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Synthesis and anti-inflammatory activity of 2'-phenyl steroidal[17, 16-*c*]pyrazoles

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Vilsmeier-Haack reaction / steroidal[17,16-*c*]pyrazoles / anti-inflammatory activity / dexamethasone

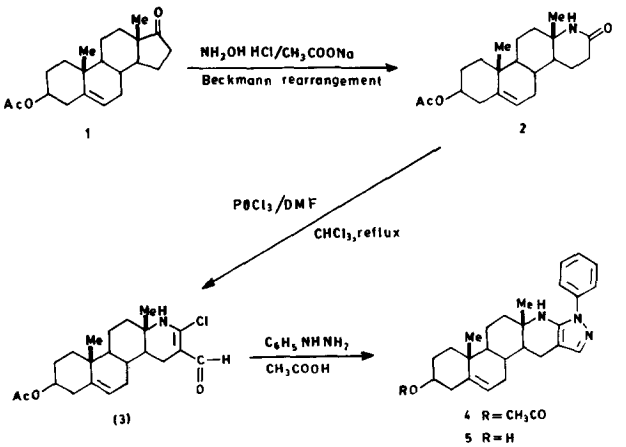
Steroidal[3,2-*c*]pyrazoles, such as 2'-phenyl-11β,17α, 21-trihydroxy-16α-methyl-4-pregneno[3,2-*c*]pyrazol-20-one 21-acetate, p-fluorophenyl analogue, cortivazol, and nivazol are potent anti-inflammatory agents [1–5]. A comprehensive review on heterosteroids and drug research has been published [6]. Heterosteroids of medicinal interest have been reported from our research laboratory [7–12]. The observation that pyrazole ring system fused to A ring of the pregnane series are potent anti-inflammatory agents, suggested us to study the effect of the pyrazole ring fused to positions 16 and 17 of androstane series. Here in this paper we report the synthesis and biological activity of steroidal pyrazoles.

Chemistry

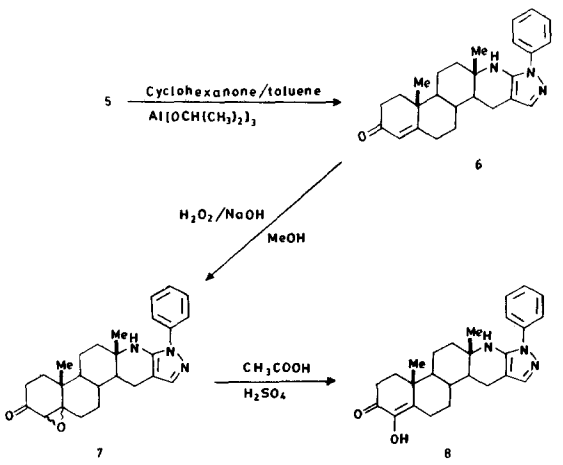
The synthesis of compounds is shown in schemes 1 and 2. The lactam 2 [13] was prepared from oxime of dehydroepiandrosterone acetate 1 by Beckmann rearrangement using thionyl chloride as the catalyst [14]. The lactam was treated with Vilsmeier-Haack reagent to obtain the 17-chloro-16-formyl-17a-aza-D-homo-5,16-androstadien-3β-yl acetate 3 [15]. The synthesis of 4 was carried out by the reaction of α,β-unsaturated β-chloroaldehyde with phenylhydrazine in catalytic amount of glacial acetic acid. The acetoxy function was hydrolysed in 4 by potassium hydroxide to obtain 5, which was subjected to Oppenauer oxidation using cyclohexanone/toluene system [16]. α,β-Unsaturated ketone 6 showed ultraviolet maximum at 243 nm and vinylic proton appeared at δ 5.73. The compound **6** on treatment with alkaline hydrogen peroxide gave the isomeric mixture of oxiranes (**7a**, **7b**) which was apparent from singlets at δ 2.96 (4α-*H*) and 3.00 (4β-*H*) in 1:1 area ratio and both together integrated for one proton, and 7.30–7.80 (m, 5 aromatic proton and 1 methine proton). The compound **8** was obtained by treatment of mixture of oxiranes with sulphuric acid in glacial acetic acid. The ultraviolet maxima appeared at 243 nm and 277 nm, which shifted to 315 nm in alkaline medium. The structure of these compounds was confirmed by elemental analysis, IR, UV, ^{1}H NMR and mass spectra.

Results and discussion

All the compounds showed an interesting profile of anti-inflammatory activity. Of the compounds tested 7 showed maximum potency with ED_{50} value of 0.35 mg/kg. All the compounds inhibited œdema in a



Scheme 1.



Scheme 2.

dose related manner. As can be seen from the data presented in table I, compound **4** with 3β-acetoxy group (ED_{50} 0.59 mg/kg) and **5** with 3β-hydroxy group (ED_{50} 0.65 mg/kg) were found to be almost equipotent. The steroidal pyrazole **6** (ED_{50} 0.37 mg/kg) bearing 4-en-3-one system was more active than the related compounds **4** and **5**. The oxirane **7** (ED_{50} 0.35 mg/kg) obtained from **6** retained the anti-inflammatory activity slightly more than 4-en-3-one derivative.

Experimental protocols

Chemistry

Melting points reported are uncorrected. NMR spectra were recorded on a EM-390, 90 MHz model NMR instrument using tetramethylsilane (TMS) as the internal standard. IR spectra were obtained with a Perkin-Elmer 137 spectrophotometer in a potassium bromide pellet. Mass spectra were recorded on a Vg Micro Mass 7070F mass spectrometer. The purity of the compounds was examined by thin-layer chromatography and by elemental analysis (C, H, N).

Preparation of 2'-phenyl-17a-aza-D-homo-5-androsteno[17,16-c]pyrazol-3β-yl acetate **4**

Glacial acetic acid (2.0 ml) was added to refluxing solution of 17-chloro-16-formyl-17a-aza-D-homo-5,16-androstadien-3β-yl acetate (0.5 g) in aldehyde-free ethanol (100 ml). Phenyl hydrazine (0.2 ml) was added to the refluxing solution and further refluxed for 6 h. The reaction mixture was concentrated to 10 ml and poured into ice-cold water (100 ml). The precipitate obtained was filtered, washed, dried and crystallised from acetone. Yield: 0.4 g (70.2%); mp = 242–245°C. $[\alpha]_D^{20}$ −166.7° (c = 0.75 in chloroform). λ_{max}^{MeOH} 245 nm (log ε 4.07). IR (KBr): 3260 (N-H stretch), 2860 (C-H stretch), 1740 (ester), 1605 (C=N ring stretch), 1520 cm^{-1} (C=C ring stretch). NMR ($CDCl_3$): δ 1.00 (3H, s); 1.03 (3H, s); 2.00 (3H, s); 3.40 (H, NH, exchangeable); 4.60 (H, m); 5.40 (H, m); 7.30–7.70 (6H, m). MS: m/z 445. Anal for $C_{28}H_{35}N_3O_2$ (C, H, N).

Table I. Anti-inflammatory activity of various compounds on carrageenin-induced paw œdema in rats (observed at 3 h).

Compd	*Dose mg/kg ip*	*Inhibition œdema (%) ± SEM*	*ED_{50} (mg/kg)*
4	0.50	45.36 ± 1.28*	
	1.00	70.64 ± 1.32*	
	2.00	73.28 ± 1.76*	0.59
5	0.50	44.00 ± 1.76*	
	1.00	62.64 ± 1.20*	
	2.00	66.26 ± 1.32*	
	4.00	68.00 ± 1.28*	0.65
6	0.25	44.24 ± 2.70*	
	0.50	53.68 ± 5.97*	
	1.00	68.08 ± 0.88*	
	2.00	76.00 ± 1.28*	0.37
7	0.25	46.69 ± 1.57*	
	0.50	53.36 ± 1.12	
	1.00	62.72 ± 2.40*	
	2.00	69.36 ± 1.68*	0.35
8	1.00	21.28 ± 1.28	
	2.00	50.65 ± 1.04*	
	4.00	58.64 ± 1.60*	
	8.00	67.26 ± 2.02*	1.95
Indomethacin	1.00	28.36 ± 0.96	
	2.00	45.34 ± 1.28*	
	4.00	58.64 ± 1.52*	
	8.00	73.36 ± 1.28*	2.61
Dexamethasone	0.10	29.28 ± 0.64	
	0.20	42.72 ± 1.92*	
	0.40	66.64 ± 1.28*	0.23
Hydrocortisone	2.50	26.72 ± 1.83	
	5.00	50.64 ± 1.68*	
	10.00	64.00 ± 1.20*	5.55

*$P < 0.001$; Student's t test *versus* control

Preparation of 2'-phenyl-17a-aza-D-homo-5-androsteno[17,16-c]pyrazol-3β-ol ***5***

A solution of 2'-phenyl-17a-aza-D-homo-5-androsteno[17,16-*c*]pyrazol-3β-yl acetate (0.5 g) in methanol (50 ml) containing potassium hydroxide (0.1 g) was refluxed for 45 min. The reaction mixture was acidified with glacial acetic acid (0.5 ml) and concentrated to induce crystallisation. The separated crystals were washed with 40% aqueous methanol and recrystallised from methanol. Yield: 0.3 g (66.2%); mp = 243–245°C. $[\alpha]_D^{20}$ – 141.3° (*c* = 0.75 in pyridine). λ_{max}^{MeOH} 246 nm (log ε 4.14). IR (KBr): 3400 (O-H stretch), 2850 (C-H stretch), 1600 (C=N ring stretch), 1515 cm^{-1} (C=C ring stretch). NMR ($CDCl_3$-DMSO-d_6): δ 1.04 (3H, s); 1.10 (3H, s); 3.40–3.60 (2H, m, 1H exchangeable); 5.43 (H, m); 7.30–7.80 (6H, m). MS: m/z 403. Anal for $C_{26}H_{33}N_3O$ (C, H, N).

Preparation of 2'-phenyl-17a-aza-D-homo-4-androsteno[17,16-c]pyrazol-3-one ***6***

A solution of 2'-phenyl-17a-aza-D-homo-5-androsteno[17,16-*c*]pyrazol-3β-ol (1.0 g) in cyclohexanone (10 ml) and toluene (100 ml) was slowly distilled as aluminium isopropoxide (1.0 g) in toluene (10 ml) was added. Distillation was continued for 30 min as 30 ml of the distillate was collected. The mixture was refluxed for 4 h and then allowed to stand overnight. It was filtered, the filtrate was steam-distilled and the residue obtained was collected, dried and crystallised from methanol. Yield: 0.56 g (56.3%); mp = 223–224°C. $[\alpha]_D^{20}$ + 100° (*c* = 0.60 in chloroform). λ_{max}^{MeOH} 243 nm (log ε 4.50). IR (KBr): 3300 (N-H stretch), 3000 (C-H stretch), 1680 (conjugated C=O), 1620 (C = N ring stretch), 1515 cm^{-1} (C=C ring stretch). NMR ($CDCl_3$): δ 1.10 (3H, s); 1.20 (3H, s); 3.66 (H, s, exchangeable); 5.73 (H, s); 7.30–7.80 (6H, m). MS: m/z 401. Anal for $C_{26}H_{31}N_3O$ (C, H, N).

Preparation of 4ξ,5-oxido-2'-phenyl-17a-aza-D-homo-5ξ-androstano[17, 16-c]pyrazol-3-one ***7***

To a stirred solution of 2'-phenyl-17a-aza-D-homo-4-androsteno[17,16-*c*]pyrazol-3-one (0.2 g) in methanol (100 ml), hydrogen peroxide (30% v/v, 1.2 ml) and aqueous sodium hydroxide (4 N, 0.4 ml) were added simultaneously maintaining the temperature below 15°C. The reaction mixture was kept for 24 h at 2–5°C, diluted with water (500 ml) and extracted with dichloromethane (3 x 25 ml). The organic layer was washed with water, dried and solvent removed. The white residue was then crystallised from methanol. Yield: 0.1 g (48.1%); mp = 248–250°C. $[\alpha]_D^{20}$ – 44.0° (*c* = 0.50 in chloroform). λ_{max}^{MeOH} 246 nm (log ε 4.11). IR (KBr): 3280 (N-H stretch), 2885 (C-H stretch), 1715 (C=O stretch), 1615 (C=N ring stretch), 1510 cm^{-1} (C=C ring stretch). NMR ($CDCl_3$): δ 1.10 (3H, s); 1.16 (3H, s); 3.00 (s) and 2.96 (s) (1:1 ratio, 1H); 3.63 (H, s, exchangeable); 7.30–7.80 (6H, m). MS: m/z 417. Anal for $C_{26}H_{31}N_3O_2$ (C, H, N).

Preparation of 4-hydroxy-2'-phenyl-17a-aza-D-homo-4-androstano[17,16-c]pyrazol-3-one ***8***

4ξ,5-Oxido-2'-phenyl-17a-aza-D-homo-5ξ-androstano[17,16-*c*]pyrazol-3-one (0.25 g) was dissolved in glacial acetic acid (5.0 ml) by warming and then cooled at 10°C. The reaction mixture was treated with a cold mixture of concentrated sulphuric acid (0.2 ml) and glacial acetic acid (2.0 ml), which turned yellow to orange and allowed to stand for 17 h at 10°C. The reaction mixture was poured into ice-cold water and the resulting precipitate was filtered, washed and dried. The residue was chromatographed over a column of silica gel (100–200 mesh). Elution with benzene-ethyl acetate (3%) gave a white residue which was crystallised from benzene. Yield: 0.06 g (24.0%); mp = 240–242°C. $[\alpha]_D^{20}$ + 193.7° (*c* = 0.80 in chloroform). λ_{max}^{MeOH} 245 nm (log ε 4.21), 277 nm (log ε 4.10); in 0.01 N KOH 245 nm (log ε 4.21) and 315 nm (log ε 4.05). IR (KBr): 3475 (O-H stretch), 3240 (N-H stretch), 1680 (conjugated C=O), 1620 (C=N ring stretch), 1510 cm^{-1} (C=C ring stretch). NMR ($CDCl_3$): δ 1.06 (3H, s); 1.23 (3H, s); 3.63 (H, s, exchangeable); 5.98 (H, s, exchangeable); 7.30–7.80 (6H, m). MS: m/z 417. Anal for $C_{27}H_{47}NO$ (C, H, N).

Pharmacology

The anti-inflammatory studies were conducted on albino rats (Wistar strain) of either sex weighing 100–200 g. The rats were randomly divided into various groups, each group consisting of a minimum of 6 animals. The suspension of the respective compounds, uniformly dispersed in distilled water by adding 0.1 ml of Tween 80, was administered to test animals intraperitoneally. The control group received the same experimental handling as test group except that equivalent doses of vehicle alone were administered by the corresponding route in place of the test compounds. Food and water were withdrawn during the test.

Carrageenin-induced œdema

Freshly prepared suspension of carrageenin, 0.1 ml (1% w/v carrageenin suspension in distilled water) was injected under the planter aponeurosis of the paw of the rats by the method of Winter *et al* [17]. Oedema was determined immediately and 30, 60, 120 and 180 min after the injection, using a mercury plethysmograph. Different doses of test compounds and the standard drug were administered 30 min before the carrageenin injection.

The per cent inhibition of inflammation after 30, 60, 120 and 180 min were calculated after the method of Newbould [18] using the following formula:

$$\% \text{ inhibition} = 100\left(1 - \frac{a-x}{b-y}\right)$$

where *x* and *a* are the mean foot volume of the rats before and after the administration of carrageenin injection respectively in the test of standard group; *y* and *b* are the mean foot volumes of the rats before and after the administration of carrageenin respectively in the control group. The results are given in table I.

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