.25 (m, 4H, $2CH_2$), 1.40 – 1.54 (m, 4H, $2CH_2$), 1.58 – 1.75 (m, 2H, CH_2), 1.90 (m, 1H, CH), 2.25 – 2.40 (m, 2H, CH_2), 2.56 (m, 1H, CH), 5.25 (m, 1H, CH-olefinic, C-3), 5.50 (m, 1H, CH-olefinic, C-4), 5.65 (m, 1H, CH-olefinic, C-6), 7.31 – 7.48 (m, 4H, Ar-H). MS m/z (%): 458 (M^+, 32), corresponding to the molecular formula $C_{29}H_{28}ClNO_2$, 460 (M^++2, 10) and 277 (100, base peak).

*Synthesis of 2-oxo-3-cyano-6-(4-chlorophenyl)androst-5-ene[17,16-c]-pyran-3-one **9***

The compound was prepared by the method given for compounds **5** using compound **7** as starting material (Table 4). IR (KBr, cm^{-1}): 2231 (CN), 1731 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 0.74 (s, 3H, CH_3, C-19), 0.79 (s, 3H, CH_3, C-18), 0.95 – 1.00 (m, 1H, CH), 1.24 – 1.30 (m, 4H, $2CH_2$), 1.42 – 1.60 (m, 6H, $3CH_2$), 1.65 – 1.92 (m, 2H, CH_2), 2.05 (m, 1H, CH), 2.32 – 2.42 (m, 2H, CH_2), 2.48 (m, 1H, CH), 5.58 (m, 1H, CH-olefinic, C-6), 7.18 – 7.33 (m, 4H, Ar-H). MS m/z (%): 474 (M^+, 19), corresponding to the molecular formula $C_{29}H_{28}ClNO_3$, 476 (M^++2, 6) and at 229 (100, base peak).

*Synthesis of 2-oxo-3-cyano-6-(4-chlorophenyl)androst-4-ene[17,16-c]-pyran-3-one (**10**) (Oppenauer Oxidation)*

To a solution of compound **7** (6.9 mmol) in a mixture of cyclohexanone (50 mL)/dry benzene (45 mL), freshly distilled aluminum isopropoxide (2 g, 9.7 mmol) in benzene (5 mL) was added. The reaction mixture was refluxed for 16 h. The reaction mixture was treated dropwise with water (4 mL) and the precipitated aluminum salt was collected by filtration. The filtrate was evaporated under reduced pressure and the obtained residue was crystallized from the proper solvent to give the corresponding oxidized derivative **10** (Table 4). IR (KBr, cm^{-1}): 2234 (CN), 1768 (C=O, enone), 1731 (C=O, ketone). ^{1}H-NMR (DMSO-d_6) δ: 0.73 (s, 3H, CH_3, C-19), 0.82 (s, 3H, CH_3, C-18), 1.00 – 1.10 (m, 1H, CH), 1.20 – 1.30 (m, 4H, $2CH_2$), 1.45 – 1.55 (m, 4H, $2CH_2$), 1.60 – 1.75 (m, 4H, $2CH_2$), 1.80 (m, 1H, CH), 1.88 – 2.05 (m, 2H, CH_2), 2.35 – 2.40 (m, 1H, CH), 5.71 (s, 1H, CH-olefinic, C-4), 7.23 – 7.38 (m, 4H, Ar-H). MS m/z (%): 474 (M^+, 100, base peak), corresponding to the molecular formula $C_{29}H_{28}ClNO_3$ and at 476 (M^++2, 35).

*Synthesis of 3-oxo-3-cyano-6-(4-chlorophenyl)androst-4,6-diene[17,16-c]-pyrane-3-one **11***

The compound was prepared according to the Wettstein method (Modified Oppenauer) [21] using compound **7** as starting material (Table 4). IR (KBr, cm^{-1}): 2234 (CN), 1777 (C=O, enone), 1731 (C=O, ketone). ^{1}H-NMR (DMSO-d_6) δ: 0.76 (s, 3H, CH_3, C-19), 0.85 (s, 3H, CH_3, C-18), 1.05 – 1.15 (m, 1H, CH), 1.25 – 1.32 (m, 2H, CH_2), 1.46 – 1.55 (m, 2H, CH_2), 1.64 – 1.76 (m, 4H, $2CH_2$), 1.75 (m, 1H, CH), 1.85 – 2.00 (m, 2H, CH_2), 2.30 – 2.45 (m, 1H, CH), 5.78 (s, 1H, CH-olefinic, C-4), 6.21 (m, 1H, CH-olefinic, C-7), 6.76 (d, 1H, CH-olefinic, C-6), 7.25 – 7.31 (m, 4H, Ar-H). MS m/z (%): 472 (M^+, 38), corresponding to the molecular formula $C_{29}H_{26}ClNO_3$, 474 (M^++2, 9) and at 292 (100, base peak).

Pharmacological Screening

Determination of acute toxicity (LD_{50})

The LD_{50} was determined by using rats. They were injected with different increasing doses of the synthesized compounds. The dose that killed 50% of the animal was calculated according to Austen et al [22].

Anti-inflammatory activity

Carrageenan-induced edema (rats paw test)

Groups of adult male albino rats (150 – 180 g), each of eight animals were orally dosed with tested compounds at a dose level of 25 – 50 mg/kg one hour before carrageenan challenge. All animals were obtained from the Animal House Colony, Research Institute of Ophthalmology, Giza, Egypt. Foot paw edema was induced by subplantar injection of 0.05 mL of 1% suspension of carrageenan in saline into the plantar tissue of one hind paw. An equal volume of saline was injected to the other hand paw and served as control. Four hours after drug administration the animals were decapitated, blood was collected, and the paws were rapidly excised. The average weight of edema was examined for the treated as well as the control group and the percentage inhibition of weight of edema was also evaluated. Prednisolone (5 mg/kg) was employed as standard reference against which the tested compounds were compared.

Estimation of plasma prostaglandin E2 (PGE2)

Heparinized blood samples were collected from rats (n = 8), plasma was separated by centrifugation at 12,000 g for 2 min at 40°C, immediately frozen, and stored at 20°C until use. The design correlate EIA prostaglandin E2 (PGE2) kit (Aldrich, Steinheim, Germany) is a competitive immuno assay for the quantitative determination of PGE2 in biological fluids. The kit uses a monoclonal antibody to PGE2 to bind, in a competitive manner, the PGE2 in the sample after a simultaneous incubation at room temperature. The excess reagents were washed away and the substrate was added, after a short incubation time the enzyme reaction was stopped, and the yellow color generated was read on a microplate reader DYNATech, MR 5000 at 405 nm (Dynatech Industries Inc., McLean, VA, USA). The intensity of the bound yellow color is inversely proportional to the concentration of PGE2 in either standard or samples.

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