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Synthesis of thiazolyl and thieno cholestane derivatives: a novel class of potent antiinflammatory steroids

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The reactivity of 5α-cholestan-3-one (**1**) towards the formation of thiazolyl derivatives of potential antiinflammatory activity was studied. Also starting with the aminothieno[2′,3′ : 2,3]cholestane derivative **8** several thiazolylthieno[2′,3′ : 2,3]cholestane derivatives were synthesized. The structures of the new compounds were established based on the analytical and spectral data and the *in vivo* antiinflammatory activities of some of these compounds were investigated.

1. Introduction

Since the discovery of cortisone and hydrocortisone as antiinflammatory agents (Hench et al. 1949), a considerable amount of research has been devoted to the development of new antiinflammatory agents of superior activity and less side effects (Avery and Woolfrey 1997; You et al. 2002, Lee et al. 1998). Steroidal heterocyclic derivatives posses several pharmaceutical activities (Penov-Gasi et al. 1998; Jindal et al. 2001; Guarna et al. 1999); several derivatives are well authenticated to have antiinflammatory activities (Hoyte et al. 2002; Kwon et al. 1995; Pathak and Jindal 1988; Hannah et al. 1995). In continuation of studies involving the synthesis of novel modified steroids of pharmacological interest (Doss et al. 1999; Doss et al. 2001; Mohamed et al. 2003; Elmegeed et al. 2004) we report herein on the synthesis of new steroidal heterocyclic derivatives with structures justifying antiinflammatory activities using 5α-cholestan-3-one **(1)** as starting material and on the *in vivo* antiinflammatory activity of some derivatives.

2. Investigations, results and discussion

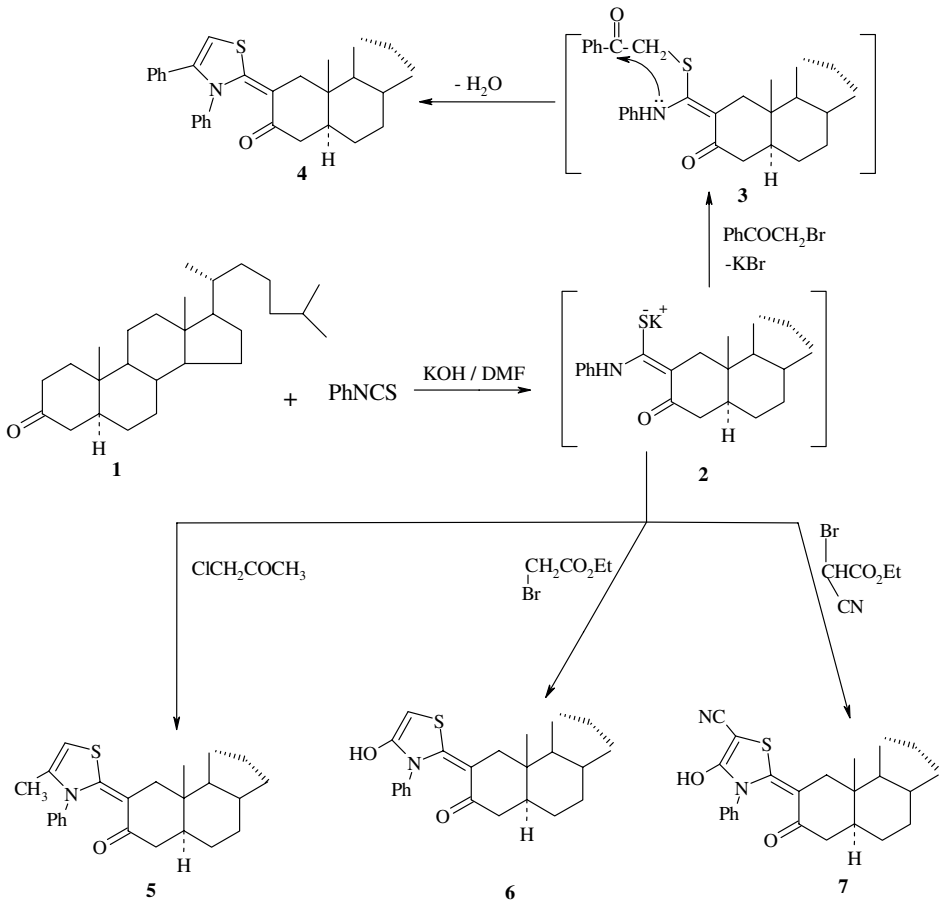
2.1. Chemistry

Several substituted thiazolyl derivatives possess antiinflammatory activities (Geronikaki et al. 2003; Farghaly et al. 2000; Lantos et al. 1984). Hence, it was of interest to synthesize thiazolyl steroids of potential antiinflammatory activity. The reaction of isothiocyanates with different compounds containing an active methylene group followed by heterocyclization of the resultant product with halogenated compounds to afford thiazole derivatives has been studied (Mohareb and Sherif 1991). In view of these observations, 5α-cholestan-3-one **(1)** reacted with phenyl isothiocyanate in dimethylformamide, containing potassium hydroxide, to afford the potassium sulfide salt **2** as a non-isolable intermediate. The latter reacted *in situ* with phenacyl bromide to give the intermediate **3** which readily loses H_2O to afford the 2-ylidenodiphenylthiazolyl-5α-cholestan-3-one derivative **4** (Scheme 1). Its isomer at C-4 of ring A was excluded (Kirk and Hartshorn 1968; Nicolaropoulos and Catsoulacos 1988). The MS of compound **4** showed $M^{+\cdot}$ at m/z 621 (32%) besides a base peak at m/z 237 for the substituted thiazolyl cation which is formed via a preceding rearrangement to liberate the 1,4-dienthiazolyl cation. The IR spectrum of compound **4** revealed a band at $\upsilon = 1695\ cm^{-1}$ corresponding to the carbonyl group. The ^1H NMR spectrum showed, beside the expected signals for the cholestane moiety, a singlet at δ 6.65 for thiazole 5-H and a multiplet (10 H) at δ 7.32-7.62 for the aromatic protons (Experimental section).

The reaction of intermediate **2** with chloroacetone under the same conditions provided 2-ylideno(4′-methyl-3′-phenylthiazolyl)-5α-cholestan-3-one derivative **5** (Scheme 1). In addition, the intermediate **2** reacted with an equimolar amount of ethyl bromoacetate to give 2-ylideno(4′-hydroxy-3′-phenylthiazolyl)-5α-cholestan-3-one derivative **6** (Scheme 1). The intermediate **2** reacted under the same conditions with ethyl cyanobromoacetate to give 2-ylideno(5′-cyano-4′-hydroxy-3′-phenylthiazolyl)-5α-cholestan-3-one **7** by KBr and EtOH elimination (Scheme 1). Structures of the products **5, 6** and **7** were confirmed by means of spectral and analytical data (Experimental section).

The reaction of 5α-cholestan-3-one **(1)** with equimolar amounts of malononitrile and sulfur in ethanolic triethylamine solution to give the aminothieno[2′,3′ : 2,3]cholestane derivative **8** has been reported (Elmegeed et al. 2004; Roy 1973) (Scheme 2). The reaction of heterocyclic amines towards carbon disulfide followed by cyclization with α-haloketones yields thiazoles with potential biological activities (Mohareb et al. 1999; Zohdi et al. 1996). Thus, compound **8** reacted with carbon disulfide in dimethylformamide, containing KOH, to afford the non-isolable intermediate, *N*-potassium thiocarbamate salt **9** (Scheme 2). The latter reacted *in situ* with phenacyl bromide to give the phenylthiazolylthieno[2′,3′ : 2,3]cholestane derivative **11** via the intermediate **10** (Scheme 2). The structure of compound **11** was based on its analytical and spectral data.

Scheme 1



The MS revealed the molecular ion at m/z 643 (48%). The IR spectrum showed absorptions at $\upsilon = 2225\ cm^{-1}$ (CN group) and $\upsilon = 1195\ cm^{-1}$ (C=S group). Moreover, the ^{1}H NMR spectrum revealed, besides the expected signals, a singlet at δ 6.62 for the thiazole 5-H and a multiplet (5H) at δ 7.42- 7.71 for the aromatic protons but no NH_2 group singlet (Experimental section).

The reaction of the intermediate **9** with chloroacetone under the same conditions provided the methylthiazolylthieno[2′,3′ : 2,3]cholestane derivative **12**. Compound **9** reacted with an equimolar amount of ethyl bromoacetate to give the hydroxythiazolylthieno[2',3':2,3]cholestane derivative **13**, and with ethyl cyanobromoacetate to give the cyanohydroxythiazolylthieno[2′,3′ : 2,3]cholestane derivative **14** (Scheme 2). Structures **12**, **13** and **14** were assigned by their elemental analysis and their spectral data (Experimental section).

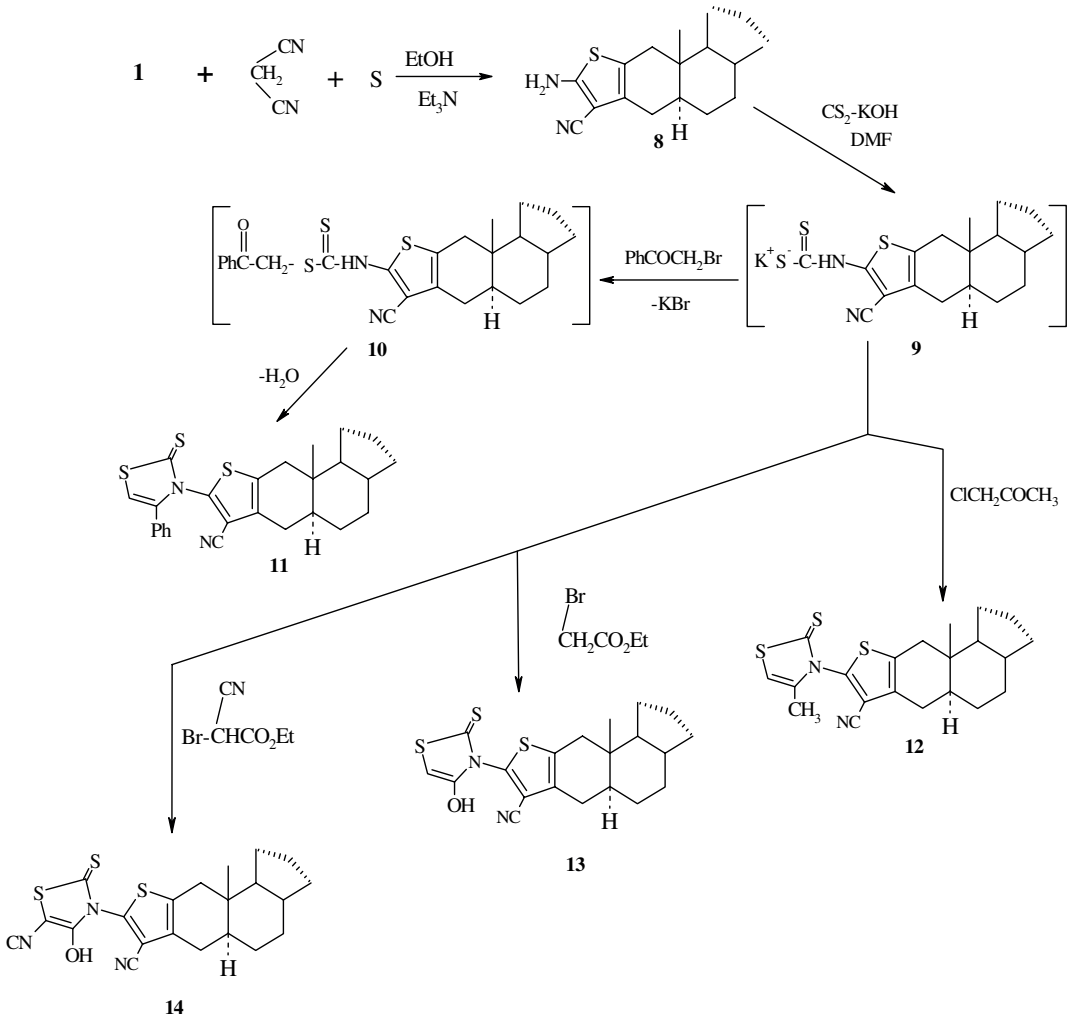
Heterocyclic steroids with a pyridine ring fused in different positions of the steroidal skeleton are endowed with useful biological activities (Guarna et al. 1999; Nicolaropoulos and Catsoulacos 1988). Therefore we synthesized these derivatives starting from compound **8**. The reactivity of the enaminonitrile moiety of compound **8** towards some active methylene reagents was studied. Thus, compound **8** reacted with an equimolar amount of ethyl acetoacetate **15** in dimethylformamide containing a catalytic amount of triethylamine to afford the corresponding aminopyrido[3′′,2′′ : 4′,5′]thieno[2′,3′ : 2,3]-5α-cholestane derivative **17** (Scheme 3). The reaction took place by elimination of ethanol to give the intermediate **16**, which readily underwent intramolecular Michael addition to give compound **17**. The MS of the latter product revealed $M^{+\cdot}$ at m/z 550 (52%). The IR spectrum of compound **17** showed, in addition to the absence of the cyano group stretching, absorptions at $\upsilon = 3425-3350\ cm^{-1}$ corresponding to the NH and NH_2 functions and two absorptions for the two C=O groups at $\upsilon = 1730$ and $1690\ cm^{-1}$. In addition to the expected signals its ^{1}H NMR spectrum revealed two types of D_2O-exchangeable singlets at δ 6.87 (2H) and at δ 9.15 (1H) corresponding to the NH_2 and NH groups (Experimental section).

The reaction of compound **8** with acetyl acetone **18** under the same conditions yielded the corresponding aminopyrido[3′′,2′′ : 4′,5′]thieno[2′,3′ : 2,3]-5α-cholestane derivative **19** (Scheme 3), its elemental and spectral data were consistent with the assigned structure (Experimental section).

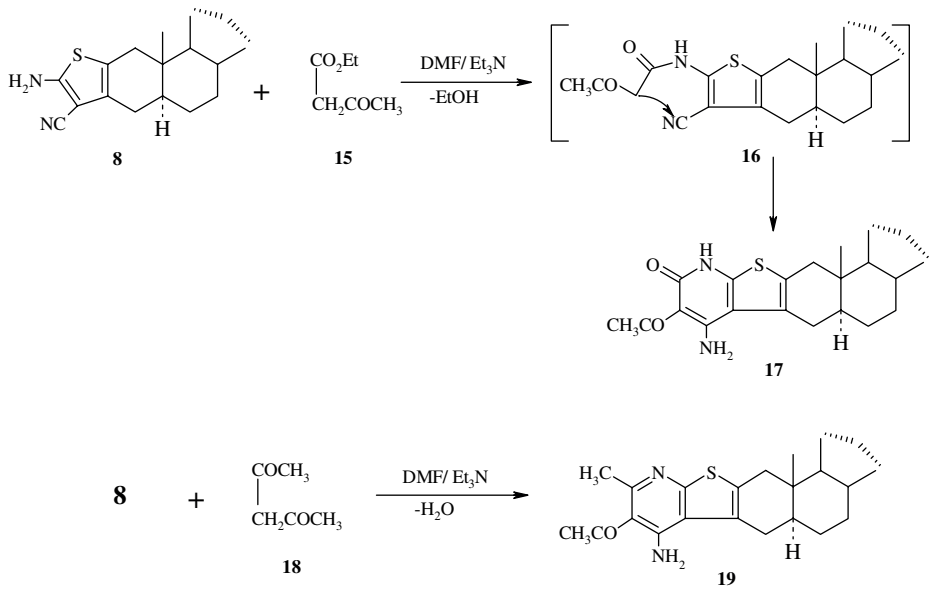
2.1. Pharmacology

The effect of systemic injections of the newly steroidal heterocyclic derivatives **4, 8, 11, 13** and **17** on oedema formation were studied using a carrageenan induced paw inflammation. Each compound was injected in three doses (15, 22.5 and 33.75 mg kg^{-1}) i.p. 30 min before sub-plantar carrageenan and decreased paw oedema in dose dependent manner compared to control group (Experimental section). Dexamethazone was given at 0.2 mg kg^{-1} i.p. 30 min before carrageenan as a control.

Scheme 2



Scheme 3



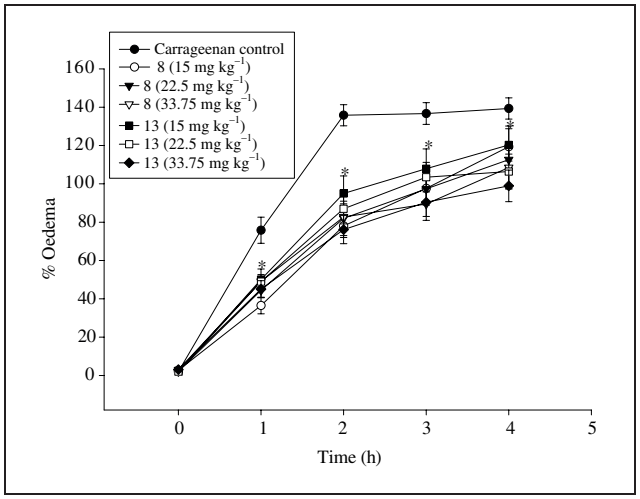


Fig. 1: Effect of systemic compounds **8** or **13** (15, 22, 5 and 33.75 mg kg^{-1}, i.p.) on the carrageenan-induced paw oedema formation. Compound **8** or **13** or vehicle was given 30 min prior to carrageenan (1%) injection and rats were evaluated for paw oedema at 1, 2, 3 and 4 h post carrabeenan. The results are expressed as a percentage change from control (pre-drug) values, each point represents the mean ± S.E. of six rats per group. (*) $P < 0.05$ vs. control value at respective time points (Duncan's multiple comparison test)

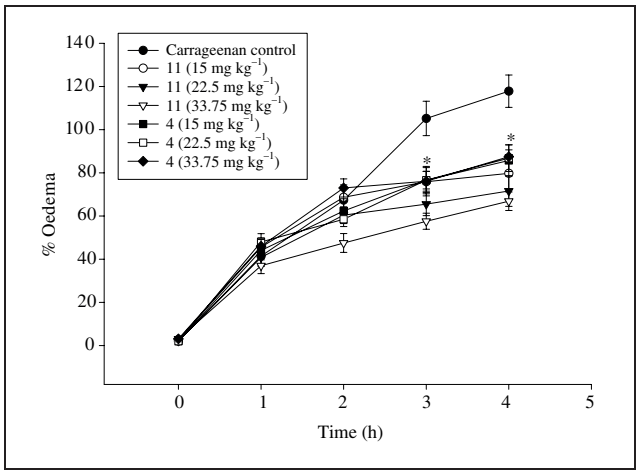


Fig. 2: Effect of systemic compounds **11** or **4** (15, 22.5 and 33.75 mg kg^{-1}, i.p.) on the carrageenan-induced paw oedema formation. Compound **11** or **4** or vehicle was given 30 min prior to carrageenan (1%) injection and rats were evaluated for paw oedema at 1, 2, 3 and 4 h post carrageenan. The results are expressed as a percentage change from control (pre-drug) values, each point represents the mean ± S.E. of six rats per group. (*) $P < 0.05$ vs. control value at respective time points (Duncan's multiple comparison test)

*2.2.1. Effect of compounds **8** and **13***

Administration of compounds **8** and **13** decreased paw oedema, and the maximal decrease in oedema was noted at 1–2 h time points for the doses 15 and 22.5 and 33.75 mg kg^{-1} of each one. Compound **8** reduced the oedema by 51.8%–42.5%, 41.4%–39.7% and 35.2%–39.2% while compound **13** reduced the oedema by 33.9%–30.1%, 35.4%–35.9% and 40.6%–44.0% for the three doses respectively, compared to the control group. In the control group, the paw thickness increased by 139.3% 4 h after injection of carrageenan (Fig. 1).

The inhibitions of oedema formation by all doses of the two tested compounds were statistically significant at 1, 2, 3 and 4 h post-carrageenan.

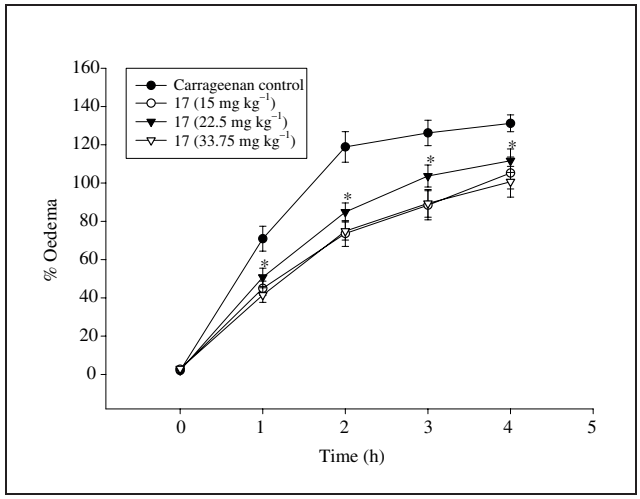


Fig. 3: Effect of systemic compounds **17** (15, 22.5 and 33.75 mg kg^{-1}, i.p.) on the carrageenan-induced paw oedema formation. Compound **17** or vehicle was given 30 min prior to carrageenan (1%) injection and rats were evaluated for paw oedema at 1, 2, 3 and 4 h post carrageenan. The results are expressed as a percentage change from control (pre-drug) values, each point represents the mean ± S.E. of six rats per group. (*) $P < 0.05$ vs. control value at respective time points (Duncan's multiple comparison test)

*2.2.2. Effect of compounds **11** and **4***

Administration of compounds **11** and **4** decreased paw oedema and the maximal decrease in oedema was noted at 3–4 h time points for the doses 15 and 22.5 and 33.75 mg kg^{-1} of each one. The maximal decrease in oedema of compound **11** was 27.9%–32.3%, 44.9%–39.2% and 45.2%–43.3% while compound **4** reduced the oedema by 27.0%–27.2%, 27.2%–26.3% and 27.7%–25.7% for the three doses respectively, compared to the control group. In the control group, the paw thickness increased by 117.8% 4 h after injection of carrageenan (Fig. 2).

The inhibitions of oedema formation by all doses of the two tested compounds were statistically significant at 3 and 4 h time points post-carrageenan.

*2.2.3. Effect of compound **17***

The maximal decrease in oedema after administration of compound **17** was 28.4%–28.6% for the dose 15 mg kg^{-1}, 36.5%–38.1% for the dose 22.5 mg kg^{-1} and 41.5%–37.0% for the dose 33.75 mg kg^{-1} at 1–2 h time points post-carrageenan vs. control. In the control group, the paw thickness increased by 131.2% 4 h after injection of carrageenan (Fig. 3).

The inhibition of oedema formation by all doses of the tested compound were statistically significant at 1, 2 and 3 h time points but at 4 h was significant only for the doses 22.5 and 33.75 mg kg^{-1} post-carrageenan (Fig. 3).

The positive control used in this study (dexamethazone) inhibited the paw oedema by 25.4%, 42.5%, 42.3% and 41.0% at 1, 2, 3 and 4 h post carrageenan.

Finally, the present study provided the first evidence for the systemic antiinflammatory effect of the newly synthesized steroidal heterocyclic derivatives.

3. Experimental

Starting steroids were purchased from Sigma. The appropriate precautions in handling moisture sensitive compounds were undertaken. All melting points were uncorrected, the IR spectra expressed in cm^{-1} and recorded in KBr pellets on a Pa-9721 IR spectrometer. ^{1}H-NMR spectra were obtained

on a Varian EM-390 90 MHz spectrometer in DMSO-d_6 as solvent and TMS as internal reference. Chemical shifts (δ) are expressed in ppm. MS were recorded on Kratos (75 eV) MS equipment. Elemental analyses were carried out by Microanalytical Data Unit at The National Research Centre, Giza, Egypt. All the elemental analyses results were in a range of +/− 0.35%. All described compounds showed the characteristic spectral data of a cyclopentanoperhydrophenanthrene moiety of the cholestane series similar to those reported in the literature (Smith 1999; Bhacca and William 1964). For the nomenclature of steroid derivatives, we used the definitive rules for the nomenclature of steroids published by the Joint Commission on the Biochemical Nomenclature (JCBN) of IUPAC 1960; 1989a; 1989b and 1993. The antiinflammatory assay of the new compounds was carried out at the laboratory of Pharmacology Department, National Research Centre, Dokki, Giza, Egypt.

3.1. Synthesis

*3.1.1. Preparation of compounds **4**, **5**, **6** and **7** (general procedure)*

To a cooled suspension of pulverized KOH (0.11 g, 0.002 mol) in DMF (30 ml), 5α-cholestan-3-one (**1** 0.77 g, 0.002 mol) was added followed by phenyl isothiocyanate (0.27 g, 0.002 mol). The mixture was stirred at room temp. overnight, then treated with the appropriate α-halogenated compound (0.002 mol) and left at room temp. for 24 h. The mixture was triturated with cold water containing hydrochloric acid (0.1 N, 2 ml). The resultant solid product so obtained in each case was collected and crystallized from the proper solvent.

3.1.1.1. 2-Ylideno(3′,4′-diphenylthiazol-2′-yl)-5α-cholestan-3-one (4)

Yellow crystals from ethanol, yield 75% (0.93 g), m.p. 165–166 °C; IR (υ/cm^{-1}): 3050 (CH-aromatic), 2965, 2850 (CH_3, CH_2), 1695 (C=O), 1615 (C=C); ^{1}H NMR (δ ppm): 3.45-3.65 (m, 1 H, 5-αH), 6.65 (s, 1 H, thiazole 5-H), 7.32-7.62 (m, 10 H, $2C_6H_5$); MS (m/z, %): 622 (M^+, 32%), 508 ($M^+-C_8H_{17}$, 20%), 384 ($M^+-C_{15}H_{11}NS$, 42%), 237 ($C_{15}H_{11}NS$, 100%).
$C_{42}H_{55}NOS$ (622.0)

3.1.1.2. 2-Ylideno(4′-methyl-3′-phenylthiazol-2′-yl)-5α-cholestan-3-one (**5**)

Orange crystals from ethanol, yield 67% (0.74 g), m.p. 187–189 °C; IR (υ/cm^{-1}): 3055 (CH-aromatic), 2983, 2870 (CH_3, CH_2), 1710 (C=O), 1625 (C=C); ^{1}H NMR (δ ppm): 1.23 (s, 3H, 4′-CH_3), 3.40-3.62 (m, 1 H, 5-αH), 6.52 (s, 1 H, thiazole 5-H), 7.42-7.60 (m, 5 H, C_6H_5); MS (m/z, %): 558 (M^+-1, 52%), 446 ($M^+-C_8H_{17}$, 26%), 77 (C_6H_5, 100%).
$C_{37}H_{53}NOS$ (559.9)

3.1.1.3. 2-Ylideno(4′-hydroxy-3′-phenylthiazol-2′-yl)-5α-cholestan-3-one (**6**)

Brown crystals from 1,4-dioxane, yield 74% (0.83 g), m.p. 207–208 °C; IR (υ/cm^{-1}): 3495–3415 (OH), 3035 (CH-aromatic), 2985, 2875 (CH_3, CH_2), 1710 (C=O), 1635 (C=C); ^{1}H NMR (δ ppm): 3.43–3.60 (m, 1 H, 5-αH), 6.45 (s, 1 H, thiazole 5-H), 7.52-7.72 (m, 5 H, C_6H_5), 9.27 (s, 1 H, OH, D_2O-exchangeable); MS (m/z, %): 561 (M^+, 34%), 546 (M^+-CH_3, 38%), 448 ($M^+-C_8H_{17}$, 46%), 384 ($M^+-C_9H_7NOS$, 100%).
$C_{36}H_{51}NO_2S$ (561.9)

3.1.1.4. 2-Ylideno(5′-cyano-4′-hydroxy-3′-phenylthiazol-2′-yl)-5α-cholestan-3-one (**7**)

Pale brown crystals from methanol, yield 70% (0.82 g), m.p. 196–197 °C; IR (υ/cm^{-1}): 3490-3405 (OH), 3040 (CH-aromatic), 2987, 2870 (CH_3, CH_2), 2210 (CN), 1700 (C=O), 1622 (C=C); ^{1}H NMR (δ ppm): 3.36–3.50 (m, 1 H, 5-αH), 7.35–7.75 (m, 5 H, C_6H_5), 9.95 (s, H, OH, D_2O-exchangeable); MS (m/z, %): 586 (M^+, 24%), 571 (M^+-CH_3, 54%), 473 ($M^+-C_8H_{17}$, 38%), 77 (C_6H_5, 100%)
$C_{37}H_{50}N_2O_2S$ (586.9)

*3.1.2. Preparation of compounds **11**, **12**, **13** and **14** (general procedure)*

To a solution of 5′-aminothieno[2′,3′ : 2,3]-5α-cholestan-4′-carbonitrile (**8**, 0.92 g, 0.002 mol) in DMF (30 ml), a solution of KOH (0.11 g, 0.002 mol) in 5 ml H_2O was added followed by carbon disulfide (0.15 g, 0.002 mol). The reaction mixture was heated in a water bath at 80 °C for 1 h, then left to cool to 20 °C; to this cold solution the appropriate α-halogenated compound (0.002 mol) was added. The reaction mixture, in each case, was stirred at room temp. for 24 h and the solid product formed upon pouring into ice containing few drops of HCl was crystallized from the proper solvent.

3.1.2.1. 5′-(4′′-Phenyl-2′′-thioxothiazol-3′′-yl)thieno[2′,3′ : 2,3]cholestan-4′-carbonitrile (**11**)

Brown crystals from 1,4-dioxane, yield 72% (0.92 g), m.p. 215–216 °C; IR (υ/cm^{-1}): 3040 (CH-aromatic), 2985, 2830 (CH_3, CH_2), 2225 (CN), 1620 (C=C), 1195 (C=S); ^{1}H NMR (δ ppm): 3.45–3.62 (m, 1 H, 5-αH), 6.62 (s, 1 H, thiazole 5-H), 7.42-7.71 (m, 5 H, C_6H_5); MS (m/z, %): 643 (M^+, 48%), 530 ($M^+-C_8H_{17}$, 30%), 77 (C_6H_5, 100%).
$C_{39}H_{50}N_2S_3$ (643.0)

3.1.2.2. 5′-(4′′-Methyl-2′′-thioxothiazol-3′′-yl)thieno[2′,3′ : 2,3]cholestan-4′-carbonitrile (**12**)

Red crystals from ethanol, yield 75% (0.87 g), m.p. 183–184 °C; IR (υ/cm^{-1}): 2980, 2850 (CH_3, CH_2), 2215 (CN), 1625 (C=C), 1198 (C=S); ^{1}H NMR (δ ppm): 1.28 (s, 3 H, 4′′-CH_3), 3.45–3.62 (m, 1 H, 5-αH), 6.48 (s, 1 H, thiazole 5-H); MS (m/z, %): 581 (M^+, 28%), 565 (M^+-CH_3, 34%), 467 ($M^+-C_8H_{17}$, 52%), 290 ($C_{19}H_{30}S$, 100%).
$C_{34}H_{48}N_2S_3$ (581.0)

3.1.2.3. 5′-(4′′-Hydroxy-2′′-thioxothiazol-3′′-yl)thieno[2′,3′ : 2,3]cholestan-4′-carbonitrile (**13**)

Pale brown crystals from 1,4-dioxane, yield 70% (0.82 g), m.p. 225–226 °C; IR (υ/cm^{-1}): 3480–3400 (OH), 2985, 2835 (CH_3, CH_2), 2220 (CN), 1630 (C=C), 1200 (C=S); ^{1}H NMR (δ ppm): 3.49–3.65 (m, 1 H, 5-αH), 6.52 (s, 1 H, thiazole 5-H), 10.25 (s, H, OH, D_2O-exchangeable); MS (m/z, %): 582 (M^+, 58%), 556 (M^+-CN, 38%), 469 ($M^+-C_8H_{17}$, 18%), 113 (C_8H_{17}, 100%).
$C_{33}H_{46}N_2OS_3$ (582.9)

3.1.2.4. 5′-(5′′-Cyano-4′′-hydroxy-2′′-thioxothiazol-3′′-yl)thieno[2′,3′ : 2,3]cholestan-4′-carbonitrile (**14**)

Pale red crystals from methanol, yield 64% (0.77 g), m.p. 211–212 °C; IR (υ/cm^{-1}): 3475–3405 (OH), 2980, 2845 (CH_3, CH_2), 2220, 2210 (2 CN), 1635 (C=C), 1195 (C=S); ^{1}H NMR (δ ppm): 3.39–3.67 (m, 1 H, C_5-αH), 10.48 (s, 1 H, OH, D_2O-exchangeable); MS (m/z, %): 607 (M^+-1, 45%), 592 (M^+-CH_3, 34%), 581 (M^+-CN, 28%), 494 ($M^+-C_8H_{17}$, 78%), 450 ($M^+-C_4HN_2OS_2$, 100%).
$C_{34}H_{45}N_3OS_3$ (608.0)

*3.1.3. Preparation of compounds **17** and **19** (general procedure)*

To a solution of compound **8** (0.92 g, 0.002 mol) in dimethylformamide (25 ml) containing a catalytic amount of triethylamine (2 ml) either ethyl acetoacetate **15** (0.26 g, 0.002 mol) or acetyl acetone **18** (0.20 g, 0.002 mol) was added. The reaction mixture was heated under reflux for 6 h, then poured into an ice/water mixture and neutralized with dil. HCl. The solid product formed, in each case, was filtered off, dried and crystallized from the appropriate solvent.

3.1.3.1. 5′′-Acetoxy-4′′-amino-6′′-oxopyrido[3′′,2′′ : 4′,5′]thieno[2′,3′ : 2,3]-5α-cholestane (**17**)

Pale brown crystals from ethanol, yield 76% (0.93 g), m.p. 222–223 °C; IR (υ/cm^{-1}): 3425–3350 (NH, NH_2), 2973, 2861 (CH_3, CH_2), 1730, 1690 (2 C=O), 1630 (C=C); ^{1}H NMR (δ ppm): 1.22 (s, 3 H, acetyl CH_3), 3.52–3.71 (m, 1 H, 5-αH), 6.87 (s, 2 H, NH_2, D_2O-exchangeable), 9.15 (s, 1 H, NH, D_2O-exchangeable); MS (m/z, %): 550 (M^+, 52%), 437 ($M^+-C_8H_{17}$, 38%), 113 (C_8H_{17}, 100%).
$C_{34}H_{50}N_2O_2S$ (550.8)

3.1.3.2. 5′′-Acetoxy-4′′-amino-6′′-methylpyrido[3′′,2′′ : 4′,5′]thieno[2′,3′ : 2,3]-5α-cholestane (19):

Brown crystals from 1,4-dioxane, yield 74% (0.81 g), m.p. 202–203 °C; IR (υ/cm^{-1}): 3420 (NH_2), 2978, 2864 (CH_3, CH_2), 1734; (C=O), 1630 (C=C); ^{1}H NMR (δ ppm): 1.26 (s, 6 H, acetyl CH_3, 6′′-CH_3), 3.50–3.67 (m, 1 H, 5-αH), 6.95 (s, 2 H, NH_2, D_2O-exchangeable); MS (m/z, %): 548 (M^+, 52%), 435 ($M^+-C_8H_{17}$, 38%), 400 ($M^+-C_8H_8N_2O$, 100%).
$C_{35}H_{52}N_2OS$ (548.9)

3.2. Pharmacological assay

Male Sprague-Dawley strain rats weighing 130–140 g were used. Food and water were provided *ad libitum*. Experiments were performed between 9:00 and 15:00 h. Rats were used only once. Equal groups of six rats were used.
Paw oedema was induced by sub-planter injection of 100 μL of 1% sterile carrageenan Lambada in saline into the right hind paw (Winter et al. 1962). Carrageenan produced visible redness and pronounced swelling that was well developed within 4 h and persisted for more than 48 h. Hind footpad thickness was measured immediately before carrageenan injection and at selected times thereafter with a micrometer calibre (Obukowicz et al. 1998). The rats received vehicle (Tween® 80) or the new compound 30 min before carrageenan administration. The oedema component of inflammation was quantified by measuring the difference in hind footpad thickness before carrageenan injection and at 1, 2, 3, and 4 h after carrageenan unless otherwise indicated.
The systemic effect of the five tested newly synthesized steroidal heterocyclic derivatives **4, 8, 11, 13** and **17** on peripheral oedema was studied. Each of which was injected in three doses (15, 22.5 and 33.75 mg kg^{-1}, 0.5 ml/rat, n = 6 per group) i.p. 30 min before sub-plantar carrageenan. The control group received vehicle in saline (0.5ml/rat, n = 6, i.p.) instead.

For control comparison, dexamethazone was given at 0.2 mg kg^{-1} i.p. 30 min before carrageenan, while the control group received the vehicle, and the oedema was evaluated 1, 2, 3 and 4 h post-carrageenan.
Data of paw oedema was expressed as percentage of change from control (pre-drug) level. Data were analysed by One-way ANOVA and multiple group comparison was done by Duncan's multiple comparison test. A probability value $P < 0.05$ was considered statistically significant.

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