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Anti-inflammatory profile of some synthesized heterocyclic pyridone and pyridine derivatives fused with steroidal structure

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Abstract—We herein report the anti-inflammatory activity of some newly synthesized heterocyclic pyridone and pyridine derivatives fused with steroidal structure. Initially the acute toxicity of the compounds was assayed via the determination of their LD_{50}. All compounds, except **3b**, **22**, and **23**, were interestingly less toxic than the reference drug (Prednisolone®). Regarding the protection against Carrageenan®-induced edema, eight compounds were found to be more potent than Prednisolone®. On the other hand, in searching for COX-2 inhibitor, the inhibition of plasma PGE2 for the compounds was determined and four compounds were found to be more potent than the reference drug. The structure assignment of the new compounds was based on chemical and spectroscopic evidence.

1. Introduction

Natural steroids and their synthetic congeners were extensively studied during the last decades.[1,2] Sex hormones, cardiacs, diuretics, antibiotics, and neuromuscular are some representative examples. In particular, the steroidal antiphlogistic activity, namely the anti-inflammatory characteristics, is still viewed as an active area of the bioorganic medicinal chemistry literature.[2,3]

In a previous work, we reported that substituted heterocyclic and steroidal derivatives act as analgesic, anticonvulsant, and antiandrogenic agents,[4–7] and their antimicrobial activity.[8–11] In addition, the androgenic, anabolic, and anti-inflammatory activities of many heterocyclic steroidal derivatives have been reported.[12] On the other hand, cyanopyridone and cyanopyridine derivatives have promising antimicrobial agent[13,14] as well as anticancer activities.[15–19] Recently, some new chiral heterocyclic compounds containing pyridine moiety have been reported as anticancer and anti-inflammatory agents.[20,21] In view of these observations and in continuation of our previous work in pyridine chemistry, we synthesized some new heterocyclic compounds containing pyridone, thiopyridone, and pyridine rings fused with steroidal nucleus and tested their pharmacological screening.

The evaluation of the anti-inflammatory potency of the synthesized compounds was realized comparable to the synthetic corticosteroid Prednisolone® (I, protection against Carrageenan®-induced edema and inhibition of plasma prostaglandin E_2), aimed at the search for COX-2 inhibitors. For the structure of Prednisolone®, see Figure 1.

2. Results and discussion

2.1. Chemistry

Arylmethylene of epiandrosterones **1a**,**b** and the dehydroepiandrosterone **2** was prepared according to the literature.[4,22] Condensation of **1a**,**b** with ethyl

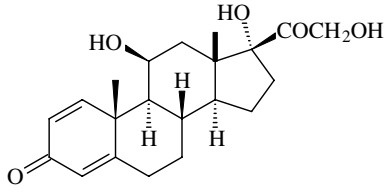

Figure 1. Chemical structure of Prednisolone® (I).

Keywords: Cyanopyridone; Thiopyridone; Cyanopyridine; Anti-inflammatory; Prednisolone®.
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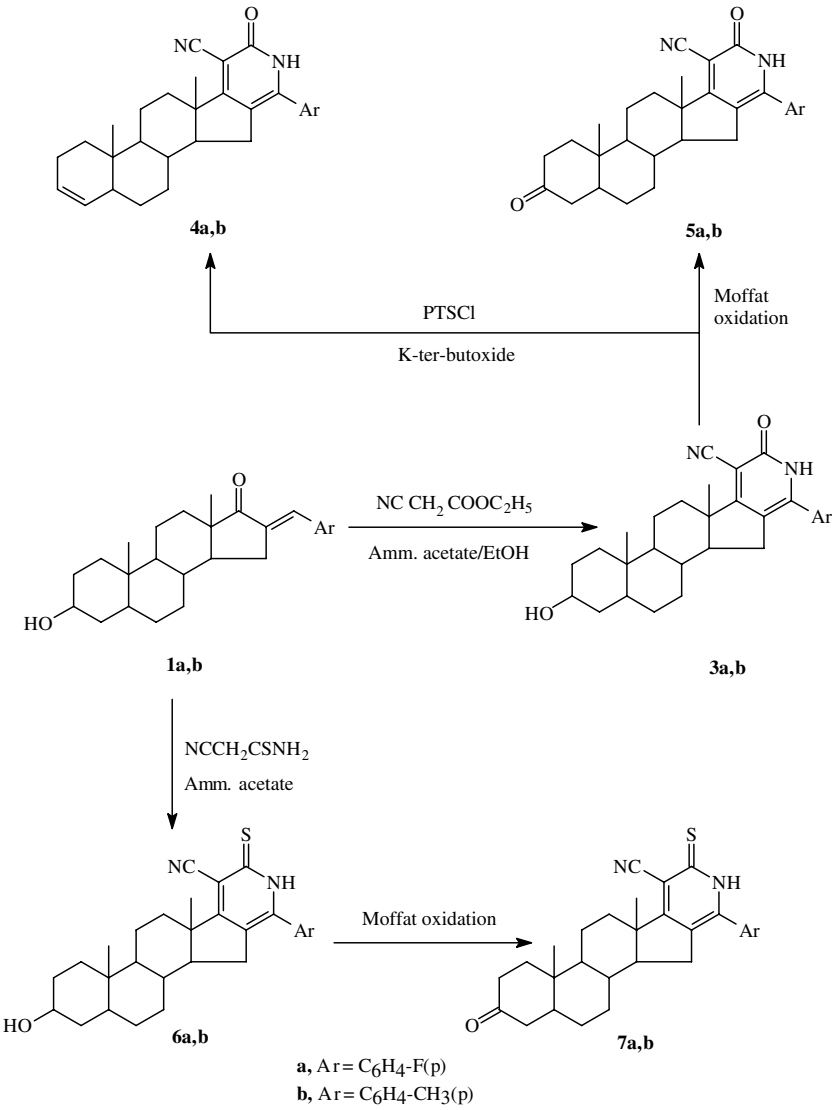
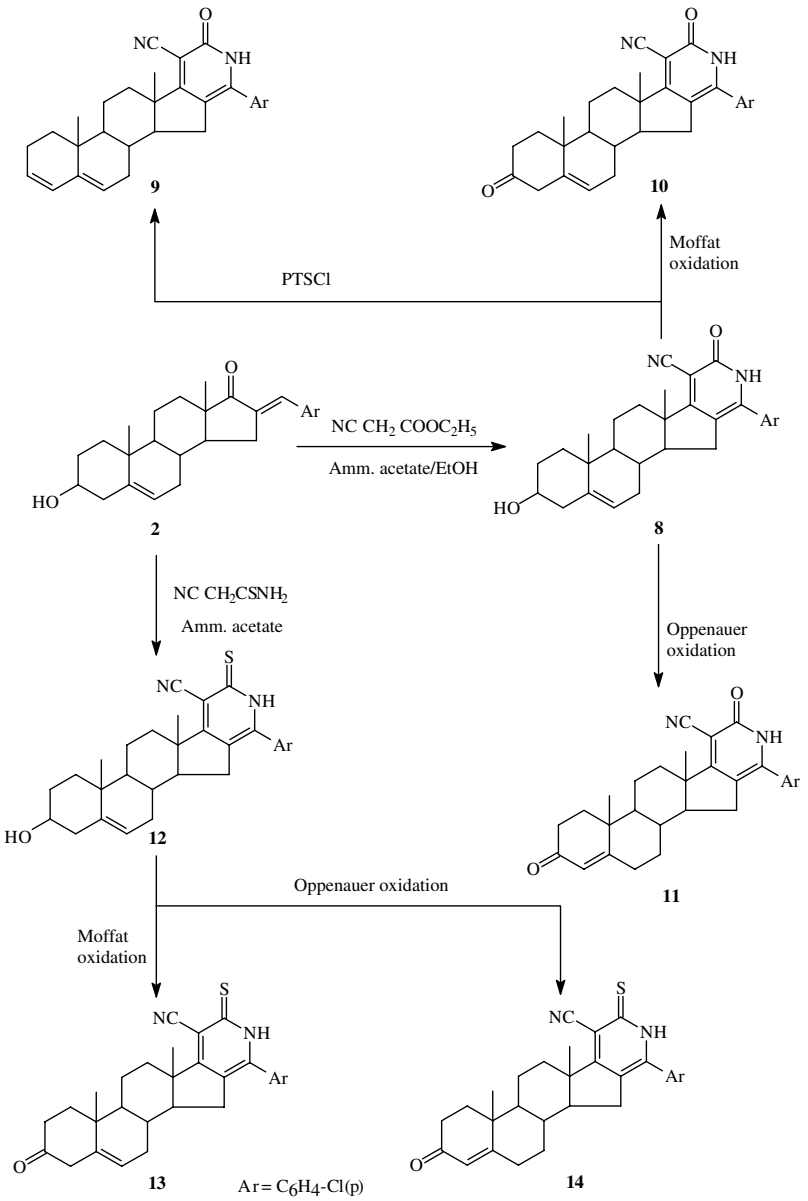
cyanoacetate in the presence of ammonium acetate in refluxing ethanol gave the corresponding androstano-cyanopyridone derivatives **3a**,**b**, which were then reacted with *p*-toluenesulfonyl chloride (PTSCl) to afford the cyclohexene (ring A) derivatives **4a**,**b**, respectively. Oxidation of compounds **3a**,**b** with Moffat oxidizing agent afforded the corresponding 3-oxo-analogues **5a**,**b**, respectively (Scheme 1). Also, condensation of compounds **1a**,**b** with cyanothioacetamide in the presence of ammonium acetate gave the corresponding thiopyridone derivatives **6a**,**b**, respectively, which were oxidized by Moffat oxidizing agent to afford 3-oxo-derivatives **7a**,**b**, respectively (Scheme 1).

The dehydroepiandrosterone derivative **2** was similarly reacted with ethyl cyanoacetate in the presence of ammonium acetate to yield the corresponding cyano-pyridone **8**, which was treated with *p*-toluenesulfonyl chloride (PTSCl) to give the corresponding cyclohexene (ring A) **9**. Moffat oxidation of compound **8** afforded the 3-oxo-analogue **10** without affecting Δ^5-ene, while, Oppenauer oxidation of **8** with aluminum isopropoxide afforded 3-oxo-analogue with delocalization of Δ^5-ene into Δ^4-ene derivative **11** (Scheme 2). On the other hand, condensation of compound **2** with cyanothioacetamide in the presence of ammonium acetate gave the corresponding androsteno-thiopyridone derivative **12**, which was oxidized with Moffat oxidizing agent to give 3-oxo-analogue **13**. Compound **12** was oxidized under modified Oppenauer conditions to afford 3-oxo-analogue with delocalization of Δ^5-ene into Δ^4-ene derivative **14** (Scheme 2).

Compounds **1a**,**b** were reacted with malononitrile in the presence of ethanolic sodium ethoxide to yield androstano-cyanoethoxypyridine derivatives **15a**,**b** via the intermediate **A**. Compounds **15a**,**b** were treated with PTSCl to give cyclohexene derivatives **16a**,**b**, respectively. Oxidation of compounds **15a**,**b** with Moffat oxidizing agent gave the corresponding 3-oxo-androstano-pyridines **17a**,**b**, which were then treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[23] in the presence



Scheme 1. Synthetic routes for compounds **3**–**7**.



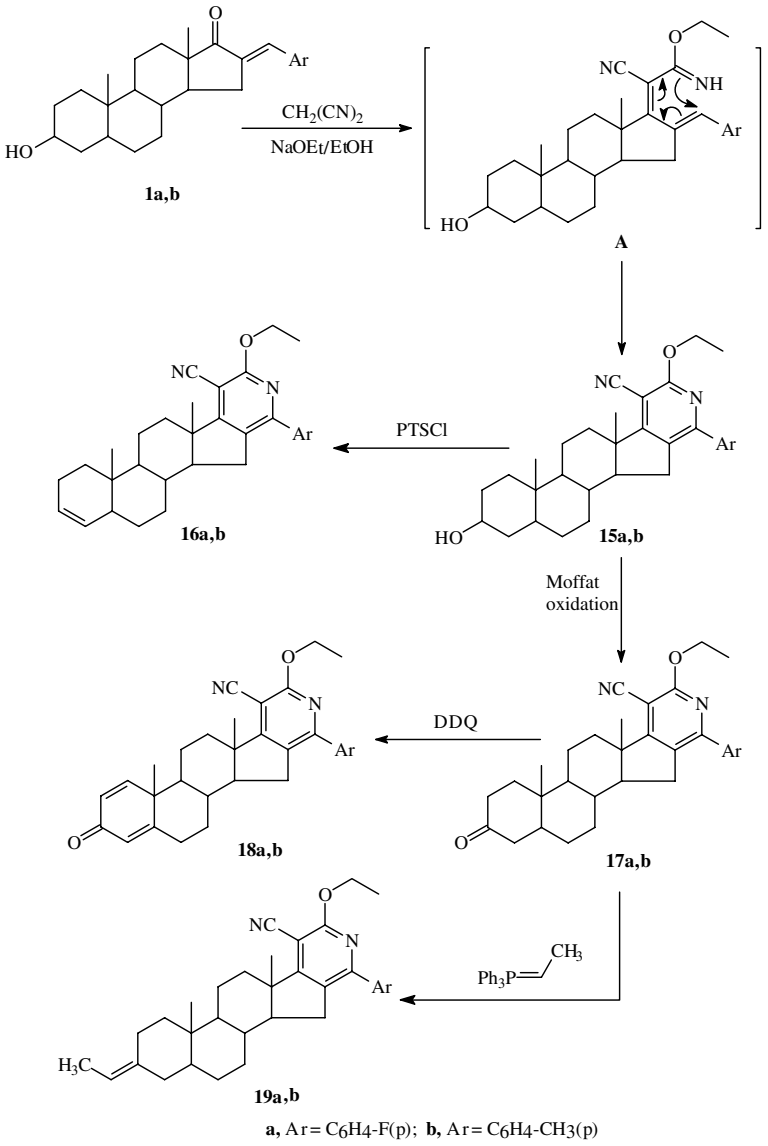
Scheme 2. Synthetic routes for compounds **8**–**14**.

of bubbled anhydrous hydrogen chloride gas in dioxane to afford the corresponding 3-oxo-$\Delta^{1,4}$-androstano-analogues **18a**,**b**, while, condensation of compounds **17a**,**b** with ethylene triphenylphosphorane ($Ph_3P{=}CHCH_3$) by heating at 60 °C in dimethylsulfoxide afforded 3-ethylene androstano-cyanoethoxypyridine derivatives **19a**,**b**, respectively (Scheme 3).

Similarly, condensation of compound **2** with malononitrile in the presence of sodium ethoxide in refluxing ethanol yielded the corresponding androsteno-cyanoethoxypyridine derivative **20**, which was oxidized by Moffat oxidizing agent to afford 3-oxo-analogue **21**. Oppenauer oxidation of **20**, with aluminum isopropoxide, resulted in the 3-oxo-analogue with delocalization of Δ^5-ene into Δ^4-ene derivative **22**, while, modified Oppenauer (Wettstein oxidation)[24] of **20** afforded the corresponding $\Delta^{4,6}$-3-oxo-analogue **23** (Scheme 4).

2.2. Pharmacological screening

2.2.1. Anti-inflammatory potency. Initially, the acute toxicity of the compounds was assayed via the determination of their LD_{50}. All compounds, except **3b**, **22**, and **23**, were interestingly less toxic than the reference drug (Table 1). The newly synthesized compounds were then pharmacologically screened on male albino rats for their anti-inflammatory potency (Tables 2 and 3). The evaluation of the anti-inflammatory activities was based on a strong biological rational, which involved two criteria present in the tested molecules. The first, is the structural similarity to Prednisolone® where ring A conjugation and the bioisosteric replacement of C-21 carbonyl with different chemical and pharmacological bioisosteric moieties. The second is the strong similarity to another clinically used drug, that contains heterocyclic rings as Meloxican (Mobic®) and Tenoxicam (Tiloctil®).



Scheme 3. Synthetic routes for compounds **15**–**19**.

2.2.2. Purpose and rational. For the determination of the antiphlogistic potency of the synthesized compounds, two standard tests were realized at 25 and 50 mg/kg rat body weight namely, the protection against Carrageenan®-induced edema according to Winter et al.[25] and the inhibition of plasma PGE2. The latter is known as a good confirming indicator for the Carrageenan®-induced rat paw edema.[26]

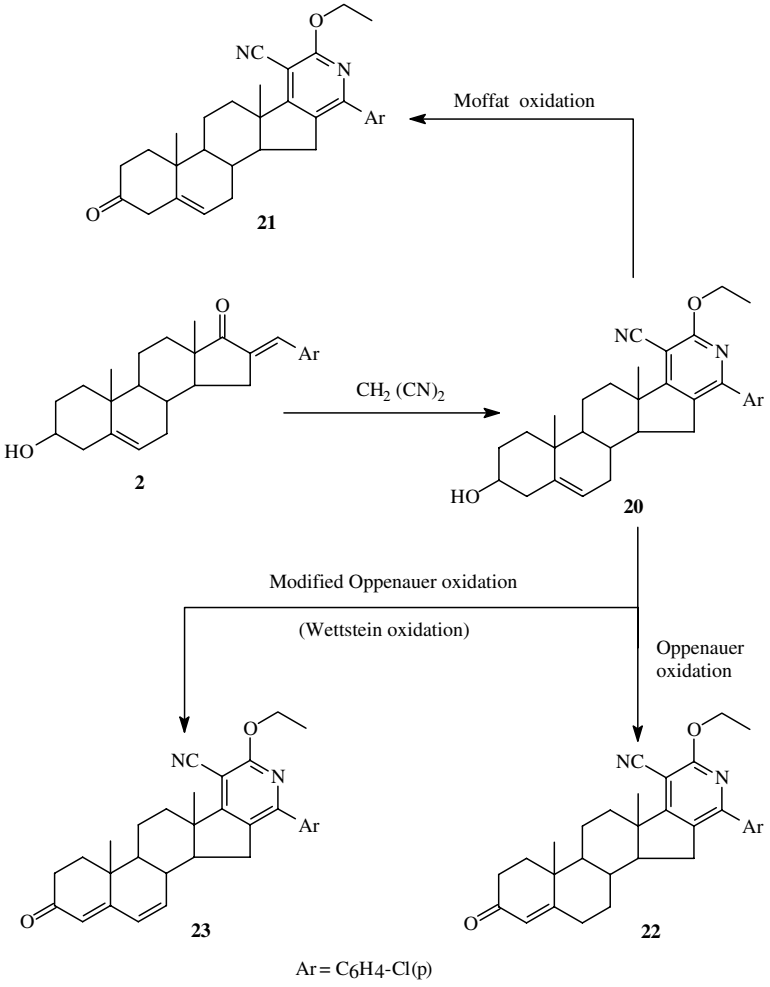
Regarding the protection against Carrageenan®-induced edema, eight compounds namely **5a**, **5b**, **6a**, **6b**, **7a**, **9**, **11**, and **12** were found to be more potent than Prednisolone®. Where, their protection percentage against carrageenan-induced edema at two dose levels 25 and 50 mg/kg was 93.44/96.18, 93.58/99.16, 92.16/99.18, 93.66/95.12, 93.12/94.15, 88.16/99.41, 88.16/98.14, and 94.66/94.71, respectively (Prednisolone® 81/93). On the other hand, the inhibition of plasma PGE2 for the compounds **5a**, **5b**, **6a**, and **9** was found to be more potent than Prednisolone® at the two tested doses levels 25 and 50 mg/kg. The inhibition percentage for the latter compounds were found as: 99.44/93.21, 92.33/96.55, 81.16/91.62, and 84.61/95.16, respectively.

2.2.3. Structure–activity relationship (SAR). From the obtained results, we can conclude [17,16-*c*]cyanopyridone fused to ring D combined with some degree of unsaturation in the steroidal scaffold is essential for anti-inflammatory activities. Pyridonethione also is essential for activity but to a less moderate extent.

3. Experimental

3.1. Synthesis

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,



Scheme 4. Synthetic routes for compounds **20**–**23**.

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. The ^{1}H NMR spectra were measured with Jeol 270 MHz (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-instrument system incorporation, 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 procedures.[4,23]

3.1.1. Synthesis of 2-oxo-3-cyano-6-(4-substituted phenyl)androsteno[17,16-*c*]pyridon-3β-ols (3a,b). A mixture of compound **1** (10 mmol) and ethyl cyanoacetate (1.13 mL, 10 mmol) in absolute ethanol (25 mL) in the presence of ammonium acetate (1.16 g, 80 mmol) 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 steroidal pyridone derivatives **3a**,**b**, respectively.

3.1.1.1. 2-Oxo-3-cyano-6-(4-fluorophenyl)androsteno-[17,16-*c*]pyridon-3β-ol (3a). Yield 75%, mp >310 °C (MeOH); IR (KBr, cm^{-1}): 3414–3377 (NH, OH), 2228 (CN), 1628–1648 (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}), 0.96–1.05 (m, 1H, CH), 1.20–1.26 (m, 4H, $2CH_2$), 1.39–1.56 (m, 6H, $3CH_2$), 1.64–1.88 (m, 4H, $2CH_2$), 1.96–2.00 (m, 1H, CH), 2.30–2.42 (m, 2H, CH_2), 2.48–2.52 (m, 1H, CH), 2.98–3.05 (m, 1H, 5α-CH), 3.54–3.58 (m, 1H, 3α-CH), 7.18–7.43 (m, 4H, Ar-H), 9.05 (s, 1H, NH, exchangeable with D_2O), 10.14 (s, 1H, OH, exchangeable with D_2O); MS *m*/*z* (%): 460 (M^+, 15), corresponding to the molecular formula $C_{29}H_{33}FN_2O_2$ and at 323 (100, base peak).

3.1.1.2. 2-Oxo-3-cyano-6-(4-methylphenyl)androsteno[17,16-*c*]pyridon-3β-ol (3b). Yield 70%, mp >256 °C (EtOAc/MeOH); IR (KBr, cm^{-1}): 3421–3348 (br, NH, OH), 2231 (CN), 1628–1648 (C=O); ^{1}H NMR (DMSO-d_6): δ 0.80 (s, 3H, CH_3, C_{19}), 0.87 (s, 3H, CH_3, C_{18}), 0.95–1.00 (m, 1H, CH), 1.22–1.27 (m, 4H, $2CH_2$), 1.36–1.54 (m, 6H, $3CH_2$), 1.65–1.84 (m, 4H, $2CH_2$), 1.92–1.96 (m, 1H, CH), 2.24–2.40 (m, 2H, CH_2), 2.44 (s, 3H, Ph–CH_3), 2.46–2.53 (m, 1H, CH), 3.00–3.08 (m, 1H, 5α-CH), 3.49–3.57 (m, 1H, 3α-CH), 7.30–7.42 (m, 4H, Ar-H), 8.98 (s, 1H, NH, exchangeable

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

Compound	LD_{50} (mg/kg)
3a	2.681 ± 0.011
3b	1.081 ± 0.012
4a	2.314 ± 0.013
4b	3.618 ± 0.011
5a	1.819 ± 0.011
5b	2.115 ± 0.012
6a	2.561 ± 0.011
6b	2.482 ± 0.013
7a	4.180 ± 0.014
7b	3.711 ± 0.011
8	1.914 ± 0.012
9	3.111 ± 0.015
10	3.068 ± 0.011
11	2.714 ± 0.013
12	3.617 ± 0.012
13	2.813 ± 0.016
14	2.817 ± 0.012
15a	2.541 ± 0.016
15b	2.011 ± 0.013
16a	2.641 ± 0.014
16b	1.983 ± 0.011
17a	2.118 ± 0.013
17b	2.694 ± 0.012
18a	2.546 ± 0.015
18b	2.000 ± 0.010
19a	1.913 ± 0.014
19b	1.858 ± 0.013
20	1.734 ± 0.011
21	1.989 ± 0.014
22	1.432 ± 0.011
23	1.521 ± 0.012
Prednisolone®	1.618 ± 0.016

with D_2O), 10.18 (s, 1H, OH, exchangeable with D_2O); MS *m/z* (%): 456 (M^+, 100, base peak), corresponding to the molecular formula $C_{30}H_{36}N_2O_2$.

3.1.2. Synthesis of 2-oxo-3-cyano-6-(4-substituted phenyl)androst-3-ene[17,16-*c*]pyridones (4a,b). A mixture of compounds **3a**,**b** (5 mmol), *p*-toluenesulfonyl chloride (PTSCl) (0.4 g, 5 mmol) and triethylamine (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, 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, 0.5 N, 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 cyclohexene cyanopyridone derivatives **4a**,**b**, respectively.

3.1.2.1. 2-Oxo-3-cyano-6-(4-fluorophenyl)androst-3-ene[17,16-*c*]pyridone (4a). Yield 56%; mp 177 °C (EtOH); IR (KBr, cm^{-1}): 3451–3381 (NH), 2223 (CN), 1625–1655 (C=O); ^1H NMR (DMSO-d_6): δ 0.77 (s, 3H, CH_3, C_{19}), 0.84 (s, 3H, CH_3, C_{18}), 0.95–0.99 (m, 1H, CH), 1.20–1.27 (m, 4H, $2CH_2$), 1.42–1.57 (m, 4H, $2CH_2$), 1.61–1.77 (m, 4H, $2CH_2$), 1.88–1.92 (m, 1H, CH), 2.30–2.40 (m, 2H, CH_2), 2.53–2.58 (m, 1H, CH), 3.18–3.26 (m, 1H, CH, C_5), 5.30–5.33 (m, 1H, CH, C_3), 5.48 (d, 1H, CH, C_4), 7.20–7.33 (m, 4H, Ar-H),

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

Compound	Dose (mg/kg)	Protection against carrageenan-induced edema (%)[a]
3a	25	—
	50	52.16 ± 0.080
3b	25	—
	50	38.66 ± 0.082
4a	25	—
	50	46.44 ± 0.079
4b	25	91.16 ± 0.091
	50	92.88 ± 0.081
5a	25	93.44 ± 0.086
	50	96.18 ± 0.083
5b	25	93.58 ± 0.090
	50	99.16 ± 0.086
6a	25	92.16 ± 0.078
	50	99.18 ± 0.077
6b	25	93.66 ± 0.080
	50	95.12 ± 0.076
7a	25	93.12 ± 0.067
	50	94.15 ± 0.078
7b	25	76.12 ± 0.068
	50	77.24 ± 0.080
8	25	65.80 ± 0.076
	50	88.16 ± 0.081
9	25	88.16 ± 0.059
	50	99.41 ± 0.075
10	25	—
	50	38.14 ± 0.054
11	25	88.16 ± 0.078
	50	98.14 ± 0.077
12	25	94.66 ± 0.068
	50	94.71 ± 0.069
14	25	52.31 ± 0.081
	50	58.17 ± 0.077
15a	25	44.15 ± 0.056
	50	72.13 ± 0.066
15b	25	54.22 ± 0.067
	50	73.14 ± 0.049
16b	25	63.88 ± 0.065
	50	84.16 ± 0.067
17b	25	55.22 ± 0.055
	50	66.15 ± 0.068
18a	25	53.16 ± 0.078
	50	65.18 ± 0.066
18b	25	55.75 ± 0.069
	50	75.13 ± 0.074
19a	25	—
	50	84.16 ± 0.066
19b	25	—
	50	44.80 ± 0.055
22	25	47.18 ± 0.080
	50	63.11 ± 0.056

Table 2 (*continued*)

Compound	Dose (mg/kg)	Protection against carrageenan-induced edema (%)[a]
23	25	58.16 ± 0.073
	50	79.13 ± 0.065
Prednisolone®	25	81.0 ± 0.100
	50	93.0 ± 0.082

[a] The tested doses were 25, 50 mg and three determinations for each dose carried out.

9.00 (s, 1H, NH, exchangeable with D_2O); MS *m/z* (%): 442 (M^+, 25), corresponding to the molecular formula $C_{29}H_{31}FN_2O$ and at 278 (100, base peak).

3.1.2.2. 2-Oxo-3-cyano-6-(4-methylphenyl)androst-3-ene[17,16-*c*]pyridone (4b). Yield 68%; mp 244 °C (MeOH); IR (KBr, cm^{-1}): 3418–3371 (NH), 2218 (CN), 1628–1665 (C═O); ^1H NMR (DMSO-d_6): δ 0.81 (s, 3H, CH_3, C_{19}), 0.87 (s, 3H, CH_3, C_{18}), 0.92–0.97 (m, 1H, CH), 1.15–1.30 (m, 4H, $2CH_2$), 1.38–1.56 (m, 4H, $2CH_2$), 1.63–1.81 (m, 4H, $2CH_2$), 1.88–1.96 (m, 1H, CH), 2.24–2.40 (m, 2H, CH_2), 2.44 (s, 3H, Ph–CH_3), 2.52–2.58 (m, 1H, CH), 3.26–3.30 (m, 1H, CH, C_5), 5.24–5.35 (m, 1H, CH, C_3), 5.47 (d, 1H, CH, C_4), 7.24–7.38 (m, 4H, Ar-H), 8.96 (s, 1H, NH, exchangeable with D_2O). MS *m/z* (%): 438 (M^+, 100, base peak), corresponding to the molecular formula $C_{30}H_{34}N_2O$.

3.1.3. Synthesis of 2-oxo-3-cyano-6-(4-substituted phenyl)androstano[17,16-*c*]pyrido-3-ones (5a,b). *Moffat method.* Appropriate compounds **3a**,**b** (2 mmol) were dissolved in mixture a of benzene (3 mL), dimethylsulfoxide (3 mL), pyridine (0.16 mL), and trifluoroacetic acid (TFA) (0.08 mL), and dicyclohexylcarbodiimide (DCC) (1.24 g, 6 mmol), was added. The reaction mixture was kept overnight at room temperature. Ether (50 mL) was added then oxalic acid (0.54 g, 6 mmol) in methanol (50 mL), after 30 min, water (50 mL) was added. The dicyclohexylurea obtained was removed by filtration. The product was extracted from filtrate with ether, washed with 5% sodium bicarbonate and then with water. The ethereal solution was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The formed residue was crystallized from the proper solvent to give the corresponding 3-oxo-analogues **5a**,**b**, respectively.

3.1.3.1. 2-Oxo-3-cyano-6-(4-fluorophenyl)androstano[17,16-*c*]pyrido-3-one (5a). Yield 58%; mp 222 °C (MeOH); IR (KBr, cm^{-1}): 3418–3337 (NH), 2231 (CN), 1735 (C═O), 1665–1625 (C═O); ^1H NMR (DMSO-d_6): δ 0.79 (s, 3H, CH_3, C_{19}), 0.86 (s, 3H, CH_3, C_{18}), 0.99–1.05 (m, 1H, CH), 1.20–1.27 (m, 4H, $2CH_2$), 1.42–1.58 (m, 6H, $3CH_2$), 1.66–1.89 (m, 4H, $2CH_2$), 1.96–1.98 (m, 1H, CH), 2.23–2.38 (m, 2H, CH_2), 2.50–2.55 (m, 1H, CH), 3.00–3.10 (m, 1H, 5α-CH), 7.28–7.40 (m, 4H, Ar-H), 8.85 (s, 1H, NH, exchangeable with D_2O); MS *m/z* (%): 458 [M^+, 100, base peak), corresponding to the molecular formula $C_{29}H_{31}FN_2O_2$.

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

Compound	Dose (mg/kg)	Inhibition of plasma PGE2 (%)[a]
3a	25	—
	50	46.61 ± 0.090
3b	25	—
	50	31.13 ± 0.085
4a	25	—
	50	32.18 ± 0.110
4b	25	88.66 ± 0.085
	50	89.99 ± 0.012
5a	25	90.44 ± 0.086
	50	93.21 ± 0.095
5b	25	92.33 ± 0.087
	50	96.55 ± 0.110
6a	25	81.16 ± 0.088
	50	91.62 ± 0.100
6b	25	78.62 ± 0.096
	50	82.66 ± 0.087
7a	25	77.41 ± 0.088
	50	81.56 ± 0.086
7b	25	72.13 ± 0.120
	50	73.54 ± 0.100
8	25	48.16 ± 0.082
	50	79.77 ± 0.079
9	25	84.61 ± 0.110
	50	95.16 ± 0.120
10	25	—
	50	31.16 ± 0.076
11	25	76.55 ± 0.078
	50	84.87 ± 0.081
12	25	82.16 ± 0.076
	50	79.15 ± 0.077
14	25	34.91 ± 0.068
	50	56.39 ± 0.076
15a	25	38.41 ± 0.110
	50	69.18 ± 0.095
15b	25	47.62 ± 0.065
	50	70.55 ± 0.087
16b	25	53.11 ± 0.088
	50	73.82 ± 0.079
17b	25	43.18 ± 0.088
	50	62.13 ± 0.078
18a	25	46.31 ± 0.090
	50	61.38 ± 0.110
18b	25	50.99 ± 0.100
	50	71.00 ± 0.098
19a	25	—
	50	77.50 ± 0.086
19b	25	—
	50	36.18 ± 0.088
22	25	41.16 ± 0.077
	50	54.17 ± 0.091

(*continued on next page*)

Table 3 (*continued*)

Compound	Dose (mg/kg)	Inhibition of plasma PGE2 (%)[a]
23	25	52.16 ± 0.082
	50	75.18 ± 0.090
Prednisolone®	25	77.0 ± 0.084
	50	91.0 ± 0.087

[a] The tested doses were 25, 50 mg and three determinations for each dose carried.

3.1.3.2. 2-Oxo-3-cyano-6-(4-methylphenyl)androstano[17,16-*c*]pyrido-3-one (5b). Yield 66%; mp 263 °C (EtOAc); IR (KBr, cm^{-1}): 3583–3491 (OH), 3408–3331 (NH), 2248 (CN), 1738 (C=O), 1667–1625 (C=O); ^1H NMR ($CDCl_3$): 0.76 (s, 3H, CH_3, C_{19}), 0.85 (s, 3H, CH_3, C_{18}), 0.98–1.10 (m, 1H, CH), 1.21–1.33 (m, 4H, $2CH_2$), 1.40–1.54 (m, 6H, $3CH_2$), 1.66–1.97 (m, 4H, $2CH_2$), 2.05–2.15 (m, 1H, CH), 2.34–2.40 (m, 2H, CH_2), 2.44 (s, 3H, Ph–CH_3), 2.49–2.53 (m, 1H, CH), 3.05–3.10 (m, 1H, 5α-CH), 7.24–7.37 (m, 4H, Ar-H), 9.00 (s, 1H, NH, exchangeable with D_2O); MS *m*/*z* (%): 454 (M^+, 22), corresponding to the molecular formula $C_{30}H_{34}N_2O_2$ and at 296 (100, base peak).

3.1.4. Synthesis of 3-cyano-2-thioxo-6-(4-substituted phenyl)androstano[17,16-*d*]pyrido-3β-ols (6a,b). A solution of **1** (10 mmol) and cyanothioacetamide (1 g, 10 mmol) in sodium ethoxide [0.920 g, 40 mmol sodium metal in 25 mL ethanol] was heated under reflux for 7 h. The reaction mixture was evaporated to dryness under reduced pressure, then washed with 10% hydrochloric acid and finally with water. The obtained product was dried and crystallized from the proper solvent to give the corresponding thioxopyridone derivatives **6a**,**b**.

3.1.4.1. 3-Cyano-2-thioxo-6-(4-fluorophenyl)androstano[17,16-*d*]pyrido-3β-ol (6a). Yield 70%; mp 256 °C (MeOH/EtOAc); IR (KBr, cm^{-1}): 3561–3489 (OH), 3418–3352 (NH), 2228 (CN), 1175 (C=S); ^1H NMR (DMSO-d_6): δ 0.75 (s, 3H, CH_3, C_{19}), 0.84 (s, 3H, CH_3, C_{18}), 0.95–1.05 (m, 1H, CH), 1.22–1.29 (m, 4H, $2CH_2$), 1.37–1.59 (m, 6H, $3CH_2$), 1.66–1.87 (m, 4H, $2CH_2$), 1.95–2.00 (m, 1H, CH), 2.28–2.41 (m, 2H, CH_2), 2.47–2.50 (m, 1H, CH), 3.00–3.15 (m, 1H, 5α-CH), 3.51–3.57 (m, 1H, 3α-CH), 7.23–7.44 (m, 4H, Ar-H), 8.56 (s, 1H, NH, exchangeable with D_2O), 10.18 (s, 1H, OH, exchangeable with D_2O); MS *m*/*z* (%): 476 (M^+, 100, base peak), corresponding to the molecular formula $C_{29}H_{33}FN_2OS$.

3.1.4.2. 3-Cyano-2-thioxo-6-(4-methylphenyl)androstano[17,16-*d*]pyrido-3β-ol (6b). Yield 62%; mp 236 °C (MeOH/EtOAc); IR (KBr, cm^{-1}): 3458–3471 (OH), 3428–3372 (NH), 2251 (CN), 1165 (C=S); ^1H NMR (DMSO-d_6): δ 0.76 (s, 3H, CH_3, C_{19}), 0.87 (s, 3H, CH_3, C_{18}), 0.92–0.99 (m, 1H, CH), 1.19–1.27 (m, 4H, $2CH_2$), 1.39–1.57 (m, 6H, $3CH_2$), 1.63–1.82 (m, 4H, $2CH_2$), 1.89–1.96 (m, 1H, CH), 2.23–2.40 (m, 2H, CH_2), 2.43 (s, 3H, Ph–CH_3), 2.53–2.57 (m, 1H, CH), 2.99–3.12 (m, 1H, 5α-CH), 3.54–3.60 (m, 1H, 3α-CH), 7.28–7.41 (m, 4H, Ar-H), 8.45 (s, 1H, NH, exchangeable with D_2O), 10.15 (s, 1H, OH, exchangeable with D_2O); MS *m*/*z* (%): 472 (M^+, 100), corresponding to the molecular formula $C_{30}H_{36}N_2OS$ and also as base peak.

3.1.5. Synthesis of 3-cyano-2-thioxo-6-(4-substituted phenyl)androstano[17,16-*d*]pyrido-3-ones (7a,b). Compounds **7a**,**b** were prepared by the method given for compounds **5** using compounds **6a**,**b** as starting materials.

3.1.5.1. 3-Cyano-2-thioxo-6-(4-fluorophenyl)androstano[17,16-*d*]pyrido-3-one (7a). Yield 65%; mp 296 °C (MeOH/EtOAc); IR (KBr, cm^{-1}): 3452–3421 (NH), 2258 (CN), 1728 (C=O), 1176 (C=S); ^1H NMR (DMSO-d_6): δ 0.74 (s, 3H, CH_3, C_{19}), 0.85 (s, 3H, CH_3, C_{18}), 1.00–1.10 (m, 1H, CH), 1.20–1.30 (m, 4H, $2CH_2$), 1.40–1.59 (m, 6H, $3CH_2$), 1.66–1.93 (m, 4H, $2CH_2$), 1.97–1.99 (m, 1H, CH), 2.26–2.38 (m, 2H, CH_2), 2.48–2.53 (m, 1H, CH), 2.99–3.10 (m, 1H, 5α-CH), 7.26–7.41 (m, 4H, Ar-H), 8.76 (s, 1H, NH, exchangeable with D_2O); MS *m*/*z* (%): 474 [M^+, 100, base peak], corresponding to the molecular formula $C_{29}H_{31}FN_2OS$.

3.1.5.2. 3-Cyano-2-thioxo-6-(4-methylphenyl)androstano[17,16-*d*]pyrido-3-one (7b). Yield 52%; mp 188 °C (MeOH/EtOAc); IR (KBr, cm^{-1}): 3358–3364 (NH), 2226 (CN), 1728 (C=O), 1175 (C=S); ^1H NMR (DMSO-d_6): δ 0.76 (s, 3H, CH_3, C_{19}), 0.87 (s, 3H, CH_3, C_{18}), 0.98–1.10 (m, 1H, CH), 1.19–1.30 (m, 4H, $2CH_2$), 1.38–1.57 (m, 6H, $3CH_2$), 1.64–1.93 (m, 4H, $2CH_2$), 2.00–2.10 (m, 1H, CH), 2.37–2.42 (m, 2H, CH_2), 2.45 (s, 3H, Ph–CH_3), 2.50–2.54 (m, 1H, CH), 3.05–3.16 (m, 1H, 5α-CH), 7.23–7.43 (m, 4H, Ar-H), 8.62 (s, 1H, NH, exchangeable with D_2O); MS *m*/*z* (%): 470 (M^+, 10), corresponding to the molecular formula $C_{30}H_{34}N_2OS$ and at 296 (100, base peak).

3.1.6. Synthesis of 2-oxo-3-cyano-6-(4-chlorophenyl)androst-5-ene[17,16-*c*]pyrido-3β-ol (8). Compound **8** was prepared by the method given for compounds **3** using compound **2** as starting material, in 76% yield; mp 201 °C (MeOH/EtOAc); IR (KBr, cm^{-1}): 3518–3428 (OH), 3376–3318 (NH), 2251 (CN), 1665–1621 (C=O); ^1H NMR (DMSO-d_6): δ 0.77 (s, 3H, CH_3, C_{19}), 0.81 (s, 3H, CH_3, C_{18}), 0.94–1.05 (m, 1H, CH), 1.22–1.27 (m, 4H, $2CH_2$), 1.36–1.55 (m, 6H, $3CH_2$), 1.64–1.78 (m, 2H, CH_2), 1.92–1.98 (m, 1H, CH), 2.26–2.49 (m, 2H, CH_2), 2.56–2.60 (m, 1H, CH), 3.54–3.62 (m, 1H, 3α-CH), 5.63–5.67 (m, 1H, CH, C_6), 7.22–7.40 (m, 4H, Ar-H), 8.97 (s, 1H, NH, exchangeable with D_2O), 10.15 (s, 1H, OH, exchangeable with D_2O); MS *m*/*z* (%): 475 (M^+, 30), corresponding to the molecular formula $C_{29}H_{31}ClN_2O_2$, at 477 (M^++2, 9) and at 278 (100, base peak).

3.1.7. Synthesis of 3-cyano-6-(4-chlorophenyl)androst-3,5-diene[17,16-*c*]pyrid-2-one (9). Compound **9** was prepared by the method given for compounds **4** using compound **8** as starting material; in 58% yield; mp 317 °C (MeOH/EtOAc); IR (KBr, cm^{-1}): 3317–3309 (NH), 2241 (CN), 1665–1621 (C=O), ^1H NMR (DMSO-d_6):

δ 0.77 (s, 3H, CH_3, C_{19}), 0.81 (s, 3H, CH_3, C_{18}), 0.91–0.98 (m, 1H, CH), 1.00–1.24 (m, 4H, $2CH_2$), 1.37–1.55 (m, 4H, $2CH_2$), 1.60–1.75 (m, 2H, CH_2), 1.90–1.96 (m, 1H, CH), 2.25–2.40 (m, 2H, CH_2), 2.52–2.58 (m, 1H, CH), 5.19–2.25 (m, 1H, CH, C_3), 5.45–5.50 (m, 1H, CH, C_4), 5.61–5.68 (m, 1H, CH, C_6), 7.24–7.44 (m, 4H, Ar-H), 8.95 (s, 1H, NH, exchangeable with D_2O). MS *m/z* (%): 457 (M^+, 18), corresponding to the molecular formula $C_{29}H_{29}ClN_2O$, 459 (M^++2, 7) and 280 (100, base peak).

3.1.8. Synthesis of 2-oxo-3-cyano-6-(4-chlorophenyl)androst-5-ene[17,16-*c*]pyrido-3-one (10). Compound **10** was prepared by the method given for compounds **5** using compound **8** as starting material, in 62% yield; mp 247 °C (MeOH); IR (KBr, cm^{-1}): 3413–3378 (NH), 2248 (CN), 1734 (C=O), 1668–1628 (C=O); ^1H NMR (DMSO-d_6): δ 0.79 (s, 3H, CH_3, C_{19}), 0.84 (s, 3H, CH_3, C_{18}), 0.93–0.98 (m, 1H, CH), 1.21–1.32 (m, 4H, $2CH_2$), 1.43–1.60 (m, 6H, $3CH_2$), 1.64–1.96 (m, 2H, CH_2), 2.05–2.10 (m, 1H, CH), 2.29–2.40 (m, 2H, CH_2), 2.44–2.48 (m, 1H, CH), 5.58–5.64 (m, 1H, CH, C_6), 7.18–7.38 (m, 4H, Ar-H), 9.05 (s, 1H, NH, exchangeable with D_2O). MS *m/z* (%): 473 (M^+, 100, base peak), corresponding to the molecular formula $C_{29}H_{29}ClN_2O_2$, 475 (M^++2, 32).

3.1.9. Synthesis of 2-oxo-3-cyano-6-(4-chlorophenyl)androst-4-ene[17,16-*c*]pyrido-3-one (11). *Oppenauer method.* To a solution of compound **8** (3.32 g, 7 mmol) in [cyclohexanone (50 mL)/dry benzene (45 mL) as mixture], freshly distilled aluminum isopropoxide (2 g, 9.7 mmol) in benzene (5 mL) was added. The reaction mixture was refluxed for 16 h, after cooling, it was treated dropwise with water (4 mL). The precipitated aluminum salt was collected by filtration, the filtrate was evaporated under reduced pressure, and the obtained residue was crystallized from methanol to give product **11**, in 55% yield; mp 207–209 °C; IR (KBr, cm^{-1}): 3406–3971 (NH), 2258 (CN), 1787 (C=O, enone), 1665–1623 (C=O, amide); ^1H NMR (DMSO-d_6): δ 0.76 (s, 3H, CH_3, C_{19}), 0.87 (s, 3H, CH_3, C_{18}), 0.96–1.00 (m, 1H, CH), 1.22–1.33 (m, 4H, $2CH_2$), 1.42–1.57 (m, 4H, $2CH_2$), 1.65–1.72 (m, 4H, $2CH_2$), 1.80–1.85 (m, 1H, CH), 1.93–2.05 (m, 2H, CH_2), 2.35–2.40 (m, 1H, CH), 5.70 (s, 1H, CH, C_4), 7.23–7.38 (m, 4H, Ar-H), 9.00 (s, 1H, NH, exchangeable with D_2O); MS *m/z* (%): 473 (M^+, 15), corresponding to the molecular formula $C_{29}H_{29}ClN_2O_2$ and at 475 (M^++2, 6) and at 296 (100, base peak).

3.1.10. Synthesis of 3-cyano-thioxo-6-(4-chlorophenyl)androst-5-one[17,16-*d*]pyrido-3β-one (12). Compounds **12** was prepared by the method given for compounds **6a**,**b** using compound **2** as starting material, in 59% yield; mp 288 °C (MeOH/EtOAc); IR (KBr, cm^{-1}): 3574–3315 (OH), 3440–3371 (NH), 2253 (CN), 1174 (C=S); ^1H NMR (DMSO-d_6): δ 0.79 (s, 3H, CH_3, C_{19}), 0.88 (s, 3H, CH_3, C_{18}), 0.90–1.00 (m, 1H, CH), 1.25–1.29 (m, 4H, $2CH_2$), 1.39–1.60 (m, 6H, $3CH_2$), 1.67–1.82 (m, 2H, CH_2), 1.90–1.95 (m, 1H, CH), 2.30–2.50 (m, 2H, CH_2), 2.58–2.62 (m, 1H, CH), 3.57–3.65 (m, 1H, 3α-CH), 5.62–5.70 (m, 1H, CH, C_6), 7.13–7.36 (m, 4H, Ar-H), 8.65 (s, 1H, NH, exchangeable with D_2O), 10.15 (s, 1H, OH, exchangeable with D_2O); MS *m/z* (%): 491 (M^+, 100, base peak), corresponding to the molecular formula $C_{29}H_{31}ClN_2OS$, at 493 (M^++2, 30).

3.1.11. Synthesis of 3-cyano-2-thioxo-6-(4-chlorophenyl)androst-5-ene[17,16-*d*]pyrido-3-one (13). Compounds **12** was prepared by the method given for compounds **5** using compound **12** as starting material, in 56% yield; mp 260 °C (EtOH); IR (KBr, cm^{-1}): 3418–3361 (NH), 2248 (CN), 1732 (C=O), 1175 (C=S); ^1H NMR (DMSO-d_6): δ 0.76 (s, 3H, CH_3, C_{19}), 0.84 (s, 3H, CH_3, C_{18}), 0.97–1.05 (m, 1H, CH), 1.19–1.32 (m, 4H, $2CH_2$), 1.44–1.62 (m, 6H, $3CH_2$), 1.69–1.95 (m, 2H, CH_2), 2.00–2.05 (m, 1H, CH), 2.30–2.40 (m, 2H, CH_2), 2.46–2.50 (m, 1H, CH), 5.60–5.68 (m, 1H, CH, C_6), 7.25–7.33 (m, 4H, Ar-H), 8.55 (s, 1H, NH, exchangeable with D_2O); MS *m/z* (%): 489 (M^+, 23), corresponding to the molecular formula $C_{29}H_{29}ClN_2OS$, 491 (M^++2, 7) and at 229 (100, base peak).

3.1.12. Synthesis of 3-cyano-2-thioxo-6-(4-chlorophenyl)androst-4-ene[17,16-*d*]pyrido-3-one (14). Compounds **14** was prepared by the method given for compound **11** using compound **12** as starting material, in 66% yield; mp 157 °C (MeOH); IR (KBr, cm^{-1}): 3418–3372 (NH), 2250 (CN), 1778 (C=O, enone), 1170 (C=S); ^1H NMR (DMSO-d_6): δ 0.73 (s, 3H, CH_3, C_{19}), 0.81 (s, 3H, CH_3, C_{18}), 0.99–1.08 (m, 1H, CH), 1.23–1.30 (m, 4H, $2CH_2$), 1.44–1.55 (m, 4H, $2CH_2$), 1.64–1.77 (m, 4H, $2CH_2$), 1.82–1.88 (m, 1H, CH), 1.92–2.00 (m, 2H, CH_2), 2.36–2.43 (m, 1H, CH), 5.72 (s, 1H, CH, C_4), 7.24–7.339 (m, 4H, Ar-H), 8.45 (s, 1H, NH, exchangeable with D_2O); MS *m/z* (%): 489 (M^+, 100, base peak), corresponding to the molecular formula $C_{29}H_{29}ClN_2OS$ and at 491 (M^++2, 35).

3.1.13. Synthesis of 2-ethoxy-3-cyano-6-(4-substituted phenyl)androst[17,16-*d*]pyridine-3β-ols (15a,b). A mixture of **1** (10 mmol) and malononitrile (0.66 g, 10 mmol) in sodium ethoxide [0.920 g, 40 mmol, sodium metal in 25 mL absolute ethanol] was refluxed for 7 h. The reaction mixture was evaporated under reduced pressure, the obtained residue solidified with water. The solid formed was collected by filtration, washed with 10% hydrochloric acid, water, dried, and crystallized from the proper solvent to give the corresponding pyridine derivatives **15a**,**b**.

3.1.13.1. 2-Ethoxy-3-cyano-6-(4-fluorophenyl)androst[17,16-*d*]pyridine-3β-ol (15a). Yield 62%; mp 210 °C (MeOH); IR (KBr, cm^{-1}): 3516–3484 (OH), 2256 (CN); ^1H NMR (DMSO-d_6): δ 0.73 (s, 3H, CH_3, C_{19}), 0.82 (s, 3H, CH_3, C_{18}), 0.95–1.00 (m, 1H, CH), 1.23–1.27 (m, 4H, $2CH_2$), 1.42–1.58 (m, 6H, $3CH_2$), 1.58 (t, 3H, CH_3-ethoxy), 1.63–1.86 (m, 4H, $2CH_2$), 1.95–1.99 (m, 1H, CH), 2.26–2.40 (m, 2H, CH_2), 2.49–2.52 (m, 1H, CH), 2.96–3.00 (m, 1H, 5α-CH), 3.50–3.55 (m, 1H, 3α-CH), 4.42 (q, 2H, CH_2-ethoxy), 7.22–7.44 (m, 4H, Ar-H), 10.12 (s, 1H, OH, exchangeable with D_2O); MS *m/z* (%): 488 (M^+, 100, base peak), corresponding to the molecular formula $C_{31}H_{37}FN_2O_2$.

3.1.13.2. 2-Ethoxy-3-cyano-6-(4-methylphenyl)androstano[17,16-*d*]pyridine-3β-ol (15b). Yield 64%; mp 216 °C (EtOH); IR (KBr, cm^{-1}): 3517–3491 (OH), 2249 (CN); ^1H NMR (DMSO-d_6): δ 0.74 (s, 3H, CH_3, C_{19}), 0.82 (s, 3H, CH_3, C_{18}), 0.94–0.98 (m, 1H, CH), 1.18–1.24 (m, 4H, $2CH_2$), 1.37–1.52 (m, 6H, $3CH_2$), 1.57 (t, 3H, CH_3-ethoxy), 1.62–1.85 (m, 4H, $2CH_2$), 1.94–1.98 (m, 1H, CH), 2.26–2.38 (m, 2H, CH_2), 2.43 (s, 3H, Ph–CH_3), 2.50–2.55 (m, 1H, CH), 3.00–3.10 (m, 1H, 5α-CH), 3.50–3.56 (m, 1H, 3α-CH), 4.43 (q, 2H, CH_2-ethoxy), 7.22–7.46 (m, 4H, Ar-H), 10.06 (s, 1H, OH, exchangeable with D_2O); MS *m*/*z* (%): 484 (M^+, 100), corresponding to the molecular formula $C_{32}H_{40}N_2O_2$ and also as base peak.

3.1.14. Synthesis of 2-ethoxy-3-cyano-6-(4-substituted phenyl)androst-3-ene[17,16-*d*]pyridines (16a,b). Compounds **16a**,**b** were prepared by the method given for compounds **4** using compound **15a**,**b** as starting materials.

3.1.14.1. 2-Ethoxy-3-cyano-6-(4-fluorophenyl)androst-3-ene[17,16-*d*]pyridine (16a). Yield 56%; mp 307 °C (MeOH); IR (KBr, cm^{-1}): 2268 (CN); ^1H NMR (DMSO-d_6): δ 0.76 (s, 3H, CH_3, C_{19}), 0.82 (s, 3H, CH_3, C_{18}), 0.92–0.96 (m, 1H, CH), 1.18–1.25 (m, 4H, $2CH_2$), 1.41–1.55 (m, 4H, $2CH_2$), 1.59 (t, 3H, CH_3-ethoxy), 1.62–1.78 (m, 4H, $2CH_2$), 1.86–1.98 (m, 1H, CH), 2.27–2.39 (m, 2H, CH_2), 2.50–2.57 (m, 1H, CH), 3.16–3.25 (m, 1H, CH, C_5), 4.40 (q, 2H, CH_2-ethoxy), 5.29–5.34 (m, 1H, CH, C_3), 5.50 (d, 1H, CH, C_4), 7.24–7.37 (m, 4H, Ar-H); MS *m*/*z* (%): 470 (M^+, 15), corresponding to the molecular formula $C_{31}H_{35}FN_2O$ and at 280 (100, base peak).

3.1.14.2. 2-Ethoxy-3-cyano-6-(4-methylphenyl)androst-3-ene[17,16-*d*]pyridine- 3β-ol (16b). Yield 68%; mp 277 °C (EtOH); IR (KBr, cm^{-1}): 2248 (CN); ^1H NMR (DMSO-d_6): δ 0.78 (s, 3H, CH_3, C_{19}), 0.83 (s, 3H, CH_3, C_{18}), 0.92–0.98 (m, 1H, CH), 1.08–1.26 (m, 4H, $2CH_2$), 1.34–1.54 (m, 4H, $2CH_2$), 1.57 (t, 3H, CH_3-ethoxy), 1.66–1.85 (m, 4H, $2CH_2$), 1.88–1.95 (m, 1H, CH), 2.23–2.42 (m, 2H, CH_2), 2.46 (s, 3H, Ph–CH_3), 2.53–2.57 (m, 1H, CH), 3.25–3.35 (m, 1H, CH, C_5), 4.40 (q, 2H, CH_2-ethoxy), 5.26–5.40 (m, 1H, CH, C_3), 5.48 (d, 1H, CH, C_4), 7.22–7.34 (m, 4H, Ar-H); MS *m*/*z* (%): 466 (M^+, 15), corresponding to the molecular formula $C_{32}H_{38}N_2O$ and at 280 (100, base peak).

3.1.15. Synthesis of 2-ethoxy-3-cyano-6-(4-substituted phenyl)androstano[17,16-*d*]pyridine-3-ones (17a,b). Compounds **17a**,**b** were prepared by the method given for compounds **5** using compound **15a**,**b** as starting materials.

3.1.15.1. 2-Ethoxy-3-cyano-6-(4-fluorophenyl)androstano[17,16-*d*]pyridine-3-one (17a). Yield 66%; mp 157 °C (EtOH/EtOAc); IR (KBr, cm^{-1}): 2251 (CN), 1723 (C═O); ^1H NMR (DMSO-d_6): δ 0.76 (s, 3H, CH_3, C_{19}), 0.86 (s, 3H, CH_3, C_{18}), 1.00–1.15 (m, 1H, CH), 1.25–1.29 (m, 4H, $2CH_2$), 1.39–1.56 (m, 6H, $3CH_2$), 1.60 (t, 3H, CH_3-ethoxy), 1.66–1.90 (m, 4H, $2CH_2$), 1.97–2.00 (m, 1H, CH), 2.25–2.42 (m, 2H, CH_2), 2.49–2.54 (m, 1H, CH), 2.99–3.10 (m, 1H, 5α-CH), 4.44 (q, 2H, CH_2-ethoxy), 7.23–7.42 (m, 4H, Ar-H); MS *m*/*z* (%): 486 [M^+, 100, base peak), corresponding to the molecular formula $C_{31}H_{35}FN_2O_2$.

3.1.15.2. 2-Ethoxy-3-cyano-6-(4-methylphenyl)androstano[17,16-*d*]pyridine-3-one (17b). Yield 54%; mp 217 °C (EtOH/EtOAc); IR (KBr, cm^{-1}): 2246 (CN), 1728 (C═O); ^1H NMR (DMSO-d_6): δ 0.77 (s, 3H, CH_3, C_{19}), 0.86 (s, 3H, CH_3, C_{18}), 0.98–1.15 (m, 1H, CH), 1.22–1.34 (m, 4H, $2CH_2$), 1.45–1.55 (m, 6H, $3CH_2$), 1.57 (t, 3H, CH_3-ethoxy), 1.65–1.94 (m, 4H, $2CH_2$), 2.00–2.10 (m, 1H, CH), 2.32–2.40 (m, 2H, CH_2), 2.45 (s, 3H, Ph–CH_3), 2.48–2.56 (m, 1H, CH), 3.00–3.15 (m, 1H, 5α-CH), 4.46 (q, 2H, CH_2-ethoxy), 7.23–7.41 (m, 4H, Ar-H); MS *m*/*z* (%): 482 (M^+, 10), corresponding to the molecular formula $C_{32}H_{38}N_2O_2$ and at 296 (100, base peak).

3.1.16. Synthesis of 2-ethoxy-3-cyano-6-(4-substituted phenyl)androst-1,4-diene[17,16-*d*]pyridine-3-ones (18a,b). Anhydrous hydrogen chloride gas was bubbled into a solution of compounds **17a**,**b** (6 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (1.8 g) in dioxane (40 mL). The reaction mixture was kept for 30 min at room temperature, the precipitated hydroquinone removed by filtration. The filtrate was extracted by ether, the ethereal solution washed with 1% sodium hydroxide then with water and dried over sodium sulfate anhydrous. The solvent was evaporated under reduced pressure, the residue formed was crystallized from ethanol to give the corresponding cyclohexyldiene derivatives **18a**,**b**, respectively.

3.1.16.1. 2-Ethoxy-3-cyano-6-(4-fluorophenyl)androst-1,4-diene[17,16-*d*]pyridine-3-one (18a). Yield 55%; mp 321 °C (EtOH); IR (KBr, cm^{-1}): 2251 (CN), 1780–1765 (CO, enone); ^1H NMR (DMSO-d_6): δ 0.78 (s, 3H, CH_3, C_{19}), 0.85 (s, 3H, CH_3, C_{18}), 0.98–1.05 (m, 1H, CH), 1.26–1.31 (m, 4H, $2CH_2$), 1.44–1.54 (m, 2H, CH_2), 1.58 (t, 3H, CH_3-ethoxy), 1.64–1.75 (m, 2H, CH_2), 1.80–1.88 (m, 1H, CH), 1.95–2.05 (m, 2H, CH_2), 2.32–2.41 (m, 1H, CH), 4.44 (q, 2H, CH_2-ethoxy), 5.70 (d, 1H, CH, C_4), 5.98 (s, 1H, CH, C_2), 7.00 (d, 1H, CH, C_1), 7.22–7.34 (m, 4H, Ar-H); MS *m*/*z* (%): 482 (45) [M^+], corresponding to the molecular formula $C_{31}H_{31}FN_2O_2$ and at 292 (100, base peak).

3.1.16.2. 2-Ethoxy-3-cyano-6-(4-methylphenyl)androst-1,4-diene[17,16-*d*]pyridine-3-one (18b). Yield 53%; mp 311 °C (EtOH); IR (KBr, cm^{-1}): 2248 (CN), 1771–1785 (C═O, enone); ^1H NMR (DMSO-d_6): δ 0.77 (s, 3H, CH_3, C_{19}), 0.87 (s, 3H, CH_3, C_{18}), 1.10–1.16 (m, 1H, CH), 1.24–1.32 (m, 4H, $2CH_2$), 1.44–1.56 (m, 2H, CH_2), 1.60 (t, 3H, CH_3-ethoxy), 1.64–1.72 (m, 2H, CH_2), 1.78–1.87 (m, 1H, CH), 1.95–2.00 (m, 2H, CH_2), 2.33–2.44 (m, 1H, CH), 2.46 (s, 3H, Ph–CH_3), 4.50 (q, 2H, CH_2-ethoxy), 5.68 (d, 1H, CH, C_4), 6.00 (s, 1H, CH, C_2), 6.96 (d, 1H, CH, C_1), 7.23–7.35 (m, 4H, Ar-H); MS *m*/*z* (%): 478 (M^+, 100, base peak), corresponding to the molecular formula $C_{32}H_{34}N_2O_2$.

3.1.17. Synthesis of 2-ethoxy-3-cyano-6-(4-substituted phenyl)androstano[17,16-*d*]pyridine-3-ethylidenes (19a,b). To a stirred solution of ethylidene triphenylphosphorane (1.2 mmol) in dimethylsulfoxide (100 mL), cycloketone derivatives (**17a**,**b**) (1 mmol) in dry benzene (60 mL) were added dropwise and then heated at 60 °C for 10–12 h. The reaction mixture was cooled, poured into ice-water, the formed product was extracted with ethyl acetate, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The obtained residue was crystallized from the proper solvent to give compounds (**19a**,**b**).

3.1.17.1. 2-Ethoxy-3-cyano-6-(4-fluorophenyl)androstano[17,16-*d*]pyridine-3-ethylidene (19a). Yield 56%; mp 287 °C (EtOH/ether); IR (KBr, cm^{-1}): 2248 (CN), 1615 (C=C); ^1H NMR (DMSO-d_6): δ 0.77 (s, 3H, CH_3, C_{19}), 0.86 (s, 3H, CH_3, C_{18}), 0.99–1.15 (m, 1H, CH), 1.23–1.29 (m, 4H, $2CH_2$), 1.40–1.56 (m, 6H, $3CH_2$), 1.59 (t, 3H, CH_3-ethoxy), 1.65–1.88 (m, 7H, $2CH_2$+CH_3), 1.98–2.05 (m, 1H, CH), 2.25–2.37 (m, 2H, CH_2), 2.50–2.54 (m, 1H, CH), 3.05–3.15 (m, 1H, 5α-CH), 4.46 (q, 2H, CH_2-ethoxy), 5.43 (q, 1H, CH=C), 7.26–7.41 (m, 4H, Ar-H); MS *m*/*z* (%): 486 [M^+, 100, base peak), corresponding to the molecular formula $C_{31}H_{35}FN_2O_2$.

3.1.17.2. 2-Ethoxy-3-cyano-6-(4-methylphenyl)androstano[17,16-*d*]pyridine-3-ethylidene (19b). Yield 63%; mp 207 °C (EtOH/EtOAc); IR (KBr, cm^{-1}): 2246 (CN), 1728 (C=O); ^1H NMR (DMSO-d_6): δ 0.77 (s, 3H, CH_3, C_{19}), 0.86 (s, 3H, CH_3, C_{18}), 1.00–1.16 (m, 1H, CH), 1.20–1.30 (m, 4H, $2CH_2$), 1.40–1.56 (m, 6H, $3CH_2$), 1.59 (t, 3H, CH_3-ethoxy), 1.68–1.98 (m, 7H, $2CH_2$+CH_3), 2.00–2.12 (m, 1H, CH), 2.36–2.42 (m, 2H, CH_2), 2.45 (s, 3H, Ph–CH_3), 2.50–2.54 (m, 1H, CH), 3.05–3.15 (m, 1H, 5α-CH), 4.46 (q, 2H, CH_2-ethoxy), 5.46 (q, 1H, CH=C), 7.21–7.42 (m, 4H, Ar-H); MS *m*/*z* (%): 482 (M^+, 10), corresponding to the molecular formula $C_{32}H_{38}N_2O_2$ and at 296 (100, base peak).

3.1.18. Synthesis of 2-ethoxy-3-cyano-6-(4-chlorophenyl)androst-5-ene[17,16-*d*]pyridine-3β-ol (20). Compounds **20** was prepared by the method given for compound **15** using compounds **2** as starting material, in 84% yield; mp 293 °C (EtOH); IR (KBr, cm^{-1}): 3204–3498 (OH), 2247 (CN); ^1H NMR (DMSO-d_6): δ 0.76 (s, 3H, CH_3, C_{19}), 0.84 (s, 3H, CH_3, C_{18}), 0.97–1.04 (m, 1H, CH), 1.24–1.31 (m, 4H, $2CH_2$), 1.42–1.55 (m, 6H, $3CH_2$), 1.60 (t, 3H, CH_3-ethoxy), 1.65–1.84 (m, 2H, CH_2), 1.95–1.98 (m, 1H, CH), 2.26–2.51 (m, 2H, CH_2), 2.56–2.60 (m, 1H, CH), 3.56–3.64 (m, 1H, 3α-CH), 4.45 (q, 2H, CH_2-ethoxy), 5.65–5.70 (m, 1H, CH, C_6), 7.30–7.45 (m, 4H, Ar-H), 10.05 (s, 1H, OH, exchangeable with D_2O); MS *m*/*z* (%): 503 (M^+, 10), corresponding to the molecular formula $C_{31}H_{35}ClN_2O_2$, at 505 (M^++2, 3) and at 279 (100, base peak).

3.1.19. Synthesis of 2-ethoxy-3-cyano-6-(4-chlorophenyl)androst-5-ene[17,16-*d*]pyridine-3-one (21). Compounds **21** was prepared by the method given for compounds **5** using compounds **20** as starting material in 55% yield; mp 195 °C (MeOH); IR (KBr, cm^{-1}): 2251 (CN), 1734 (C=O); ^1H NMR ($CDCl_3$): δ 0.79 (s, 3H, CH_3, C_{19}), 0.89 (s, 3H, CH_3, C_{18}), 0.95–1.02 (m, 1H, CH), 1.22–1.32 (m, 4H, $2CH_2$), 1.45–1.67 (m, 6H, $3CH_2$), 1.61 (t, 3H, CH_3-ethoxy), 1.65–1.92 (m, 2H, CH_2), 1.99–2.05 (m, 1H, CH), 2.30–2.40 (m, 2H, CH_2), 2.43–2.50 (m, 1H, CH), 4.44 (q, 2H, CH_2-ethoxy), 5.55–5.62 (m, 1H, CH, C_6), 7.18–7.33 (m, 4H, Ar-H); MS *m*/*z* (%): 501 (M^+, 19), corresponding to the molecular formula $C_{31}H_{33}ClN_2O_2$, 503 (M^++2, 6) and at 229 (100, base peak).

3.1.20. Synthesis of 2-ethoxy-3-cyano-6-(4-chlorophenyl)androst-4-ene[17,16-*d*]pyridine-3-one (22). Compounds **22** was prepared by the method given for compound **11** using compounds **20** as starting material in 54% yield; mp 167 °C (MeOH); IR (KBr, cm^{-1}): 2254 (CN), 1778–1765 (C=O, enone); ^1H NMR (DMSO-d_6): δ 0.77 (s, 3H, CH_3, C_{19}), 0.87 (s, 3H, CH_3, C_{18}), 1.00–1.10 (m, 1H, CH), 1.21–1.32 (m, 4H, $2CH_2$), 1.44–1.55 (m, 4H, $2CH_2$), 1.59 (t, 3H, CH_3-ethoxy), 1.62–1.75 (m, 4H, $2CH_2$), 1.79–1.85 (m, 1H, CH), 1.97–2.00 (m, 2H, CH_2), 2.33–2.43 (m, 1H, CH), 4.46 (q, 2H, CH_2-ethoxy), 5.68–5.74 (s, 1H, CH, C_4), 7.23–7.38 (m, 4H, Ar-H); MS *m*/*z* (%): 501 (M^+, 100, base peak), corresponding to the molecular formula $C_{31}H_{33}ClN_2O_2$ and at 503 (M^++2, 35).

3.1.21. Synthesis of 2-ethoxy-3-cyano-6-(4-chlorophenyl)androst-4,6-diene[17,16-*d*]pyridine-3-one (23). The compound was prepared according to modified Oppenauer (Wettstein method)[24] using compound **20** as starting material in 49% yield; mp 174 °C (EtOH); IR (KBr, cm^{-1}): 2238 (CN), 1775–1780 (C=O, enone); ^1H NMR (DMSO-d_6): δ 0.74 (s, 3H, CH_3, C_{19}), 0.81 (s, 3H, CH_3, C_{18}), 0.99–1.10 (m, 1H, CH), 1.22–1.32 (m, 2H, CH_2), 1.46–1.56 (m, 2H, CH_2), 1.59 (t, 3H, CH_3-ethoxy), 1.66–1.78 (m, 4H, $2CH_2$), 1.82–1.87 (m, 1H, CH), 1.94–2.00 (m, 2H, CH_2), 2.28–2.44 (m, 1H, CH), 4.47 (q, 2H, CH_2-ethoxy), 5.76 (s, 1H, CH, C_4), 6.18–6.21 (m, 1H, CH, C_7), 6.75 (d, 1H, CH, C_6), 7.21–7.34 (m, 4H, Ar-H); MS *m*/*z* (%): 499 (M^+, 16), corresponding to the molecular formula $C_{31}H_{31}ClN_2O_2$, 501 (M^++2, 6) and at 292 (100, base peak).

3.2. Pharmacological screening

3.2.1. Determination of acute toxicity (LD_{50}). The LD_{50} for compounds were determined by injected different gradual increased doses of the tested compounds to adult male albino rats, then calculating the dose corresponding to 50% animal death, according to Austen and Brocklehurst.[27]

3.2.2. Anti-inflammatory activity. Carrageenan®-induced rat's paw.

3.2.2.1. Procedure. Groups of adult male albino rats (150–180 g), each of 8 animals were orally dosed with tested compounds at a dose level of 25–50 mg/kg 1 h before Carrageenan® challenge. Foot paw edema was induced by subplentar 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 hind 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 calculated.

Prednisolone® (5 mg/kg) was employed as standard reference against which the tested compounds were compared.

3.2.2.2. Calculation and evaluation. Thirty minutes after the rats are challenged by subcutaneous injection of 0.05 mL of 1% solution of carrageenan into the planter side of the left hind paw. The paw is marked with ink at the level of the lateral malleolus. The paw volume was measured by a sensitive method developed by Webb and Griswold[28] that calculated by interfacing a mettler DeltaRange top-loading balance with a microcomputer.

$$\% \text{ protection} = (A - B) \times 100/A$$

$A =$ the paw volume of the non $-$ treated group
$B =$ the paw volume of the treated group

3.2.3. Estimation of plasma prostaglandin E2 (PGE2).

3.2.3.1. Procedure. Heparinized blood samples were collected from rats obtained from the previous anti-inflammatory examined groups ($n = 8$), plasma was separated by centrifugation at 12000 g for 2 min at 40 °C and immediately stored frozen at −2 °C until use.

The design correlate EIA prostaglandin E2 (PGE2) kit (Merck, Darmstadt, 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 (DYNATCh, MR 5000) at 405 nm. The intensity of the bound yellow color is inversely proportional to the concentration of PGE2 in either the standard or samples.

3.2.3.2. Calculation and evaluation. The PGE2 was calculated for the treated and control groups and then the PGE2 percentage inhibition was determined by the following equation:

$$\% \text{ inhibition} = (A - B) \times 100/A$$

$A =$ PGE2 in the control group
$B =$ PGE2 in the treated group

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