Full Paper

Evaluation of Anti-inflammatory, Anti-nociceptive, and Anti-ulcerogenic Activities of Novel Synthesized Thiazolyl and Pyrrolyl Steroids

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[6] Blessing and mercy to the soul of our dear fellow (late) Marian G. William.

Developing new therapeutic agents that can overcome gastrointestinal injury and at the same time could lead to an enhanced anti-inflammatory effect becomes an urgent need for inflammation patients. Thiazolyl and pyrrolyl steroids were synthesized via straight forward and efficient methods and their structures were established based on their correct elemental analysis and compatible IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectral data. The dihydrothiazolyl-hydrazonoprogesterone **12** and the aminopyrrolylprogesterone **16a** showed anti-inflammatory, antinociceptive, and anti-ulcerogenic activity with various intensities. Edema were significantly reduced by both doses of tested compounds (25 and 50 mg/kg) at 2, 3, and 4 h post-carrageenan. The high dose of compound **16a** was the most effective in alleviating thermal pain. Gastric mucosal lesions, caused in the rats by the administration of ethanol or indomethacin (IND), were significantly inhibited by each of the two tested compounds. These results provide a unique opportunity to develop new anti-inflammatory drugs which devoid the ulcerogenic liabilities associated with currently marketed drugs.

Keywords: Anti-inflammatory / Anti-nociceptive / Non-ulcerogenic / Pyrrole / Steroids / Thiazoles

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Introduction

Steroids have attracted much attention because of their wide spectrum of special biological activities. They can regulate a variety of biological processes and thus can be processed as drugs for treatment of many diseases. Glucocorticoids (GC) are widely used for the treatment of chronic inflammatory diseases such as asthma, rheumatoid arthritis, inflammatory bowel disease and autoimmune diseases. Although the beneficial effects of GC in the management of inflammatory and allergic conditions have been appreciated for over 50 years, complications arising from the steroid therapy have imposed limitations on the clinical use of this class of drugs [1]. A considerable research effort has been devoted to the structural modifications of glucocorticoids, with the hope of increasing their potencies while minimizing their propensity to elicit systemic adverse effects [2, 3].

Modified steroids have attracted a great deal of attention. Their preparation is a stimulating challenge to the organic chemist, often demanding development of new and generally useful reactions. Moreover, the biological properties of modified steroids have proved to be of interest [4–6]. There has been considerable interest in the synthesis and biological study of several heterocyclic steroids as high potent anti-inflammatory agents [7–9]. The incorporation of an azole ring to the 2,3-positions of various steroids was effective in the production of a variety of compounds possessing anti-inflammatory proper-

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ties [10, 11]. Developing new therapeutic agents that can overcome gastrointestinal injury and at the same time could lead to an enhanced anti-inflammatory effect becomes an urgent need for inflammation patients.

In light of the above discoveries, and in continuation of studies involving the synthesis of novel biologically active modified steroids [9, 12, 13]. In the present study, some novel steroid hybrids incorporating the pyrrole or thiazole moiety through different linkages have been developed and it is expected to exhibit anti-inflammatory, anti-nociceptive, and/or non-ulcerogenic activities.

Results and discussion

Chemistry

The present study was designed to synthesize new steroidal heterocyclic derivatives with structures justifying non-ulcerogenic, anti-inflammatory, and/or anti-nociceptive activities. Pyrroles and azoles represent molecular frameworks that serve as platform for developing pharmaceutical agents for various applications. Many derivatives of these rings proved to be anti-inflammatory and/or anti-nociceptive agents [14–16].

The reactivity of progesterone **(1)** towards hydrazines was studied in the aim to form amino steroids. Thus, progesterone reacted with hydrazine hydrate **2** (70%) in refluxing absolute ethanol/acetic acid solution to form 20-hydrazonoprogesterone **3** in 80% yield (Scheme 1). The reaction of compound **3** with equimolar amount of phenylisothiocyanate **4** under reflux in absolute ethanol/triethylamine solution gave the corresponding 20-phenylthiosemicarbazono-progesterone **5** (Scheme 1). The structure of compound **5** was established based on its elemental and spectral data. The IR spectrum revealed the presence of two NH groups stretching at $\nu = 3590$–$3350\ cm^{-1}$ and C=S stretching at $\nu = 1195\ cm^{-1}$. Moreover, the ^{1}H-NMR spectrum of compound **5** showed a multiple signal at $\delta = 7.15$–7.38 ppm (5H) for the aromatic protons and showed also two D_2O-exchangeable singlets at $\delta = 9.19$ and 9.45 ppm for the two NH protons. The ^{13}C-NMR showed, beside the expected signals due to the steroid moiety, signals at $\delta = 186.0$ (s, C=S), 137.4 (s), 126.4 (d), 129.5 (d), 123.3 (d) (C-phenyl). The mass spectrum of compound **5** showed molecular ion peak ($M^+ + 1$) at $m/z = 464$ (38%).

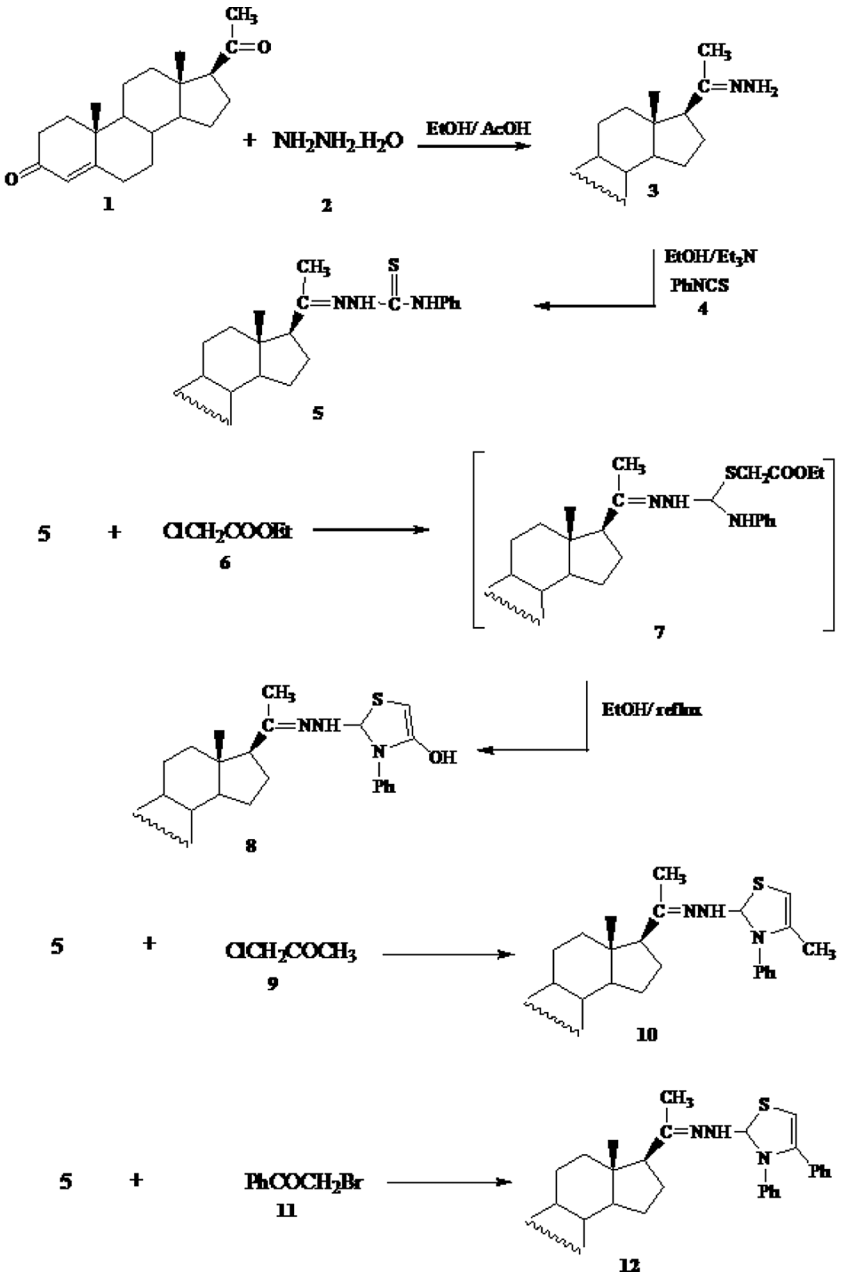
The azole moiety often shows some special biological activity when it is incorporated to some biologically active compounds [17, 18]. The basicity and hydrophilicity of an azole in theory might alter the biological function of a steroid [19]. The reaction of compound **5** with ethyl chloroacetate **6** in absolute ethanol afforded the corresponding dihydrothiazolylhydrazonoprogesterone derivative **8**. The reaction takes place via the intermediacy of compound **7** followed by intramolecular cyclization to afford compound **8** in 81% yield (Scheme 1). Under the same experimental conditions, compound **5** reacted also with either chloroacetone **9** or α-bromoacetophenone **11** to afford the corresponding dihydrothiazolylhydrazonoprogesterone derivatives **10** and **12**, respectively (Scheme 1). The proposed structures of compounds **8**, **10**, and **12** were confirmed based on their correct analytical data and compatible IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectra data (see Section Materials and Methods). For example the ^{13}C-NMR spectrum of compound **12** showed signals at $\delta = 76.4$ (d), 102.0 (d), 134.7 (s) (C-thiazole), 117.4 (d), 129.0 (d), 113.6 (d), 144.7 (s), 134.6 (s), 126.4 (d), 128.5 (d), 120.3 (d) (C-phenyl).

The reaction of 20-hydrazonoprogestrone **3** with equimolar amount of α-bromoacetophenone **11** in refluxing dioxane/piperidine solution afforded the corresponding 20-(2′-oxo-2′-phenylethylhydrazono)progesterone derivative **13** in 70% yield (Scheme 2). The behavior of compound **13** towards some active methylene reagents was investigated in the aim of forming new pyrrolyl steroids. Thus, compound **13** reacted with either malononitrile **14a** or ethyl cyanoacetate **14b** to afford the corresponding aminopyrrolylprogesterone derivatives **16a** in 82% and **16b** in 81% yield, respectively (Scheme 2). The reaction takes place via the non-isolable intermediates **15a** and **15b**, respectively, which readily undergo intramolecular cyclization to afford the isolated products **16a** and **16b**. All the elemental and spectral data of the latter products are in accordance with proposed structures (see Section Materials and Methods).

Anti-inflammatory Assay

The effect of systemic injection of the newly modified steroids **12** or **16a** on edema formation was studied using a carrageenan induced paw inflammation. Each compound was injected subcutaneously (s.c.), in either one of two doses (25 and 50 mg/kg), 30 min before sub-planter carrageenan. Each of the two tested compounds decreased paw edema in dose dependent manner compared to control group (predrug) (Table 1). Indomethacin (IND) was given at 18 mg/kg, s.c., 30 min before carrageenan as positive control. Data are expressed as mean ± S.E., $n = 6$ per group. The values in parenthesis in Table 1 indicate the percentage (%) of increase in paw volume (edema) from basal (zero time) values.

The edema response was significantly reduced by the administration of compound **12** at a low dose of 25 mg/kg compared with the control group at 2, 3 and 4 h post-carrageenan (−29, −37.8, −42.9%). The higher dose (50 mg/kg) significantly inhibited edema by −34.2, −34.6, and −42.6% at 2-h, 3-h, and 4-h time points, respectively. On the other hand, the small dose of compound **16a** inhibited the edema response by −27.4 and −38.7 at 3 and 4h post-carrageenan. Also the higher dose inhibited the



Scheme 1. Synthesis of compounds **3**, **5**, **8**, **10** and **12**.

edema response by −30.3, −39.2% at 3-h and 4-h time points (Fig. 1).

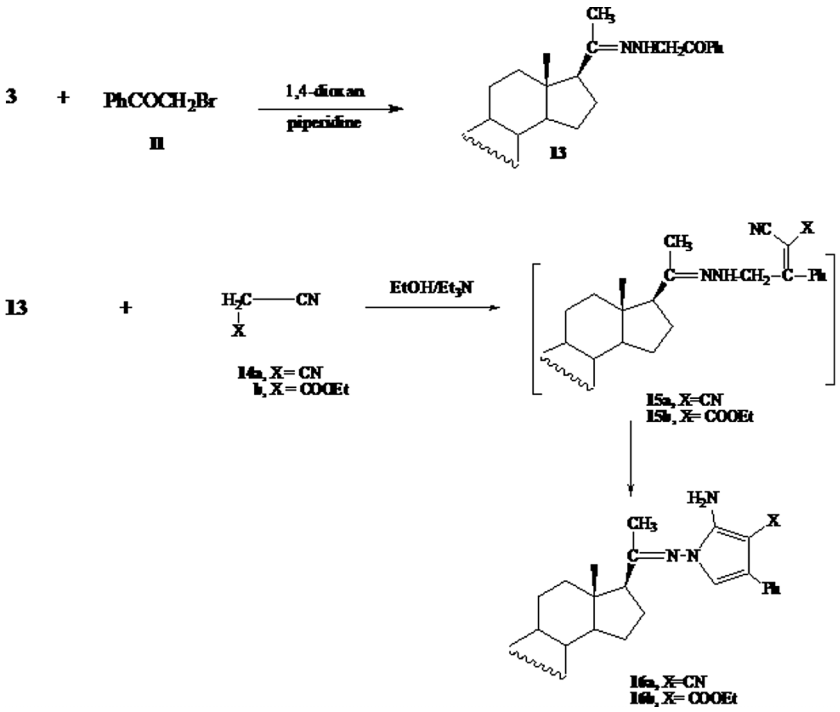
Tests of Anti-nociceptive Studies

Effect of tested compounds on thermal pain

The hot plate latency was significantly increased denoting analgesic effect after one hour of the administration of compounds **12** or **16a** at both doses administered (25 and 50 mg/kg) compared to the saline treated group. The anti-nociceptive effect was marked with compounds **12** at 25 mg/kg and compound **16a** at 50 mg/kg with increase in hot-plate latency by 36.0% and 46.0% after one hour of drug administration (Table 2, Fig. 2).

Effect of tested compounds on acetic acid-induced writhing

Each of compounds **12** and **16a** significantly reduced the number of abdominal writhes induced by i.p. administration of dilute acetic acid in mice (Table 3, Fig. 3). The degree of



Scheme 2. Synthesis of compounds **13** and **16a,b**.

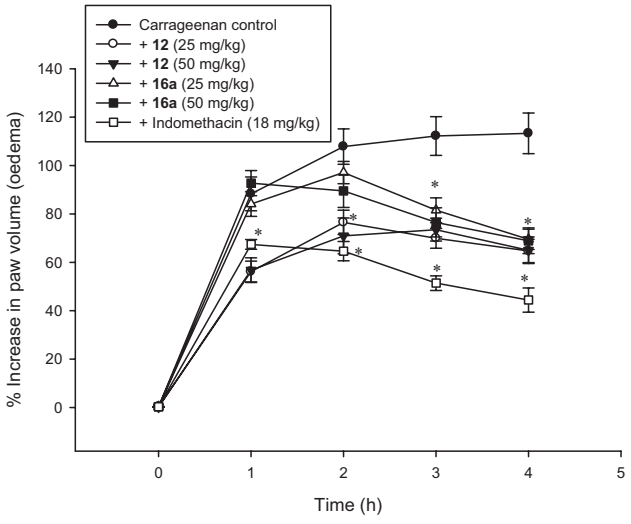


Figure 1. Effect of tested compounds on the carrageenan paw edema formation. Tested compounds were given (25 and 50 mg kg^{-1}, i.p.) 30 min prior to carrageenan injection and rats were evaluated for paw edema at 1, 2, 3, and 4 h post carrageenan. The results are expressed as a percentage change from control (pre-drug) values, each point represents the mean ± S.E. of six rats per group. Asterisks indicate significant change from the control group at the corresponding time point.

inhibition of the writhing response by these compounds ranged from −55.0% to −77.5% as compared to the saline-treated control group. The higher dose (50 mg/kg) of either of compound or and **16a** was the more effective in this respect. The degree of inhibition of the writhing response of the high dose of compound **16a** (−77.5%) was significantly higher than that of IND (−52.5%).

Testes of Gastric Ulcerogenic Studies

In order to evaluate the potential anti-ulcerogenic properties of the tested compounds **12** and **16a**, we examined the effect of the high dose (50 mg/kg) on the development of gastric mucosal lesions caused by ethanol or the non-steroidal anti-inflammatory drug IND. The number and severity of gastric mucosal lesions caused in the rats by the administration of 96% ethanol (Table 4, Fig. 4) or IND (Table 5, Fig. 5) were significantly inhibited by either one of the two tested compounds administered at a 50 mg/kg dose in the study.

Materials and methods

Synthetic methods, analytical and spectral data

Starting steroid, progesterone, was purchased from Sigma Company, USA. All solvents were dried by distillation prior to using. All melting points were measured using an Electrothermal apparatus and are uncorrected. The IR

Table 1. The anti-inflammatory effect of the tested compounds on carrageenan induced paw edema

Group	Basal	1 h	2 h	3 h	4 h
Control (saline)	0.28 ± 0.006	0.53 ± 0.02 (88.3 ± 7.0)	0.6 ± 0.018 (107.8 ± 7.6)	0.59 ± 0.013 (112.2 ± 7.3)	0.60 ± 0.013 (113.3 ± 8.4)
12 (25 mg/kg)	0.34 ± 0.014	0.527 ± 0.03 (56.2 ± 4.3)*	0.600 ± 0.016 (76.5 ± 5.0)	0.58 ± 0.024 (69.8 ± 4.1)*	0.555 ± 0.010 (64.7 ± 4.8)*
12 (50 mg/kg)	0.32 ± 0.009	0.50 ± 0.04 (56.8 ± 5.1)	0.547 ± 0.02 (70.9 ± 7.5)*	0.556 ± 0.014 (73.4 ± 4.6)*	0.528± 0.008 (65.0 ± 5.4)*
16a (25 mg/kg)	0.311 ± 0.007	0.568 ± 0.032 (84.1 ± 5.1)	0.613 ± 0.014 (97.1 ± 4.6)	0.565 ± 0.017 (81.5 ± 5.1)*	0.526 ± 0.004 (69.5 ± 4.2)*
16a (25 mg/kg)	0.318 ± 0.004	0.61 ± 0.01 (92.7 ± 5.2)	0.601 ± 0.021 (89.5 ± 6.8)	0.56 ± 0.021 (78.2 ± 6.0)*	0.54 ± 0.01 (68.9 ± 5.3)*
IND (18 mg/kg)	0.288 ± 0.004	0.48 ± 0.02 (67.4 ± 5.6)*	0.47 ± 0.01 (64.6 ± 4.10)*	0.435 ± 0.019 (51.4 ± 4.3)*	0.415 ± 0.015 (44.4 ± 2.9)*

Results are expressed as percentage change for control (pre-drug) values. Data are expressed as mean ± S.E., $n = 6$ rats/group. Asterisks indicate significant change from control value. The s.c. administration of all tested compounds inhibited the carrageenan induced paw edema (two-way ANOVA; treatment effect: $F13,280 = 30$; $P < 0.001$; time effect: $F3,280 = 23.8$; $P < 0.001$, time x drug effect: $F39,280 = 3$; $P < 0.001$). The values in parenthesis indicate the percentage (%) of increase in paw volume from basal (zero time) values.

spectra were recorded in (KBr discs) on a shimadzu FT-IR 8201 PC spectrometer and expressed in cm^{-1}. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded with Jeol instrument (Japan), at 270 and 125 MHz, respectively, in DMSO-d_6 or $CDCl_3$ as solvent and chemical shifts were recorded in ppm relative to TMS. The spin multiplicities were abbreviated by the letters: s – singlet, d – doublet, t – triplet, q – quartet, and m – multiplet (more than quartet). Mass spectra were recorded on a GCMS-QP 1000 Ex spectra mass spectrometer operating at 70 eV. Elemental analyses were carried out by the Microanalytical Data Unit at the National Research Center, Giza, Egypt and the Microanalytical Data Unit at Cairo University, Giza, Egypt. The reactions were monitored by thin layer chromatography (TLC) which was carried out using Merck 60 F254 aluminum sheets and visualized by UV light (254 nm). The mixtures were separated by preparative TLC and gravity chromatography. 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 [20, 21]. All described compounds showed the characteristic spectral data of cyclopentanoperhydrophenanthrene nuclei of pregnene series and were similar to those reported in literatures [22, 23].

Table 2. Percentage increase in hot plate latency in mice treated with tested compounds in comparison with tramadol

Drug	0 time (basal)	1 h	% Change
Saline	13.2 ± 0.6	14.65 ± 1.1	
12 (25 mg/kg)	14.25 ± 0.51	19.38 ± 1.4	36.0*
12 (50 mg/kg)	12.2 ± 0.64	16.48 ± 1.2	35.1*
16a (25 mg/kg)	13.55 ± 1.2	18.28 ± 1.1	34.9*
16a (50 mg/kg)	13.0 ± 1.1	18.98 ± 0.9	46.0**
Tramadol (20 mg/kg)	12.86 ± 1.1	20.3 ± 1.6	57.9**

Data are expressed as mean ± S.E., $n = 6$ per group. * = $P < 0.05$, ** = $P < 0.01$ vs. corresponding basal values.

*Synthesis of 20-hydrazonopregn-4-en-3-on (**3**)*

To a mixture of compound **1** (0.65 g, 2 mmol) and hydrazine hydrate (70%) **2** (0.1 g, 2 mmol) in absolute ethanol (30 mL), glacial acetic acid (3.0 mL) was added. The reaction mixture

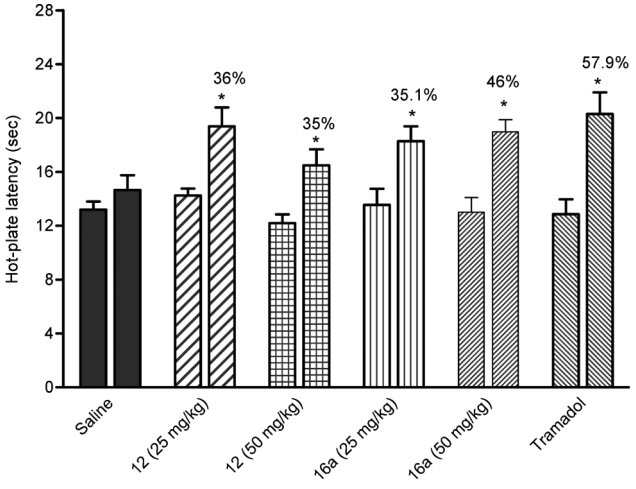


Figure 2. Reaction time on the hot- plate in seconds after the administration of tested compounds at doses of 25 and 50 mg/kg. Shown are basal (pre-drug: first column) and 60 min (post-drug: second column) values. The percentage change from basal (pre-drug) values is shown ($n = 6$/group). Asterisks indicate significant change from the saline control group at the respective time point (ANOVA and Duncan's multiple comparison tests).

Table 3. Effect of tested compounds on the number of writhes in the acetic acid test in mice

Group	Number of abdominal constrictions/30 min	% Inhibition *vs.* control
Saline	80.0 ± 5.3	
12 (25 mg/kg)	36.0 ± 3.5*	55.0%
12 (50 mg/kg)	18.0 ± 1.4*	77.5%
16a (25 mg/kg)	35.0 ± 2.7*	56.3%
16a (50 mg/kg)	28.0 ± 3*	65.0%
IND (18 mg/kg)	38 ± 2.8*	52.5%

Data are expressed as means and S.E.M. ($n = 6$/group). IND: indomethacin. * = $P < 0.05$ vs. control values.

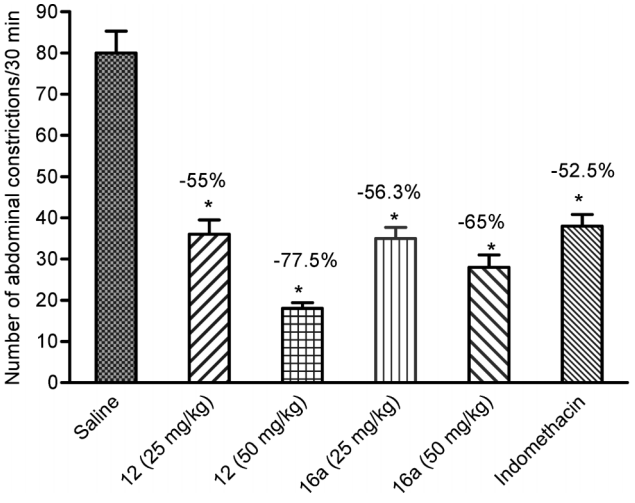


Figure 3. Effect of tested compounds on acetic acid-induced writhing. Test compounds were administered at 25 and 50 mg/kg and the number of abdominal writhes induced by i.p. injection of acetic acid in mice was determined over 30 min period (mean ± S.E. of 6 mice/group). The percent decrease in the number of writhes from the saline control group is represented above the respective group bar. Asterisks indicate significant change from the saline control group (ANOVA and Duncun's multiple comparison tests).

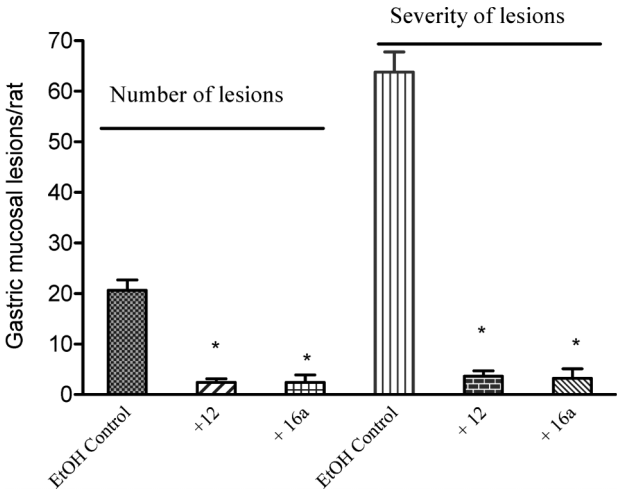


Figure 4. Effect of test compounds administrated at 50 mg/kg, on the number and severity of gastric mucosal lesions caused by s.c. injection of EtOH (96%) in rats. The percentage decrease in the number or severity of gastric lesions from the EtOH (96%) control group is represented above the respective group bar. Asterisks indicate significant change from the corresponding EtOH (96%) control group (ANOVA and Duncan's multiple comparison tests).

was heated under reflux for 3 h until all the reactants had disappeared as indicated by TLC. The reaction mixture was left to cool at room temperature, poured over ice/water mixture and extracted with diethyl ether (3 × 20 mL). The organic layer was dried over anhydrous calcium chloride. Removal of the solvent *in vacuo* afforded the corresponding product, which was crystallized from dioxane to give pale brown crystals of compound **3**, yield 0.52 g (80%), mp 175–177°C, IR (KBr, cm^{-1}): ν = 3342 (NH_2), 2956–2878 (CH-aliphatic), 1702 (C-3, C=O), 1668 (C=N). ^{1}H-NMR (DMSO-d_6, ppm): δ = 0.87 (s, 3H, CH_3-19), 1.09 (s, 3H, CH_3-18), 1.38 (s, 3H, CH_3), 6.24 (s, 2H, NH_2, D_2O-exchangeable). ^{13}C-NMR (DMSO-d_6, ppm): δ = 19.1 (t, C-1), 37.0, (t, C-2), 198.8 (s, C-3), 124.3 (d, C-4), 160.9 (s, C-5), 35.7 (t, C-6), 31.0 (t, C-7), 37.4 (d, C-8), 41.3 (d, C-9), 43.0 (s, C-10), 24.9 (t, C-11), 36.2 (t, C-12), 42.7 (s, C-13), 56.0 (d, C-14), 27.6

Table 4. Effect of the tested compounds on gastric mucosal injury caused by 96% ethanol in rats

Group	Number of lesions/rat	Severity of lesions/rat
Ethanol control	20.6 ± 2.1	63.8 ± 4.0
Ethanol + **12**	2.4 ± 0.7*	3.6 ± 1.1*
Ethanol + **16a**	2.4 ± 1.5*	3.2 ± 1.9*

Statistical comparison of the difference between the ethanol control group and other treated groups is indicated by asterisks; * = $P < 0.05$, NS = not significant vs. control values.

Table 5. Effect of the tested compounds on gastric mucosal injury caused by indomethacin in rats

Group	Number of lesions/rat	Severity of lesions/rat
IND (control)	3.6 ± 0.6	5.3 ± 0.8
IND + **12**	1.2 ± 0.7*	1.8 ± 0.9*
IND + **16a**	2.0 ± 0.4*	2.6 ± 0.5*

Statistical comparison of the difference between the indomethacin (IND) control group and other treated groups is indicated by asterisks; * = $P < 0.05$ vs. control values

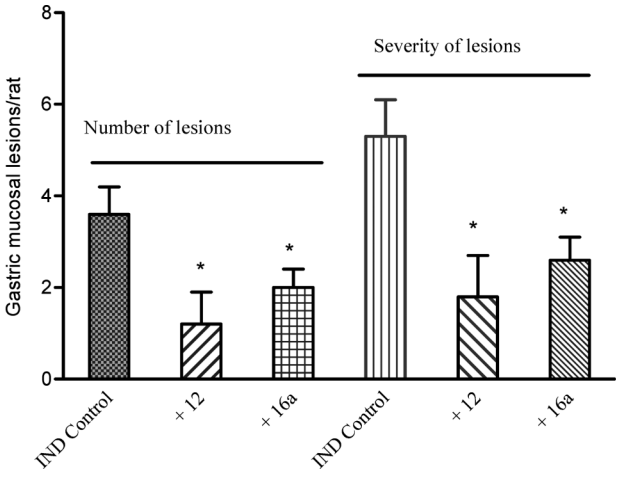


Figure 5. Effect of tested compounds administrated at 50 mg/kg on the number and severity of gastric mucosal lesions caused by s.c. injection of IND in rats. The percentage decrease in the number or severity of gastric lesions from the indomethacin (IND) control group is represented above the respective group bar. Asterisks indicate significant change from the corresponding IND control group (ANOVA and Duncan's multiple comparison tests).

(t, C-15), 23.8 (t, C-16), 30.2 (d, C-17), 23.1 (q, C-18), 24.8 (q, C-19), 164.0 (s, C-20), 13.7 (q, C-21). MS (EI): *m/z* (%): 328 (M^+, 29), 313 (M^+-CH_3), 271 ($C_{19}H_{27}O$, 100). Calcd. for $C_{21}H_{32}N_2O$ (328.491): C, 76.78; H, 9.82; N, 8.53; found: C, 76.52; H, 9.53; N, 8.35%.

*Synthesis of hydrazono-N-phenylmethanethioamide-pregn-4-en-3-one (**5**)*

A mixture of equimolar amounts of compound **3** (0.65 g, 2 mmol) and phenylisothiocyanate **4** (0.27 g, 2 mmol) in dioxane (30 mL), containing a catalytic amount of pipredine (0.5 mL) was heated under reflux for 4 h until all the reactants mixture had disappeared as indicated by TLC. The reaction mixture after cooling at room temperature was poured into ice/water mixture and neutralized with dilute hydrochloric acid. The formed solid product was filtered off, dried, and crystallized from absolute ethanol to yield 0.71 g (78%) of compound **5**, yellowish brown crystals, m.p. 190–192°C, IR (ν, cm^{-1}): 3590–3350 (2 NH), 3050 (CH-aromatic), 2985, 2864 (CH-aliphatic), 1715 (C=O), 1645 (C=N), 1595 (C=C), 1195 (C=S). ^{1}H-NMR ($CDCl_3$, ppm): 0.75 (s, 3H, CH_3-19), 0.92 (s, 3H, CH_3-18), 1.15 (s, 3H, 21-CH_3), 5.78 (s, 1H, C_4-H), 7.15–7.38 (m, 5H, C_6H_5), 9.19, 9.45 (2 s, 2H, 2 NH, D_2O-exchangeable). ^{13}C-NMR ($CDCl_3$, ppm): δ = 21.2 (t, C-1), 36.4, (t, C-2), 197.4 (s, C-3), 124.0 (d, C-4), 158.9 (s, C-5), 35.3 (t, C-6), 32.3 (t, C-7), 37.7 (d, C-8), 42.3 (d, C-9), 45.2 (s, C-10), 26.9 (t, C-11), 36.3 (t, C-12), 42.8 (s, C-13), 57.2 (d, C-14), 27.8 (t, C-15), 23.2 (t, C-16), 31.2 (d, C-17), 23.2 (q, C-18), 24.3 (q, C-19), 156.0 (s, C-20), 13.9 (q, C-21), 186.0 (s, C=S), 137.4 (s), 126.4 (d), 129.5 (d), 123.3 (d) (C-phenyl). MS (EI): *m/z* (%): 465 (M^+ + 2, 18), 464 (M^+ + 1, 38), 312 (60), 272 (40), 92 (100). Calcd. for $C_{28}H_{37}N_3OS$ (463.678): C, 72.53; H, 8.04; N, 9.06; S, 6.92; found: C, 72.76; H, 8.26; N, 9.27; S, 6.75%.

*Synthesis of 20-thiazolylhydrazonopregn-4-ene-3-one derivatives (**8**, **10**, and **12**)*

To a solution of compound **5** (0.92 g, 2 mmol) in absolute ethanol (30 mL) either ethylchloroacetate **6** (0.24 g, 2 mmol), chloroacetone **9** (0.18 g, 2 mmol), or α-bromoacetophenone **11** (0.4 g, 2 mmol) were added. The reaction mixture was heated under reflux for 6–8 h until all the reactants mixture had disappeared as indicated by TLC. The reaction mixture was left to cool at room temperature, poured over an ice/water mixture and neutralized with dilute hydrochloric acid and extracted with anhydrous diethyl ether (3–4 × 20 mL). The organic layer was dried over anhydrous calcium chloride. Removal of the solvent *in vacuo* afforded the corresponding product, which was crystallized from the appropriate solvent.

*20-[(4′-Hydroxy-3′-phenyl-2′,3′-dihydrothiazol-2′-yl)-hydrazono]pregn-4-ene-3-one (**8**)*

Orange crystals from absolute ethanol, yield 0.81 g (81%), mp 96–98°C, IR (KBr, cm^{-1}): ν = 3495–3415 (NH, OH), 3035 (CH-aromatic), 2985, 2875 (CH-aliphatic), 1710 (C-3, C=O), 1663 (C=N), 1635 (C=C); ^{1}H-NMR (DMSO-d_6, ppm): 0.78 (s, 3H, CH_3-19), 0.97 (s, 3H, CH_3-18), 1.13 (s, 3H, 21-CH_3), 4.82 (s, 1H, thiazole 2′-H), 5.23 (s, 1H, OH, D_2O-exchangable), 5.80 (s, 1H, C_4-H), 6.45 (s, 1H, thiazole 5′-H), 7.52–7.72 (m, 5H, C_6H_5), 9.27 (s, 1H, NH, D_2O-exchangeable). MS (EI): *m/z* (%): 507 (M^+ + 2, 14), 505 (M^+, 34), 490 (M^+-CH_3, 32), 328 (M^+-C_9H_7NOS, 100), 77 (63). Calcd. for $C_{30}H_{39}N_3O_2S$ (505.276): C, 71.25; H, 7.77; N, 8.31; S, 6.34; found: C, 71.52; H, 7.52; N, 8.56; S, 6.16%.

*20-[(4′-Methyl-3′-phenyl-2′,3′-dihydrothiazol-2′-yl)-hydrazono]pregn-4-ene-3-one (**10**)*

Brown crystals from dioxane, yield 0.82 g (82%), mp 132–134°C, IR (KBr, cm^{-1}): ν = 3435 (NH), 3038 (CH-aromatic), 2980, 2872 (CH-aliphatic), 1704 (C-3, C=O), 1667 (C=N), 1618 (C=C); ^{1}H-NMR (DMSO-d_6, ppm): δ = 0.82 (s, 3H, CH_3-19), 1.02 (s, 3H, CH_3-18), 1.16 (s, 3H, 21-CH_3), 1.29 (s, 3H, 4′-CH_3), 5.02 (s, 1H, thiazole 2′-H), 5.68 (s, 1H, C_4-H), 6.42 (s, 1H, thiazole 5′-H), 7.32–7.67 (m, 5H, C_6H_5), 9.27 (s, 1H, NH, D_2O-exchangeable). MS (EI): *m/z* (%): 505 (M^+ + 2, 23), 503 (M^+, 54), 489 (M^+-CH_3, 30), 271 ($C_{19}H_{27}O$, 55), 77 (100). Calcd. for $C_{31}H_{41}N_3OS$ (503.741): C, 73.91; H, 8.20; N, 8.34; S, 6.37; found: C, 73.69; H, 8.03; N, 8.60; S, 6.18%.

*20-[(4′,3′-Diphenyl-2′,3′-dihydrothiazol-2′-yl)-hydrazono]pregn-4-ene-3-one (**12**)*

Yellowish brown crystals from absolute ethanol, yield 0.79 g (78%), mp 127–128°C, IR (KBr, cm^{-1}): ν = 3443 (NH), 3042 (CH-

aromatic), 2983, 2868 (CH-aliphatic), 1709 (C-3, C=O), 1665 (C=N), 1603 (C=C); ^{1}H-NMR (DMSO-d_6, ppm): $\delta = 0.85$ (s, 3H, CH_3-19), 1.04 (s, 3H, CH_3-18), 1.18 (s, 3H, 21-CH_3), 5.12 (s, 1H, thiazole 2′-H), 5.73 (s, 1H, C_4-H), 6.50 (s, 1H, thiazole 5′-H), 6.92–7.60 (m, 10H, $2C_6H_5$), 9.92 (s, 1H, NH, D_2O-exchangeable). ^{13}C-NMR (DMSO-d_6, ppm): $\delta = 35.2$ (t, C-1), 34.7, (t, C-2), 198.2 (s, C-3), 124.6 (d, C-4), 171.9 (s, C-5), 32.7 (t, C-6), 31.3 (t, C-7), 35.4 (d, C-8), 51.3 (d, C-9), 37.8 (s, C-10), 22.5 (t, C-11), 35.4 (t, C-12), 42.2 (s, C-13), 56.3 (d, C-14), 27.4 (t, C-15), 23.5 (t, C-16), 32.8 (d, C-17), 21.1 (q, C-18), 22.7 (q, C-19), 164.4 (s, C-20), 18.20 (q, C-21), 76.4 (d), 102.0 (d), 134.7 (s) (C-thiazole), 117.4 (d), 129.0 (d), 113.6 (d), 144.7 (s), 134.6 (s), 126.4 (d), 128.5 (d), 120.3 (d) (C-phenyl). MS (EI): *m*/*z* (%): 567 ($M^+ + 2$, 10), 566 ($M^+ + 1$, 45), 550 (M^+-CH_3, 32), 271 ($C_{19}H_{27}O$, 67), 77 (100). Calcd. for $C_{36}H_{43}N_3OS$ (565.312): C, 76.42; H, 6.77; N, 7.43; S, 5.67; found: C, 76.69; H, 7.01; N, 7.63; S, 5.82%.

*Synthesis of 20-(2′-oxo-2′-phenylethylhydrazono)pregn-4-ene-3-one (**13**)*

A solution of equimolar amounts of compound **3** (0.65 g, 2 mmol) and α-bromoacetophenone **11** (0.40 g, 2 mmol) in dioxane (30 mL) containing a catalytic amount of piperidine (0.5 mL) was heated under reflux for 6 h until all the reactants mixture had disappeared as indicated by TLC. The reaction mixture, cooled, poured over an ice/water mixture and neutralized with dilute hydrochloric acid. The solid product was filtered off, dried, and crystallized from methanol.

Compound **13**: Brown crystals, yield 0.63 g (70%), mp 145–147°C, IR (KBr, cm^{-1}): $\nu = 3394$ (NH), 2930–2859 (CH-aliphatic), 1698, 1712 (2 C=O), 1656 (C=N). ^{1}H-NMR ($CDCl_3$, ppm): $\delta = 0.98$ (s, 3H, CH_3-19), 1.07 (s, 3H, CH_3-18), 1.19 (s, 3H, 21-CH_3), 3.95 (s, 2H, CH_2COPh), 5.80 (s, 1H, C_4-H), 7.04–7.91 (m, 5H, C_6H_5), 8.94 (s, 1H, NH, D_2O-exchangeable). MS (EI): *m*/*z* (%): 446 (M^+, 63), 431 (M^+-CH_3, 32), 341 (M^+-COPh, 100), 105 (75). Calcd. for $C_{29}H_{38}N_2O_2$ (446.624): C, 77.99; H, 8.58; N, 6.27; found: C, 77.78; H, 8.36; N, 6.52%.

*Synthesis of pyrrolyl progesterone derivatives (**16a,b**)*

General procedure

To a solution of compound **13** (0.89 g, 2 mmol) in absolute ethanol (30 mL) containing a catalytic amount of triethylamine (0.5 mL), an equimolar amount of malononitrile **14a** (0.13 g, 2 mmol) or ethyl cyanoacetate **14b** (0.22 g, 2 mmol) was added. The reaction mixture was heated under reflux for 5 h until all the reactants mixture had disappeared as indicated by TLC. The reaction mixture was left to cool at room temperature, poured over an ice/water mixture and neutralized with dilute hydrochloric acid. The solid product that formed, in each case, was filtered off, dried and crystallized from the appropriate solvent.

*20-[1′-(2″-Amino-3″-cyano-4″-phenyl-1″H-pyrrol-17-yl)-ethylidenamino]pregn-4-en-3-on (**16a**)*

Brown crystals from ethanol (70%), yield 0.81 g (82%), mp 230–232°C, IR (KBr, cm^{-1}): $\nu = 3353$ (NH_2), 2947–2875 (CH-aliphatic), 2225 (CN), 1697 (C-3, C=O), 1647 (C=N), 1612 (C=C). ^{1}H-NMR ($CDCl_3$, ppm): $\delta = 0.98$ (s, 3H, CH_3-19), 1.07 (s, 3H, CH_3-18), 1.17 (s, 3H, 21-CH_3), 5.80 (s, 1H, C_4-H), 6.15 (s, 2H, NH_2, D_2O-exchangeable), 6.52 (s, 1H, pyrrole 5′-H), 6.72–7.25 (m, 5H, C_6H_5). ^{13}C NMR (DMSO-d_6, ppm): $\delta = 35.1$ (t, C-1), 34.0, (t, C-2), 198.4 (s, C-3), 125.3 (d, C-4), 172.9 (s, C-5), 32.7 (t, C-6), 31.4 (t, C-7), 35.4 (d, C-8), 50.3 (d, C-9), 37.0 (s, C-10), 22.5 (t, C-11), 37.4 (t, C-12), 42.2 (s, C-13), 56.3 (d, C-14), 27.0 (t, C-15), 22.8 (t, C-16), 31.2 (d, C-17), 22.1 (q, C-18), 23.8 (q, C-19), 165.4 (s, C-20), 13.7 (q, C-21), 124.7 (s), 114.5 (d), 113.4 (s), 106.0 (s) (C-pyrrole), 117.4 (s, CN), 127.0 (d), 129.6 (d), 128.5 (d), 136.3 (s) (C-phenyl). MS (EI): *m*/*z* (%): 495 ($M^+ + 1$, 54), 479 (M^+-CH_3, 30), 271 ($C_{19}H_{27}O$, 39), 77 (100). Calcd. for $C_{32}H_{38}N_4O$ (494.670): C, 77.70; H, 7.74; N, 11.33; found: C, 77.52; H, 7.95; N, 11.53%.

*20-[1′-(2″-Amino-3″-ethoxycarbonyl-4″-phenyl-1″H-pyrrol-17-yl)ethylidenamino]-pregn-4-en-3-on (**16b**)*

Pale brown crystals from methanol, yield 0.88 g (81%), mp 170–172°C, IR (KBr, cm^{-1}): $\nu = 3348$ (NH_2), 2957–2883 (CH-aliphatic), 1735 (C=O, ester), 1698 (C-3, C=O), 1647 (C=N), 1605 (C=C). ^{1}H-NMR ($CDCl_3$, ppm): $\delta = 0.93$ (s, 3H, CH_3-19), 1.02 (s, 3H, CH_3-18), 1.13 (s, 3H, 21-CH_3), 1.32 (t, 3H, CH_3-ester), 4.25 (q, 2H, CH_2-ester), 5.82 (s, 1H, C_4-H), 6.18 (s, 2H, NH_2, D_2O-exchangeable), 6.48 (s, 1H, pyrrole 5′-H), 6.82–7.35 (m, 5H, C_6H_5). MS (EI): *m*/*z* (%): 541 (M^+, 62), 526 (M^+-CH_3, 30), 271 ($C_{19}H_{27}O$, 60), 77 (100). Calcd. for $C_{34}H_{43}N_3O_3$ (541.723): C, 75.38; H, 8.00; N, 7.76; found: C, 75.22; H, 8.17; N, 7.92%.

Pharmacological assay

Animals

Sprague-Dawley strain rats weighing 120–130 g or Swiss albino mice 20–25 g body weight were used throughout the experiments, supplied by the Animal House Colony of the National Research Centre, Cairo, Egypt, and acclimated for one week in a specific pathogen-free (SPF) barrier area where temperature 25 ± 1°C and humidity 55%. Animals were controlled constantly with a 12 h light/dark cycle at the National Research Centre animal facility breeding colony. Animals were individually housed with *ad libitum* access to standard laboratory diet and tap water. All animal procedures were performed after approval from the Ethics Committee of the National Research Centre and in accordance with the recommendations for the proper care and use of laboratory animals (NIH publication No. 85–23, revised 1985).

Testes of inflammation: carrageenan-induced paw edema assay

Paw edema was induced by sub-plantar injection of 100 μL of 1% sterile carrageenan lambda in saline into the right hind paw of rats [24]. Contralateral paw received an equal volume of saline. Paw volume was determined immediately before carrageenan injection and at selected times thereafter using a plethysmometer (Ugo Basile, Milan, Italy). The edema component of inflammation was quantified by measuring the paw volume (mL) at zero time (before carrageenan injection) and at 1, 2, 3, and 4 h after carrageenan injection and comparing it with the pre-injection value for each animal. Edema was expressed as a percentage of change from control (pre-drug, zero time) values. The effect of systemic administration of compounds **12** and **16a** (25 or 50 mg/kg, s.c., 0.2 mL, $n = 6$/group) given 30 min before induction of inflammation by subplantar carrageenan was studied. The control group of carrageenan-treated rats received an equal volume of saline 30 min before subplantar carrageenan injection ($n = 6$ each). Another group administered IND (18 mg/kg, s.c.) served as control positive.

Tests of nociception

Hot plate assay

The hot plate test was performed using an electronically controlled hot plate (Ugo Basile, Italy) heated to 53°C (± 0.1°C). Each mouse was placed unrestrained on hot plate for the baseline measurement just prior to saline or drug administration. Different groups of mice ($n = 6$/group) were given compounds **12** or **16a** (25 or 50 mg/kg; 0.2 ml, orally), tramadol (20 mg/kg, 0.2 ml, orally) (control + ve) or saline (control − ve). Measurements were then taken 60 min after drug administration. The experimenter was blind to doses. Latency to lick a hind paw or jump out of the apparatus was recorded for the control and drug-treated groups. The cut-off time was 30 s.

Acetic acid induced writhing

Separate groups of 6 mice each were administered vehicle (saline), compounds **12** or **16a** (25 or 50 mg/kg; 0.5 mL, orally) or IND (18 mg/kg, 0.5 mL, orally). After 60 min, mice received an i.p. injection of 0.6% acetic acid (0.2 mL) [25]. The number of writhes (constrictions of abdomen, twisting of trunk, and extension of hind legs) during 30 min observation period following acetic acid injection was compared with the control group and drug-treated groups.

Gastric ulcerogenic study

Gastric mucosal damage was evoked in rats by the administration of IND (20 mg/kg, 0.2 mL, s.c.). The effect of compounds **12** or **16a** (50 mg/kg; 0.5 mL, orally) administered at time of IND injection was studied. Rats were killed 24 h after IND administration. In other experiments, the effect of tested compounds (50 mg/kg; 0.5 ml, s.c.) on gastric damage caused by ethanol (96%) was evaluated. Rats were fasted for 18 h, but allowed water *ad libitum*. They were administered either saline (control) or one of the tested compounds 30 min prior to ethanol (96%, 1 mL, p.o.). Rats were killed 1 h after ethanol administration, stomachs excised, opened along the greater curvature, rinsed with saline, extended on a plastic board and examined for mucosal lesions. The number and severity of mucosal lesions were noted and lesions were scaled as described by Mózsik et al. [26].

Statistical analysis

Data are expressed as mean ± SE. Data were analyzed by one-way analysis of variance, followed by a Tukey's multiple range tests for *post hoc* comparison of group means. When there were only two groups a two-tailed Student's t test was used. For all tests, effects with a probability of $P < 0.05$ were considered to be significant and that with a probability of $P < 0.001$ were considered to be highly significant.

Conclusion

This study described a straight forward and efficient synthesis of novel modified steroids containing fused pyrrole or thiazole nucleus in addition to the pharmacophoric features of the steroid moiety. The novel synthesized modified steroids **12** and **16a** showed anti-inflammatory, antinociceptive and anti-ulcerogenic activities with various intensities. Edema was significantly reduced by both doses of tested compounds (25 and 50 mg/kg) at 2, 3, and 4 h post-carrageenan. The high dose of the pyrrolyl steroid **16a** was the most effective in alleviating thermal pain. Gastric mucosal lesions caused in the rat by the administration of 96% ethanol or IND was significantly inhibited by either one of the two tested compounds. These results provide a unique opportunity to develop new anti-inflammatory drugs which devoid the ulcerogenic liabilities associated with currently marketed drugs. Finally, the encouraging results of the anti-inflammatory, anti-nociception and anti-ulcerogenic activity displayed by these compounds may be of interest for further derivatization and further drug-ability and physical studies in the hope of finding new potent prescriptions.

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