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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of progesterone derivatives and evaluation of their efficiency as pneumococcal vaccines

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Abstract Progesterone (**1**) was used as a template to develop new anticancer compounds. Ring D modification of **1**, through its reaction with active methylene derivatives, gave the condensate derivatives **3a**, **b**. The latter compounds underwent heterocyclization reactions through the reaction with either hydrazine hydrate or phenyl hydrazine to give the pyrazole derivatives **6a–d**, respectively. The reaction of **1** with bromine gave the α-bromo derivative **7**, which in turn reacted with potassium cyanide to yield the cyanoacetyl derivative **8**. Compound **8** has been subjected to a series of reactions that produced benzylidene, aryl hydrazine, pyrazole, and pyran derivatives. The newly synthesized products were tested for their efficiency as pneumococcal vaccines and the results were promising.

Keywords Pneumococcal vaccines · Progesterone · Pyrazole · Thiophene · Pyran · Arylhydrazone · Benzylidene

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Introduction

Found in animals, plants and microorganisms, steroids are classified as organic compounds. Estradiol, Progesterone, Epitestosterone, and Testosterone (sex hormones) are some common steroids that are found in animals. It is found that a steroidal skeleton is involved in the chemical structure of a large number of therapeutic drugs, such as Dexamethasone and Betamethasone. The positive effects of the administration of exogenous steroids include muscle growth, appetite stimulation, increased red blood cell production, and bone density (Kintz et al., 2001; Bhasin et al., 1996; Page et al., 2008). In the field of medicine, steroids have been used in treating a variety of illnesses including tropical diseases, inflammation, anemia, breast cancer, muscle regeneration, and also osteoporosis in postmenopausal women (Basaria et al., 2001). In spite of that, steroids exhibit serious environmental toxicity and have several health hazards (Pagonis et al., 2006). The fact that minor structural changes in steroids can cause extensive changes in their biological activity has for long aroused the curiosity of medicinal chemists. Naturally occurring steroidal nuclei have been modified in several ways with the aim of finding more active compounds that are free from the undesirable side effects, and of recognizing the structural and stereochemical features required for the display of specific, selective physiological activity. The replacement of one or more carbon atoms in a steroid molecule with heteroatoms results in substantial modifications of its biological activity (Morand and Lyall, 1968; Huisman, 1971; Huisman and Speckamp, 1976). Wolff and Zanati have reported that some A-ring hetero-androstanes exhibit androgenic activity similar to that of testosterone (Wolff and Zanati, 1970a, b). A large number of compounds with oxygen, nitrogen, or sulfur in the nucleus of the steroidal skeleton have been

prepared since 1970 and their activities studied (Zanati and Wolff, 1971; Salvi et al., 1976; Rosen et al., 1980; Ouali, 2006, 2007; Ouali and Rocheblave, 2008). These modified steroids have been found to possess various biological activities, such as analgesic properties (Taylor and Lenard, 1967). These results have motivated organic chemists to synthesize novel compounds that have therapeutic potential. It is known that progesterone completely prevents the androgenic action of testosterone on the diameter of the pigmented spot (Vermorken et al., 1980). In addition, progesterone stimulates the growth of sebaceous tissue from flam organs in the same way that testosterone and dihydrotestosterone do (Vermorken et al., 1980).

As a continuation of our previous work (Mohareb and Hanaa, 2007; Mohareb and El-Omran, 2012; Mohareb et al., 2012) on the synthesis of different bioactive fused heterocyclic derivatives of progesterone and pregnenolone, in this article we describe the employment of progesterone in the formation of heterocyclic ring systems. Our strategy involves the study of the use of the acetyl group in attaching a heterocyclic ring system to ring D. Moreover, we provide the experimental details of the newly synthesized compounds followed by the results of testing their efficiency as pneumococcal vaccines.

Results and discussion

Recently, our research group was involved in a series of heterocyclic transformations using pregnenolone, through subjecting it to the Knoevenagel reaction with some cyanomethylene compounds to obtain reagents capable of further heterocyclization (Mohareb et al., 2013). In this paper, we carry out the same procedure, but using progesterone instead of pregnenolone. Thus, the reaction of progesterone with either malononitrile (**2a**) or ethyl cyanoacetate (**2b**), in the presence of ammonium acetate, gave the condensate products **3a** and **3b**, respectively. Their structures were verified based on analytical and spectral data. Thus, the ^{13}C NMR spectrum of **3a** showed beside the expected signals due to progesterone, two signals at δ 116.5 and δ 117.3 indicating the presence of the two CN groups, and two signals at δ 83.6, 178.0 corresponding to the C=C group. Compounds **3a** and **3b** reacted with elemental sulfur in the presence of triethylamine to give the thiophene derivatives **4a** and **4b**, respectively. Formation of the latter products follows the well-known pattern of Gewald's thiophene synthesis (McKibben et al., 1999; Balamurugan et al., 2009). On the other hand, the reaction of either **3a** or **3b** with either hydrazine hydrate or phenyl hydrazine gave the pyrazole derivatives **6a–d**, respectively. Their analytical and spectral data were consistent with their respective structures (Scheme 1).

The reaction of progesterone with bromine in acetic acid gave the corresponding α-bromoacetyl derivative, **7**. The latter readily underwent nucleophilic displacement when reacted with potassium cyanide to give the corresponding α-cyanoacetyl derivative **8**, with a high yield. This has encouraged us to study the latter's reactivity toward several chemical reagents. Thus, its reaction with aromatic diazonium salts **9a–c** in basic ethanol at 0–5 °C, gave the arylhydrazone derivatives **10a–c**, respectively.

On the other hand, the reaction of compound **8** with either hydrazine hydrate (**5a**) or phenylhydrazine (**5b**) gave the pyrazole derivatives **11a** and **11b**, respectively (Scheme 2). The ^{1}H NMR spectrum was used to confirm the structure of compound **11a** which showed a singlet (D_2O exchangeable) at δ 4.82 ppm, another at δ 6.53 and a third at δ 8.22 indicating the presence of the NH_2 group, the pyrazole H-4 and the NH group, respectively. Moreover, the ^{13}C NMR spectrum that showed beside the expected peaks, a peak at δ 119.2 indicating the CN group and three peaks at δ 114.6, 152.4, and 156.8 corresponding to the pyrazole carbon atoms.

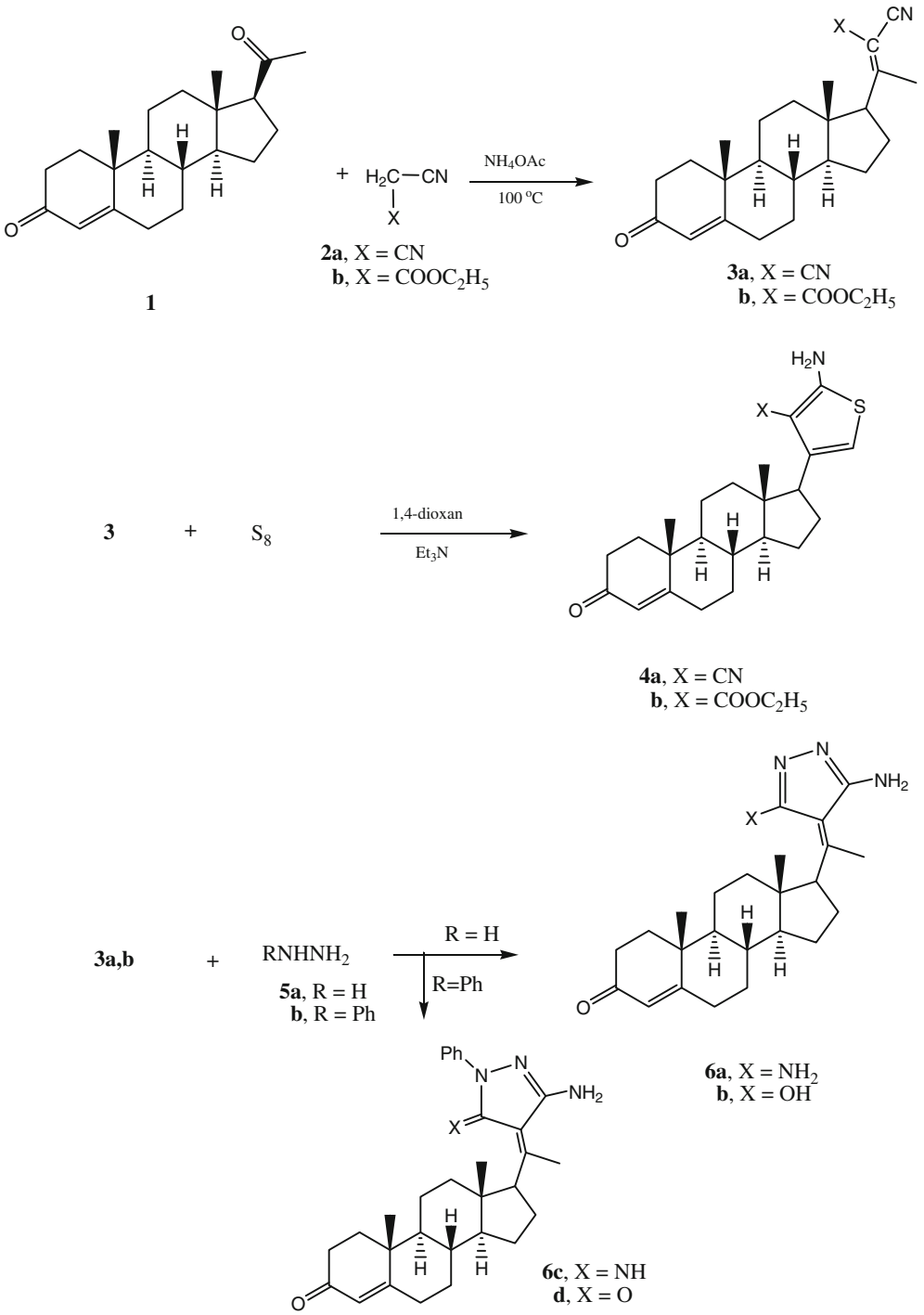
Compound **8** reacted with aromatic aldehydes **12a–c** to give the aromatic ylidene derivatives **13a–c**, respectively, the structures of which were based on analytical and spectral data (see spectral data). Compounds **13a–c** reacted with either of the cyanomethylene derivatives **2a** or **2b** to give the pyran derivatives **15a–d**. Formation of the latter products took place through the intermediate formation of **14a–d** followed by the intramolecular Michael addition of the enolic OH group to the cyano group (Scheme 3). The ^{1}H NMR and ^{13}C NMR spectra provided proof of their respective structures. Thus, the ^{1}H NMR spectrum of **15a** showed the presence of a singlet at δ 4.62 corresponding to the NH_2 group, a singlet at δ 6.53 corresponding to the pyran H-4 and a multiplet at δ 7.28–7.38 indicating the phenyl protons. Moreover, the ^{13}C NMR spectrum showed the presence of two signals at δ 116.8 and 117.3 corresponding to the two CN groups.

Compounds **3**, **5a–d**, **7a**, **7b**, **9a–d**, and **11b** exhibit stereoisomerism and could exist as E and Z forms due to the presence of the π-electron conjugation. Trials to obtain pure isomers were carried out using flash chromatography of the crude compounds (0.5 mmol) in a hexane–acetone mixture (3:1). Thin layer chromatography examination of the reaction mixture revealed minor products probably due to the presence of other isomers (Stulov et al., 2010).

Determination of antibacterial activity

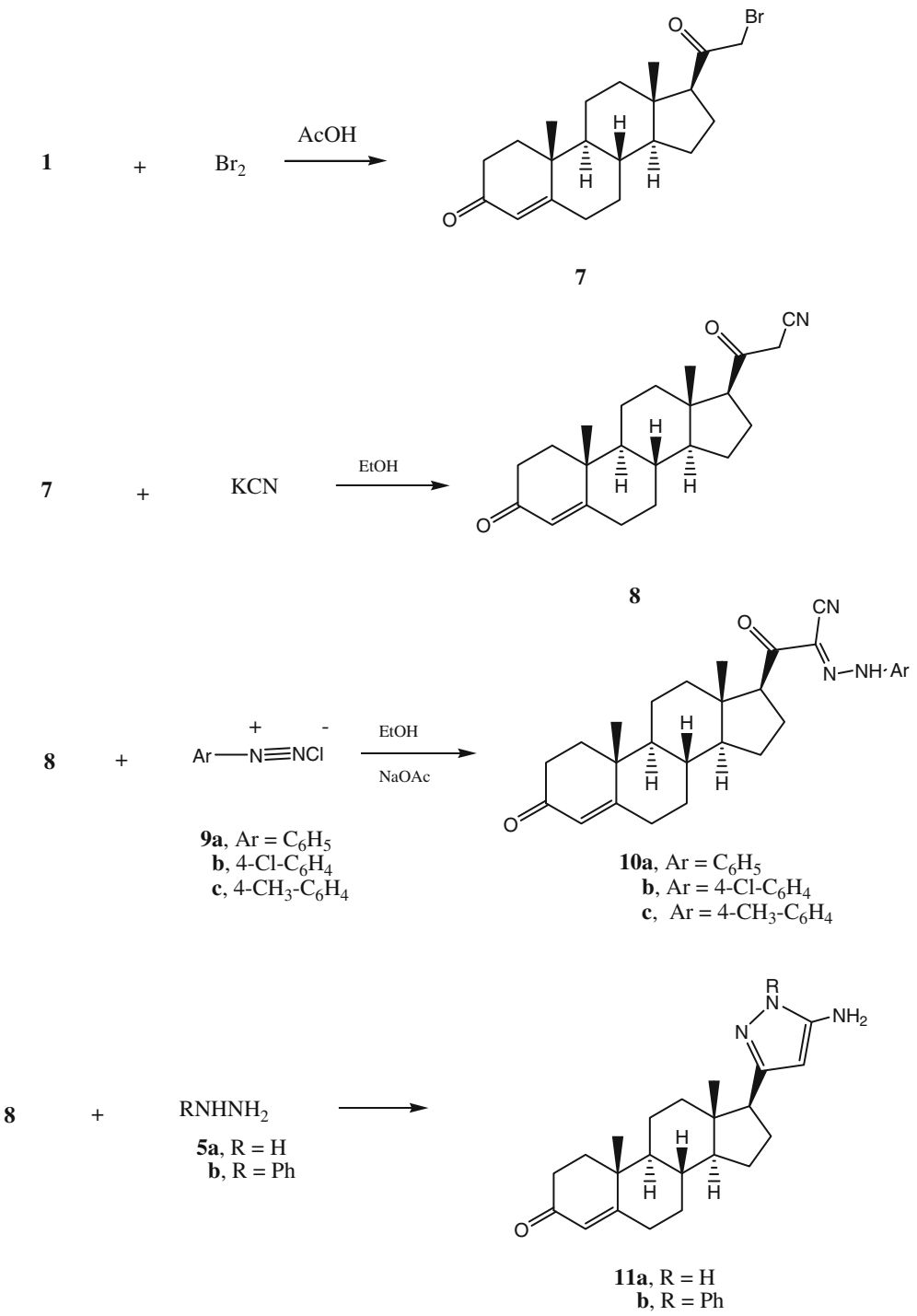
Microorganisms

Different serotypes of Streptococcus pneumonia clinical isolates were used; AV3 (serotype 3), AV6 (serotype 6B),



Scheme 1 Synthesis of compounds **3a**,**b**; **4a**,**b** and **6a–d**

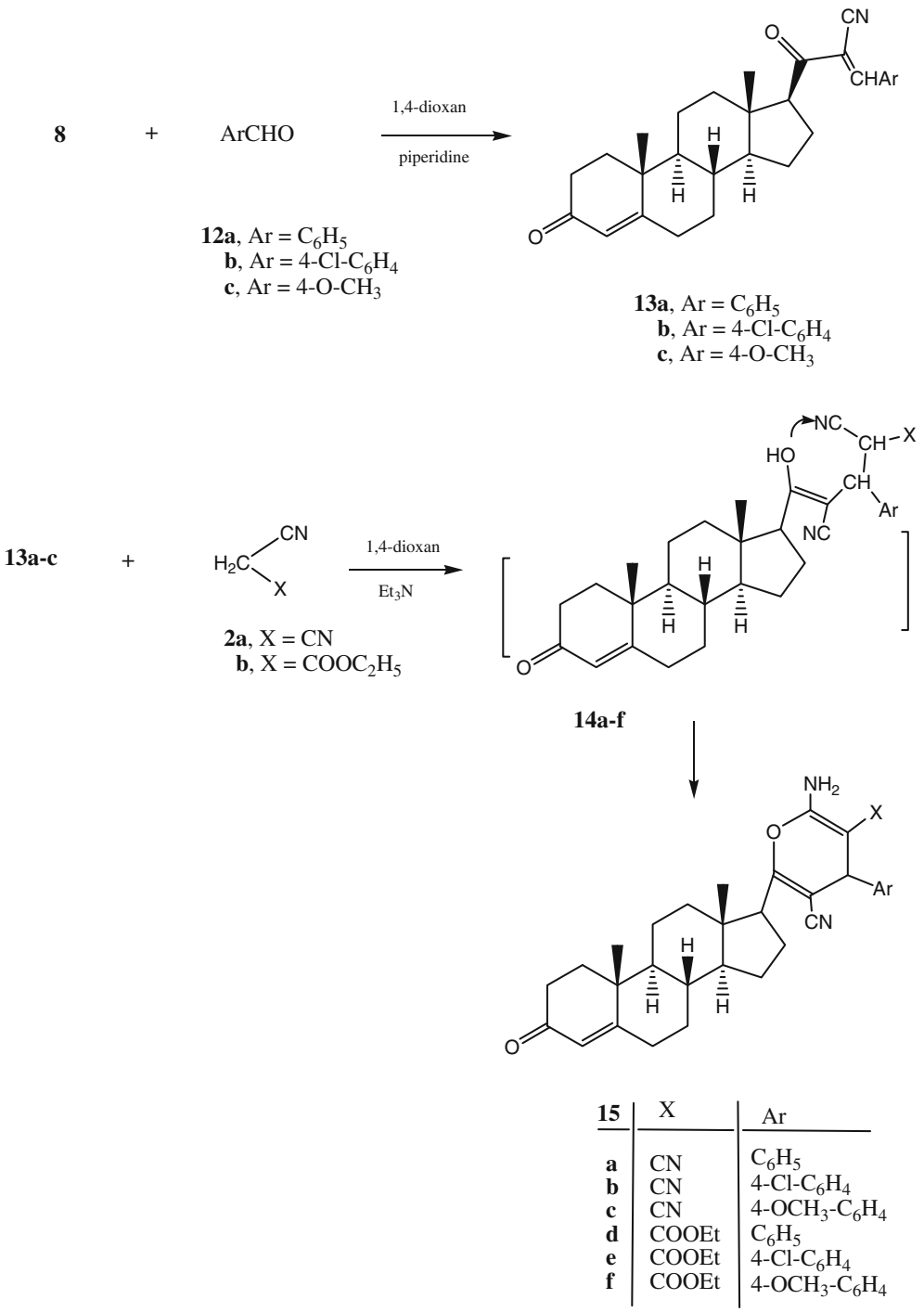
AV14 (serotype 14), and AV23 (serotype 23F). The strains were maintained at −70 °C in a Brain Heart Infusion (BHI) containing 30 % (v/v) glycerol in CERELA (CONICET). Bacterial inocula were prepared from an overnight culture in a 5 % sheep blood Columbia Agar (BioMérieux Brazil S.A.) at 37 °C until the log phase was reached. Then, each suspension was diluted in a sterile 0.9 % NaCl solution to obtain a cell suspension of 107 CFU/ml (colony forming units/ml).



Scheme 2 Synthesis of compounds **7**; **8**; **10a–c** and **11a–b**

Determination of minimum inhibitory concentrations (MIC)

Samples' MIC-defined as the lowest concentration of sample capable of inhibiting visible growth after incubation-, were determined against the different Streptococcus pneumonia strains (Villena et al., 2010) by the agar macro dilution method in accordance with the Clinical Laboratory Standards Institute (CLSI, NCCLS) guidelines. Zuccagnia punctata extract, HF, DHF, and DHC were sequentially diluted in DMSO and added to Mueller–Hinton Agar supplemented with a 5 % sheep blood medium at



Scheme 3 Synthesis of compounds **13a–c** and **15a–b**

concentrations of 12.5, 25, 50, 100, 200, and 400 μg GAE/ml for the crude extract and concentrations of 0.1, 1, 10, 100, and 500 μg/ml for the isolated compounds.

After cooling and drying, the plates were inoculated in spots with 2 μl of each bacterial cell suspension (5×10^4 CFU). A growth control of each tested strain and a solvent control were prepared. The inoculated plates were incubated for 20–24 h in a 5 % CO_2 environment at 37 ± 2 °C. MIC values were also determined for commercial antibiotics (amoxicillin, GlaxoSmithKline S.A.), and essays were performed in triplicates.

Animals

Infant mice were selected for their high susceptibility to pneumococcal respiratory infection. All experiments were

carried out in compliance with the Guide for Care and Use of Laboratory Animals and approved by the Ethical Committee of Animal Care at CERELA under the bioethical allowance number BIOT-CRL/11. 3 weeks old Swiss albino mice, each weighing 20 ± 3 g, were used for the in vivo experiments. They were kept in a temperature-controlled room under a 12-h luminous–12-h dark cycle. Animals had access to commercial solid food and water ad libitum.

Experimental streptococcus pneumonia infection

The therapeutic effects of the standardized Zuccagnia punctata extract and the flavonoids isolated from them were previously determined in a Streptococcus pneumonia infection model in mice (Racedo et al., 2006). Freshly grown colonies of the Streptococcus pneumonia strain AV6 (serotype 6B) were suspended in Todd Hewitt Broth (THB) (Laboratorios Britania, Argentina) and incubated at 37 °C until the log phase was reached. The pathogen was nasally introduced to mice by dropping 25 µl of an inoculum containing 106 CFU of Streptococcus pneumonia into each nostril (Villena et al., 2010).

Treatment protocol

Mice were distributed into groups of nine mice each one: G1 control (C), G2 Streptococcus pneumoniae-infected (SI), G3 Streptococcus pneumoniae-infected + Zuccagnia punctata extract (0.25 mg/mice) (SIZp 0.25), G4 Streptococcus pneumoniae-infected + Zuccagnia punctata extract (0.5 mg/mice) (SIZp 0.5), G5 Streptococcus pneumoniae-infected + Zuccagnia punctata extract (1.0 mg/mice) (SIZp 1.0), G6 Streptococcus pneumoniae-infected + HF (1 mg/mice) (SIHF), and G7 Streptococcus pneumoniae-infected + DHC (1 mg/mice) (SIDHC). Mice without infections treated with different concentrations of extract and flavonoids were included. The antibiotics (Zuccagnia punctata extract or flavonoids) in DMSO were administered orally once a day for 7 days postinfection (p.i.); and twice a day for 7 days p.i., Treatment of positive control was performed with two doses of amoxicillin sodium salt (Clamoxyl® 1 g; GlaxoSmithKline S.A.): 0.5 and 2.0 mg/mice. Amoxicillin was administered once a day for 7 days. In all groups, the treatments of animals with drug started at 24 h p.i. At days 3, 5, and 7 p.i. three mice of each group were sacrificed, and lung and blood samples were obtained for bacterial cell counts. The serum was used by biochemical analysis. The results are means of three independent experiments.

In vitro antibacterial activity

The antimicrobial efficacy of Zuccagnia punctata extract, DHC, HF, DHF, and commercial antibiotic, against the four different serotypes of Streptococcus pneumonia strains, was evaluated by the agar macro dilution method. The MIC values of the samples against Streptococcus pneumonia strains are shown in Table 1. All natural products showed in vitro antimicrobial activity against each of the tested strains with MIC values ranging from 50 to 500 µg/ml. Streptococcus pneumonia AV6 and AV23 strains were more sensitive to natural products than the Streptococcus pneumonia AV14 strain. The highest antibacterial activity against the various Streptococcus pneumonia serotypes was achieved with DHC, followed by HF and DHF. The MIC values of DHC were similar to those observed with the Zuccagnia punctata extract.

The antibacterial activity (MIC) of the newly synthesized compounds against the Streptococcus pneumonia strains showed that compounds **3a**, **4a**, **6d**, **8**, **10b**, **13b**, and **15c** are the most active compounds toward the four

Table 1 Antibacterial activity (MIC) of obtained compound against streptococcus pneumonia strains

Compd. no.	MIC (mg/ml)			
	AV3	AV6	AV14	AV23
3a	R	50	R	50
3b	100	R	100	100
4a	R	50	R	100
4b	500	100	R	100
6a	200	100	300	100
6b	R	R	100	100
6c	500	100	200	100
6d	R	R	100	R
7	100	R	100	50
8	R	100	100	R
10a	200	500	100	100
10b	R	100	100	R
10c	100	100	400	200
11a	400	50	300	100
11b	100	50	200	R
13a	300	100	500	100
13b	R	R	100	R
13c	R	100	200	50
15a	100	R	300	200
15b	R	R	200	100
15c	R	R	100	200
15d	50	100	200	100
15e	100	R	R	200
15f	200	300	R	R
Amoxicillin	R	R	R	R

MIC Minimum inhibitory concentrations, compounds were diluted in DMSO, *R* resistant until 400 or 500 µg/ml for Z. puntata extract, Breakpoint for amoxicillin are less than −2.0 µg/l, 4.0 µg/ml (intermediate) and >/8.0 µg/ml (resistant)

Streptococcus pneumonia strains, and that such activities are very close to that of the reference amoxicillin.

Experimental procedure

Synthetic methods, analytical and spectral data

The starting steroid, progesterone, was purchased from Sigma Company, USA. All solvents were dried by distillation prior to using. Melting points were recorded on a Buchi melting point apparatus D-545, and IR spectra (KBr disks) were recorded on a Bruker Vector 22 instrument. NMR spectra were recorded on a Bruker DPX200 instrument in $CDCl_3$ and DMSO with TMS as internal standard for protons and solvent signals as internal standard for carbon spectra. Chemical shift values are provided in δ (ppm). Mass spectra were recorded on an EIMS (Shimadzu) and an ESI-esquire 3000 Bruker Daltonics instrument. Elemental analyses were carried out at the Micro analytical Data Unit at the National Research Center, Giza, Egypt and the Micro analytical Data Unit at Cairo University, Giza, Egypt. The progress of all reactions was monitored by TLC on 2 × 5 cm pre-coated silica gel 60 F254 plates 0.25 mm thick (Merck). The purity of the tested compounds was evaluated using HPLC (Waters Associates, Milford, MA, USA) employing an ultraviolet detector (215 nm). All described compounds showed spectral data that are characteristic of cyclopentanoperhydrophenanthrene nuclei of Pregnene and Androstane series and were similar to those reported in literature (IUPAC, 1989a, b).

*Synthetic procedure for the etylidene derivatives **3a, b***

To dry solid progesterone (**1**) (0.362 g, 1 mmol) either malononitrile (0.066 g, 1 mmol) or ethyl cyanoacetate (0.107 g, 1 mmol) was added, followed by the addition of ammonium acetate (0.25 g). The reaction mixture was heated at 80 °C for 4 h then left to cool down. The solid product obtained upon trituration of the formed product by ethanol was collected by filtration. The filtrate was then dried and checked for purity through TLC. Thin layer chromatography revealed a single spot proving the presence of a sole product.

*2-(1-((8S,9S,10R,13S,14S)-10,13-dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)ethylidene)malononitrile (**3a**)* HPLC purity = 96 % (C-18 NovaPak column; MeCN:MeOH:H_2O/45:20:35), t_r = 18 min; white crystals from EtOAc: hexane (88 %), m.p. 220–223 °C; IR (KBr) cm^{-1}: 2932 (CH_2), 2227, 2220 (2 CN), 1688 (CO), 1639 (C=C); ^1H NMR ($CDCl_3$): δ 0.82 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.61–1.93 (m, 6H, $3CH_2$), 2.20–2.38 (m, 3H, 3CH), 2.66 (s, 3H, CH_3), 2.82 (t, J = 8.6 Hz, 1H, CH), 3.01–3.49 (m, 10H, $5CH_2$), 5.83 (s, 1H, CH=C); ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.6, 21.6 (C-18, C-19), 23.0 (ethylidene CH_3), 31.9 (C-16), 37.2 (C-15), 43.8 (C-11), 45.1 (C-12), 49.3 (C-1), 49.3 (C-6), 50.1 (C-2), 57.2 (C-7), 71.5, 83.0, 84.2 (C-9, C-8, C-14), 116.5, 117.3 (C=C), 119.0, 120.6 (2 CN), 121.3 (C-4), 168.3 (C-5), 178.0 (C-17), 187.4 (C-3).

*Ethyl 2-cyano-3-((8S,9S,10R,13S,14S)-10,13-dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)but-2-enoate (**3b**)* HPLC purity = 92 % (C-18 NovaPak column; MeCN:MeOH:H_2O/50:40:10) t_r = 22 min; white crystals from ethanol (73 %), m.p. 160–162 °C; IR (KBr) cm^{-1}: 2936 (CH_2), 2222 (CN), 1686, 1688 (2 CO), 1636 (C=C); ^1H NMR ($CDCl_3$): δ 0.80 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.12 (t, 3H, J = 6.77 Hz, ester CH_3), 1.62–1.93 (m, 6H, $3CH_2$), 2.20–2.39 (m, 3H, 3CH), 2.84 (t, J = 7.25 Hz, 1H, CH), 3.02–3.48 (m, 10H, $5CH_2$), 4.21 (q, J = 6.77 Hz, 2H, ester CH_2), 5.84 (s, 1H, CH=C); ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.6, 21.6 (C-18, C-19), 14.8 (ester CH_3), 23.3 (ethylidene CH_3), 31.6 (C-16), 37.7 (C-15), 43.4 (C-11), 45.1 (C-12), 49.3 (C-1), 49.7 (C-6), 50.1 (C-2), 57.4 (C-7), 60.2 (ester CH_2), 71.5, 83.0, 84.2 (C-9, C-8, C-14), 116.6, 119.2 (C=C), 120.6 (CN), 121.3 (C-4), 168.6 (C-5), 178.0) 187.4 (ester CO, C-3); MS: m/e = 409 (M^+); Anal. Calcd. for $C_{26}H_{35}NO_3$: C, 76.25; H, 8.61; N, 3.42. Found C, 76.41; H, 8.48; N, 3.58.

*Synthetic procedure of the thiophene derivatives **4a, b***

To a solution of either **3a** (0.362 g, 1 mmol) or **3b** (0.409 g, 1 mmol) in 1,4-dioxane (30 ml) containing a catalytic amount of triethylamine, sulfur (0.032 g, 1 mmol) was added. The reaction mixture was heated under reflux for 1 h, left at room temperature overnight, poured onto an ice-water mixture, and then the formed solid product was collected by filtration.

*2-Amino-4-((8S,9S,10R,13S,14S)-10,13-dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13, 14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)thiophene-3-carbonitrile(**4a**)* HPLC purity = 96 % (C-18 NovaPak column; MeCN:MeOH:H_2O/45:20:35), t_r = 20 min; pale yellow crystals from EtOAc: hexane (88 %), m.p. 220–223 °C; IR (KBr) cm^{-1}: 3484, 3438 (NH_2), 3020 (CH thiophene), 2932 (CH_2), 2227 (CN), 1687 (CO), 1639 (C=C); ^1H NMR (DMSO-d_6): δ 0.83 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.62–1.93 (m, 6H, $3CH_2$), 2.20–2.39 (m, 3H, 3 CH), 2.81 (t, J = 7.12 Hz, 1H, CH),

3.20–3.45 (m, 10H, $5CH_2$), 4.38 (s, 2H, D_2O exchangeable, NH_2), 5.85 (s, 1H, CH=C), 6.36 (s, 1H, thiophene H-5); ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.2, 21.6 (C-18, C-19), 31.9 (C-16), 37.3 (C-15), 43.8 (C-11), 45.1 (C-12), 49.0 C-1), 50.1 (C-2), 57.2 (C-7), 71.5, 83.0, 84.5 (C-9, C-8, C-14), 120.6 (CN), 121.3 (C-4), 126.2, 128.3, 132.8, 134.5, 140.2 (thiophene C), 168.0 (C-5), 184.3 (C-3); MS: *m/e* = 394 (M^+); Anal. Calcd. for $C_{24}H_{30}N_2OS$: C, 73.06; H, 7.66; N, 7.10; S, 8.13. Found C, 72.86; H, 6.83; N, 7.31; S, 7.84.

*Ethyl 2-amino-4-((8S,9S,10R,13S,14S)-10,13-dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13, 14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)thiophene-3-carboxylate (**4b**)* HPLC purity = 94 % (C-18 NovaPak column; MeCN:MeOH:H_2O/60:30:10) t_r = 22 min; white crystals from ethanol (78 %), m.p. 122–124 °C; IR (KBr) cm^{-1}: 3488, 3422 (NH_2), 3022 (thiophene CH), 2936, 2877 (CH_3, CH_2), 1684, 1689 (2 CO), 1638 (C=C); ^1H NMR ($CDCl_3$): δ 0.82 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.14 (t, 3H, J = 7.58 Hz, ester CH_3), 1.60–1.93 (m, 6H, $3CH_2$), 2.21–2.39 (m, 3H, 3 CH), 2.80 (t, J = 7.25 Hz, 1H, CH), 3.22–3.50 (m, 10H, $5CH_2$), 4.21 (q, J = 7.58 Hz, 2H, ester CH_2), 4.58 (s, 2H, D_2O exchangeable, NH_2), 5.84 (s, 1H, CH=C), 6.36 (s, 1H, thiophene H-5), ^{13}C NMR (500 MHz, $CDCl_3$): δ 14.8 (ester CH_3), 13.3, 21.6 (C-18, C-19), 31.9 (C-16), 37.9 (C-15), 43.4 (C-11), 45.1 (C-12), 49.4 (C-1), 49.7 (C-6), 50.4 (C-2), 58.5 (C-7), 71.5, 83.2, 84.2 (C-9, C-8, C-14), 60.4 (ester CH_2), 121.8 (C-4), 126.8, 128.6, 130.8, 135.4 (thiophene C), 168.2 (C-5), 178.3, 187.2 (ester CO, C-3); MS: *m/e* = 441 (M^+); Anal. Calcd. for $C_{26}H_{35}NO_3S$: C, 70.71; H, 7.99; N, 3.17; S, 7.26. Found C, 70.52; H, 8.31; N, 2.93; S, 7.06.

*Synthetic procedure of the pyrazole derivatives **6a–d***

To a solution of either **3a** (0.362 g, 1 mmol) or **3b** (0.409 g, 1 mmol) in 1,4-dioxane (30 ml) either hydrazine hydrate or phenyl hydrazine was added. The reaction mixture was heated under reflux for 2 h, then poured onto an ice-water mixture containing a few drops of concentrated hydrochloric acid. The formed solid product was collected by filtration and crystallized from the appropriate solvent.

*(8S,9S,10R,13S,14S)-17-(1-(3,5-diamino-4H-pyrazol-4-yl) ethyl)-10,13-dimethyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3(2H)-one (**6a**)* HPLC purity = 94 % (C-18 NovaPak column; MeOH: H_2O/65: 35), t_r = 24 min; pale yellow crystals from ethanol (88 %), m.p. 180–183 °C; IR (KBr) cm^{-1}: 3455–3421 ($2NH_2$), 2938 (CH_2), 1687 (CO), 1642 (C=N), 1622 (C=C); ^1H NMR (DMSO-d_6): δ 0.80 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 2.66 (s,3H,CH_3), 1.60–1.91 (m, 6H, $3CH_2$), 2.22–2.39 (m, 3H, 3CH), 2.83 (t, J = 7.12 Hz, 1H, CH), 3.01–3.49 (m, 10H, $5CH_2$), 4.42, 4.81 (2 s, 4H, D_2O exchangeable, $2NH_2$), 5.86 (s, 1H, CH=C); ^{13}C NMR (DMSO-d_6) δ 13.0, 21.4 (C-18, C-19), 23.3 (ethyl CH_3), 31.9 (C-16), 37.3 C-15), 43.8 (C-11), 45.0 (C-12), 49.0 (C-1), 49.6 (C-6), 50.2 (C-2), 56.5 (C-7), 71.8, 83.0, 84.4 (C-9, C-8, C-14), 121.7 (C-4), 102.8, 106.7 (ylidene C=C), 150.8, 153.2, 158. 2 (pyrazole C), 168.2 (C-5), 189.6 (C-3); MS: *m/e* = 396 (M^+); Anal. Calcd. for $C_{24}H_{34}N_4O$: C, 73.06; H, 8.69; N, 14.20. Found C, 72.96; H, 8.91; N, 14.39.

*(8S,9S,10R,13S,14S)-17-((E)-1-(3-amino-5-hydroxy-4H-pyrazol-4-yl)ethyl)-10,13-dimethyl-6,7,8,9,10,11,12,13, 14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3 (2H)-one (**6b**)* HPLC purity = 90 % (C-18 NovaPak column; MeOH:H_2O/80:20), t_r = 26 min; yellow crystals from ethanol (73 %), m.p. 166–168 °C; IR (KBr) cm^{-1}: 3477–3337 (OH, NH_2), 2934, 2894 (CH_3, CH_2), 1688 (CO), 1640 (C=N); ^1H NMR (DMSO-d_6): δ 0.81 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 1.63–1.87 (m, 6H, $3CH_2$), 2.20–2.39 (m, 3H, 3CH), 2.68 (s, 3H,CH_3), 2.80 (t, J = 7.02 Hz, 1H, CH), 3.10–3.46 (m, 10H, $5CH_2$), 4.43 (s, 2H, D_2O exchangeable, NH_2), 5.84 (s, 1H, CH=C), 10.05 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.3, 21.6 (C-18, C-19), 23.3 (ethyl CH_3), 31.9 (C-16), 37.3 (C-15), 43.8 (C-11), 45.0 (C-12), 49.3 (C-1), 49.6 (C-6), 50.3 (C-2), 57.9 (C-7), 71.5, 83.0, 84.6 (C-9, C-8, C-14), 103.0, 106.5 (C=C), 121.7 (C-4), 130.6, 144.2, 153. 6 (pyrazole C), 168.0 (C-5), 189.8 (C-3); MS: *m/e* = 395 (M^+); Anal. Calcd. for $C_{24}H_{33}N_3O_2$: C, 72.88; H, 8.41; N, 10.62. Found C, 72.68; H, 8.22; N, 10.53.

*(8S,9S,10R,13S,14S)-17-((E)-1-(3,5-diamino-1-phenyl-1H-pyrazol-4(5H)-yl)ethyl)-10,13-dimethyl-6,7,8,9,10,11,12,13, 14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3 (2H)-one (**6c**)* HPLC purity = 96 % (C-18 NovaPak column; MeCN:MeOH:H_2O/70:20:10), t_r = 23 min; yellow crystals from ethanol (70 %), m.p. 152–154 °C; IR (KBr) cm^{-1}: 3582, 3420 ($2NH_2$), 2930,1896 (CH_3, CH_2), 1686 (CO), 1673 (C=N), 1636 (C=C); ^1H NMR (DMSO-d_6): δ 0.82 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.60–1.87 (m, 6H, $3CH_2$), 2.20–2.36 (m, 3H, 3CH), 2.67 (s, 3H,CH_3), 2.82 (t, J = 6.12 Hz, 1H, CH), 3.10–3.46 (m, 10H, $5CH_2$), 3.54 (m, 1H, CH), 4.42, 5.21 (2 s, 4H, D_2O exchangeable, $2NH_2$), 5.86 (s, 1H, CH=C), 7.32–7.44 (m, 5H, C_6H_5). ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.2, 21.8 (C-18, C-19), 23.3 (ethyl CH_3), 31.6 (C-16), 37.4 (C-15), 44.0 (C-11), 44.9 (C-12), 49.3 (C-1), 49.6 (C-6), 50.3 (C-2), 57.2 (C-7), 71.5, 83.2, 84.8 (C-9, C-8, C-14), 121.6 (C-4), 124.3, 126.8, 128.3, 138.6 (C_6H_5), 156.3, 158.9 (pyrazole C), 168.2 (C-5), 177.7 (exocyclic C=N), 189.6 (C-3); MS: *m/e* = 472 (M^+); Anal. Calcd. for $C_{30}H_{38}N_4O$: C, 76.56; H, 8.14; N, 11.90. Found C, 76.52; H, 8.42; N, 12.15.

*(8S,9S,10R,13S,14S)-17-(1-(5-amino-3-hydroxy-1-phenyl-1H-pyrazol-4-yl)ethyl)-10,13-dimethyl-6,7,8,9,10,11,12,13, 14,15,16,17-dodecahydro-1H-cyclopenta[a]-phenanthren-3(2H)-one (**6d**)* HPLC purity = 92 % (C-18 NovaPak column; MeCN:MeOH:H_2O/55:35:10), t_r = 20 min; yellow crystals from ethanol (66 %), m.p. 230–233 °C; IR (KBr) cm^{-1}: 3540, 3433 (OH, NH_2), 2928, 2889 (CH_3, CH_2), 1688 (CO), 1633 (C=N); ^{1}H NMR (DMSO-d_6): δ 0. 81 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.62–1.87 (m, 6H, 4.44 (s, 2H, CH_2), 2.68 (s, 3H, CH_3), 3.10–3.48 (m, 10H, 5CH_2), 4.48 (s, 2H, NH_2), 5.86 (s, 1H, CH=C), 7.28–7.38 (m,5H, C_6H_5), 8.22 (s, 1H, OH); ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.1, 21.8 (C-18, C-19), 23.4 (ethyl CH_3), 31.6 (C-16), 37.6 (C-15), 43.9 (C-11), 45.0 (C-12), 49.3 (C-1), 49.6 (C-6), 50.6 (C-2), 57.2 (C-7), 71.5, 83.2, 84.8 (C-9, C-8, C-14), 121.8 (C-4), 129.6, 130.4, 138.6, 143.8 (C_6H_5), 150.8, 168.4 (C-5), 190.2 (C-3); MS: *m/e* = 473 (M^+); Anal. Calcd. for $C_{30}H_{37}N_3O_2$: C, 76.40; H, 7.91; N, 8.91. Found C, 76.26; H, 8.07; N, 9.04.

*(8S,9S,10R,13S,14S,17S)-17-(2-bromoacetyl)-10,13-dimethyl-6,7,8,9,10,11,12,13,14, 15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3(2H)-one (**7**)*

To a solution of progesterone (**1**) (0.362 g, 1 mmol) in acetic acid (10 ml) at 60 °C, bromine (0.050 g, 1 mmol) was added drop wise and stirred at room temperature for 1 h. The solvent was then evaporated under vacuum, and the remaining product triturated with diethyl ether and collected by filtration.

HPLC purity = 92 % (C-18 NovaPak column; MeOH:H_2O/90:10), t_r = 22 min; pale yellow crystals from ethanol (73 %), m.p. 66 °C; IR (KBr) cm^{-1}: 2923 (CH_2), 1689, 1684 (2CO) 1638 (C=C); ^{1}H NMR (DMSO-d_6): δ 0.81 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.62–1.87 (m, 6H, 3CH_2), 2.20–2.36 (m, 3H, 3CH), 2.82 (t, J = 7.23 Hz, 1H, CH), 3.53 (m, 1H, CH), 3.13–3.51 (m, 10H, 5CH_2), 3.88 (s, 2H, CH_2), 5.82 (s, 1H, CH=C); ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.0, 21.8 (C-18, C-19), 31.8 (C-16), 38.0 (C-15), 37.6 (C-15), 44.4 (C-11), 44.9 C-12), 49.4 (C-1), 49.6 (C-6), 50.6 (C-2), 57.0 (C-7), 66.8 ($\underline{CH_2}$Br), 71.6, 77.2, 83.2, 84.5 (C-9, C-8, C-14, C-17), 121.6 (C-4), 168.2 (C-5), 177.8, 190.0 (acetyl CO, C-3); MS: *m/e* = 392 (M^+); Anal. Calcd. for $C_{21}H_{29}BrO_2$: C, 64.12; H, 7.43; Br, 20.31. Found C, 64.37; H, 7.68; Br, 20.63.

*3-((8S,9S,10R,13S,14S,17S)-10,13-dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14, 15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)-3-oxopropanenitrile (**8**)*

A solution of compound **7** (0.392, 1 mmol) in ethanol (30 ml) was heated in a boiling water bath to which a concentrated solution of potassium cyanide (0.064 g, 1 mmol) in water (1 ml) was added. The whole reaction mixture was stirred at 60 °C for 2 h then poured onto an ice-water mixture containing three drops of concentrated hydrochloric acid, and the formed solid product was collected by filtration.

HPLC purity = 93 % (C-18 NovaPak column; MeOH:H_2O/80:10), t_r = 24 min; pale yellow crystals from ethanol (58 %), m.p. 140–142 °C; IR (KBr) cm^{-1}: 2926 (CH_2), 2224 (CN), 1687, 1682 (2 CO), 1641 (C=C); ^{1}H NMR (DMSO-d_6): δ 0.80 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.60–1.89 (m, 6H, 3CH_2), 2.21–2.34 (m, 3H, 3CH), 2.80 (t, J = 6.52 Hz, 1H, CH), 3.10–3.48 (m, 10H, 5CH_2), 3.52 (m, 1H, CH), 4.45 (s, 2H, CH_2), 5.85 (s, 1H, CH=C); ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.0, 21.6 (C-18, C-19), 31.4 (C-16), 37.6 (C-15), 43.5 (C-11), 44.9 (C-12), 49.4 (C-1), 50.8 (C-2), 57.0 (C-7), 63.8 ($\underline{CH_2}$CN), 71.6, 77.2, 83.2, 84.5 (C-9, C-8, C-14, C-17), 119.1 (CN), 121.8 (C-4), 168.0 (C-5), 177.6, 189.4 (acetyl CO, C-3); MS: *m/e* = 339 (M^+); Anal. Calcd. for $C_{22}H_{29}NO_2$: C, 77.84; H, 8.61; N, 4.13. Found C, 78.04; H, 8.41; N, 4.42.

*Synthesis of the arylhydrazo derivatives **10a–c***

To a solution of compound **8** (0.339, 1 mmol) in ethanol (30 ml) containing sodium acetate (2.5 g), either benzenediazonium chloride (0.01 mol), 4-chlorobenzenediazonium chloride (1 mmol) or 4-methylbenzenediazonium chloride (1 mmol) [prepared by adding sodium nitrite solution (0.007 g, 1 mmol) to a cold solution of the appropriate aniline or its derivative (1 mmol) in concentrated hydrochloric acid (3 ml, 18 M) with continuous stirring] was added with stirring. The reaction mixture was kept at room temperature for 1 h and the formed solid product, in each case, was collected by filtration.

*2-((8S,9S,10R,13S,14S,17S)-10,13-dimethyl-3-oxo-2,3,6,7, 8,9,10,11,12,13,14,15,16, 17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)-2-oxo-N′-phenylacetohydrazonoyl cyanide (**10a**)* HPLC purity = 96 % (C-18 NovaPak column; CH_3CN:MeOH:H_2O/35:55:10), t_r = 22 min; Orange crystals from ethanol (71 %), m.p. 190–193 °C; IR (KBr) cm^{-1}: 3463–3321 (NH), 3055 (CH aromatic), 2928 (CH_2), 2220 (CN), 1710, 1686 (2CO), 1650 (C=N), 1636 (C=C); ^{1}H NMR (DMSO-d_6): δ 0.81 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.60–1.88 (m, 6H, 3CH_2), 2.21–2.36 (m, 3H, 3CH), 2.82 (t, J = 7.42 Hz, 1H, CH), 3.10–3.46 (m, 10H, 5CH_2), 3.54 (m, 1H, CH), 5.88 (s, 1H, CH=C), 7.30–7.38 (m, 5H, C_6H_5), 8.42 (s, 1H, D_2O exchangeable, NH): ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.0, 21.6 (C-18, C-19), 31.4 (C-16), 37.6 (C-15), 44.7 (C-11), 44.6 (C-12), 49.8 (C-1), 49.9 (C-6), 50.8 (C-2), 57.2 (C-7), 71.8, 77.4, 83.0, 84.7 (C-9, C-8, C-14, C-17), 119.8 (CN), 121.7 (C-4), 120.8, 124.3, 126.3, 127.6 (C_6H_5), 158.3 (C=N), 168.2 (C-5),

177.7, 189.8 (acetyl CO, C-3); MS: *m/e* = 443 (M^+); Anal. Calcd. for $C_{28}H_{33}N_3O_2$: C, 75.81; H, 7.50; N, 9.47. Found C, 75.64; H, 7.74; N, 9.21.

*N′-(4-Chlorophenyl)-2-((8S,9S,10R,13S,14S,17S)-10,13-dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)-2-oxoaceto hydrazonoyl cyanide (**10b**)* HPLC purity = 90 % (C-18 NovaPak column; CH_3CN:MeOH:H_2O/40:50:10), t_r = 24 min; Orange crystals from ethanol (66 %), m.p. 120–123 °C; IR (KBr) cm^{-1}: 3472–3329 (NH), 3058 (CH aromatic), 2932 (CH_2), 2224 (CN), 1705, 1684 (2CO), 1638 (C=C); ^{1}H NMR (DMSO-d_6): δ 0.80 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.60–1.88 (m, 6H, $3CH_2$), 2.23–2.37 (m, 3H, 3CH), 2.80 (t, J = 7.42 Hz, 1H, CH), 3.12–3.49 (m, 10H, $5CH_2$), 3.56 (m, 1H, CH), 5.86 (s, 1H, CH=C), 7.26–7.36 (m, 4H, C_6H_4), 8.40 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.3, 21.6 (C-18, C-19), 31.4 (C-16), 37.6 (C-15), 43.7 (C-11), 44.5 (C-12), 49.2 (C-1), 49.8 (C-6), 50.3 (C-2), 57.0 (C-7), 71.6, 77.7, 83.2, 84.9 (C-9, C-8, C-14, C-17), 119.4 (CN), 121.5 (C-4), 122.6, 124.3, 133.6, 134.8 (C_6H_4), 158.6 (C=N), 168.4 (C-5), 177.6, 189.6 (acetyl CO, C-3); MS: *m/e* = 477 (M^+); Anal. Calcd. for $C_{28}H_{32}ClN_3O_2$: C, 70.35; H, 6.75; N, 8.79. Found C, 70.46; H, 6.55; N, 8.86.

*N′-(4-methylphenyl)-2-((8S,9S,10R,13S,14S,17S)-10,13-dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)-2-oxoaceto hydrazonoyl cyanide (**10c**)* HPLC purity = 93 % (C-18 NovaPak column; MeOH:H_2O/80:20), t_r = 18 min; Orange crystals from ethanol (80 %), m.p. 144–146 °C; IR (KBr) cm^{-1}: 3465–3320 (NH), 3060 (CH aromatic), 2932 (CH_2), 2223 (CN), 1698, 1686 (2CO), 1660 (C=N); ^{1}H NMR (DMSO-d_6): δ 0.80 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.60–1.88 (m, 6H, $3CH_2$), 2.23–2.37 (m, 3H, 3CH), 2.80 (t, J = 7.42 Hz, 1H, CH), 2.89 (s, 3H, CH_3), 3.10–3.46 (m, 10H, $5CH_2$), 5.86 (s, 1H, CH=C), 7.28–7.44 (m, 4H, C_6H_4), 8.37 (s, 1H, D_2O exchangeable, NH): ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.0, 21.4, 24.3 (C-18, C-19, 4-$\underline{CH_3}$-phenyl), 31.4 (C-16), 37.6 (C-15), 43.8 (C-11), 44.8 (C-12), 49.3 (C-1), 49.4 (C-6), 50.7 (C-2), 57.5 (C-7), 71.8, 77.5, 83.0, 84.5 (C-9, C-, C-14, C-17), 119.8 (CN), 121.7 (C-4), 124.3, 126.3, 133.6, 134.8 (C_6H_4), 168.6 (C-5), 173.5 (C=N), 177.7, 189.4 (acetyl CO, C-3); MS: *m/e* = 457 (M^+); Anal. Calcd. for $C_{29}H_{35}N_3O_2$: C, 76.12; H, 7.71; N, 9.18. Found C, 76.07; H, 7.59; N, 9.33.

*Synthesis of the pyrazole derivatives **11a**, **b***

To a solution of compound **8** (0.339, 1 mmol) in ethanol (30 ml), either hydrazine hydrate (0.050 g, 1 mmol) or phenyl hydrazine (0.108 g, 1 mmol) was added. The reaction mixture was heated under reflux for 4 h, poured onto an ice-water mixture containing one drop of concentrated hydrochloric acid, and the formed solid product was collected by filtration.

*(8S,9S,10R,13S,14S,17S)-17-(5-amino-1H-pyrazol-3-yl)-10,13-dimethyl-6,7,8,9, 10,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3(2H)-one (**11a**)* HPLC purity = 91 % (C-18 NovaPak column; MeOH:H_2O/90:10), t_r = 20 min; yellow crystals from ethanol (68 %), m.p. 120–122 °C; IR (KBr) cm^{-1}: 3471–3313 (NH_2, NH), 3052 (CH pyrazole), 2930 (CH_2), 1684 (CO), 1636 (C=C); ^{1}H NMR (DMSO-d_6): δ 0.80 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.61–1.86 (m, 6H, $3CH_2$), 2.20–2.37 (m, 3H, 3CH), 2.81 (t, J = 7.42 Hz, 1H, CH), 3.11–3.49 (m, 10H, $5CH_2$), 3.53 (m, 1H, CH), 4.82 (s, 2H, D_2O exchangeable, NH_2), 5.82 (s, 1H, C-4), 6.53 (s, 1H, pyrazole H-3), 8.22 (s, 1H, D_2O exchangeable, NH): ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.1, 21.4 (C-18, C-19), 31.5 (C-16), 37.6 (C-15), 43.8 (C-11), 44.8 (C-12), 49.2 (C-1), 49.8 (C-6), 50.7 (C-2), 57.0 (C-7), 71.5, 77.7, 83.3, 84.5 (C-9, C-8, C-14, C-17), 119.2 (CN), 114.6, 152.4, 156.8 (pyrazole C), 168.8 (C-5), 190.0 (C-3); MS: *m/e* = 353 (M^+); Anal. Calcd. for $C_{22}H_{31}N_3O$: C, 74.75; H, 8.84; N, 11.89. Found C, 74.84; H, 9.14; N, 12.09.

*(8S,9S,10R,13S,14S,17S)-17-(5-amino-1-phenyl-1H-pyrazol-3-yl)-10,13-dimethyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3(2H)-one (**11b**)* HPLC purity = 93 % (C-18 NovaPak column; CH_3CN/MeOH:H_2O/20:70:10), t_r = 18 min; yellow crystals from ethanol (73 %), m.p. 180–184 °C; IR (KBr) cm^{-1}: 3477, 3323 (NH_2), 3044 (CH pyrazole), 2928 (CH_2), 1638 (C=C); ^{1}H NMR (DMSO-d_6): δ 0.82 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.61–1.86 (m, 6H, $3CH_2$), 2.20–2.37 (m, 3H, 3CH), 2.83 (t, J = 7.89 Hz, 1H, CH), 3.09–3.49 (m, 10H, $5CH_2$), 4.82 (s, 2H, D_2O exchangeable, NH_2), 5.82 (s, 1H, CH=C), 6.53 (s, 1H, pyrazole H-3), 7.29–7.39 (m, 5H, C_6H_5); ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.0, 21.4 (C-18, C-19), 31.5 (C-16), 37.6 (C-15), 43.8 (C-11), 44.9 (C-12), 49.4 (C-1), 49.8 (C-6), 50.7 (C-2), 57.2 (C-7), 71.8, 77.7, 83.5, 84.6 (C-9, C-8, C-14, C-17), 121.3 (C-4), 124.9, 133.6, 135.6 (C_6H_5), 142.3, 153.0, 156.2 (pyrazole C), 168.8 (C-5), 190.0 (C-3); MS: *m/e* = 429 (M^+); Anal. Calcd. for $C_{28}H_{35}N_3O$: C, 78.28; H, 8.21; N, 9.78. Found C, 78.52; H, 8.04; N, 9.53.

*Synthesis of the arylidene derivatives **13a–c***

To a solution of compound **8** (0.339 g, 1 mmol) in 1,4-dioxane (10 ml) containing piperidine (0.250 ml), either benzaldehyde (0.106 g, 1 mmol), 4-chlorobenzaldehyde (0.140 g, 1 mmol) or 4-methoxybenzaldehyde (0.136 g, 1 mmol) was added. The whole reaction mixture was

heated under reflux for 2 h, evaporated under vacuum, and the remaining product triturated with ethanol and collected by filtration.

*2-((8S,9S,10R,13S,14S,17S)-10,13-dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14, 15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-17-carbonyl)-3-phenylacrylonitrile (**13a**)* HPLC purity = 96 % (C-18 NovaPak column; MeOH:H_2O/80:20), t_r = 20 min; yellow crystals from ethanol (80 %), m.p. 166–168 °C; IR (KBr) cm^{-1}: 3064 (CH aromatic), 2927 (CH_2), 2220 (CN), 1687, 1683 (2CO), 1636 (C=C); ^{1}H NMR (DMSO-d_6): δ 0.80 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.60–1.88 (m, 6H, 3CH_2), 2.20–2.38 (m, 3H, 3CH), 2.82 (t, J = 7.26 Hz, 1H, CH), 3.09–3.49 (m, 10H, 5CH_2), 5.82 (s, 1H, CH=C), 6.01 (s, 1H, ylidene CH), 7.26–7.39 (s, 5H, C_6H_5); ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.0, 21.4 (C-18, C-19), 31.5 (C-16), 37.6 (C-15), 43.8 (C-11), 49.4 (C-1), 49.9 (C-6), 50.7 (C-2), 57.2 (C-7), 71.8, 77.7, 83.5, 84.6 (C-9, C-8, C-14, C-17), 121.4 (C-4), 116.4 (ylidene CH), 119.8 (CN), 124.9, 127.9, 134.2, 135.6 (C_6H_5), 152.2 (ylidene C), 168.4 (C-5), 166.9, 189.6 (acetyl CO, C-3); MS: *m/e* = 427 (M^+); Anal. Calcd. for $C_{29}H_{33}NO_2$: C, 81.46; H, 7.78; N, 3.28. Found C, 81.72; H, 7.94; N, 3.52.

*3-(4-Chlorophenyl)-2-((8S,9S,10R,13S,14S,17S)-10,13-dimethyl-3-oxo-2,3,6,7,8,9, 10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-17-carbonyl)acrylonitrile (**13b**)* HPLC purity = 94 % (C-18 NovaPak column; CH_3CN:MeOH:H_2O/20:60:20), t_r = 22 min; yellow crystals from ethanol (63 %), m.p. 80 °C; IR (KBr) cm^{-1}: 3052 (CH aromatic), 2927 (CH_2), 2222 (CN), 1688, 1684 (2CO), 1636 (C=C): ^{1}H NMR (DMSO-d_6): δ 0.80 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.60–1.88 (m, 6H, 3CH_2), 2.20–2.38 (m, 3H, 3CH), 2.82 (t, J = 7.83 Hz, 1H, CH), 3.03–3.51 (m, 10H, 5CH_2), 5.83 (s, 1H, CH=C), 6.01 (s, 1H, ylidene CH), 7.30–7.38 (2d, 4H, J = 4.66 Hz, C_6H_4); ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.0, 21.4 (C-18, C-19), 31.5 (C-16), 37.6 (C-15), 43.8 (C-11), 44.4 (C-12), 49.3 (C-1), 49.8 (C-6), 50.7 (C-2), 57.3 (C-7), 71.8, 77.9, 83.6, 84.9 (C-9, C-8, C-14, C17), 106.8 (ylidine CH), 119.0 (CN), 121.6 (C-4), 124.9, 126.5, 127.9, 134.2, 141.8 (C_6H_4), 151.6 (ylidene C), 168.2 (C-5), 166.3, 189.8 (acetyl CO, C-3); MS: *m/e* = 461 (M^+); Anal. Calcd. for $C_{29}H_{32}ClNO_2$: C, 75.39; H, 6.98; Cl, 7.67; N, 3.03. Found C, 75.60; H, 7.25; Cl, 7.92; N, 3.27.

*2-((8S,9S,10R,13S,14S,17S)-10,13-dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14, 15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-17-carbonyl)-3-(4-methoxyphenyl) acrylonitrile (**13c**)* HPLC purity = 90 % (C-18 NovaPak column; CH_3CN:MeOH:H_2O/20:60:20), t_r = 20 min; yellow crystals from ethanol (68 %), m.p. 130–133 °C; IR (KBr) cm^{-1}: 3058 (CH aromatic), 2927 (CH aromatic), 2220 (CN), 1687, 1686 (2CO), 1638 (C=C); ^{1}H NMR (DMSO-d_6): δ 0.80 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.60–1.88 (m, 6H, 3CH_2), 2.20–2.36 (m, 3H, 3CH), 2.81 (t, J = 6.22 Hz, 1H, CH), 3.02 (s, 3H, OCH_3), 3.12–3.52 (m, 10H, 5CH_2), 5.80 (s, 1H, CH=C), 6.01 (s, 1H, ylidene CH), 7.31–7.42 (m, 4H, C_6H_4); ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.0, 21.6, (C-18, C-19) 28.6 (OCH_3), 31.5 (C-16), 37.6 (C-15), 43.6 (C-11), 44.9 (C-12), 49.4 (C-1), 49.8 (C-6), 50.7 (C-2), 57.3 (C-7), 71.6, 77.9, 83.3, 84.8 (C-9, C-8, C-14, C-17), 106.9 (ylidene CH), 119.2 (CN), 122.6 (C-4), 124.9, 126.5, 134.2, 138.8 (C_6H_4), 149.3 (ylidene C), 168.2 (C-5), 166.4, 189.9 (acetyl CO, C-3); MS: *m/e* = 457 (M^+); Anal. Calcd. for $C_{30}H_{35}NO_3$: C, 78.74; H, 7.71; N, 3.06. Found C, 78.82; H, 7.41; N, 3.32.

*Synthesis of the pyran derivatives **15a–f***

To a solution of **13a** (0.427 g, 1 mmol), **13b** (0.461 g, 1 mmol) or **13c** (0.427 g, 1 mmol) in 1,4-dioxane (20 ml) containing triethylamine (0.50 ml), **2a** (0.066 g, 1 mmol) or **2b** (0.107 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h, poured into an ice-water mixture, and the formed solid product collected by filtration.

*2-Amino-6-((8S,9S,10R,13S,14S)-10,13-dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14, 15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)-4-phenyl-4H-pyran-3,5-dicarbonitrile (**15a**)* HPLC purity = 93 % (C-18 NovaPak column; CH_3CN:MeOH:H_2O/30:60:10), t_r = 15 min; yellow crystals from ethanol (73 %), m.p. 192–194 °C; IR (KBr) cm^{-1}: 3482, 3336 NH_2), 3061 (CH aromatic), 2922 (CH_2), 2222, 2218 (2CN), 1688 (CO), 1641 (C=C); ^{1}H NMR (DMSO-d_6): δ 0.80 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.60–1.86 (m, 6H, 3CH_2), 2.20–2.36 (m, 3H, 3CH), 2.80 (t, J = 7.23 Hz, 1H, CH), 3.12–3.50 (m, 10H, 5CH_2), 4.62 (s, 2H, D_2O exchangeable, NH_2), 5.80 (s, 1H, CH=C), 6.53 (s, 1H, pyran H-4), 7.28–7.38 (m, 5H, C_6H_5); ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.0, 21.8 (C-18, C-19), 31.5 (C-16), 37.3 (C-15), 43.6 (C-11), 44.9 (C-12), 49.3 (C-1), 49.8 (C-6), 50.7 (C-2) 57.2 (C-7), 71.8, 77.3, 83.3, 84.9 (C-9, C-8, C-14, C-17)), 116.8, 117.3 (2CN), 122.8 (C-4), 124.9, 126.5, 128.6, 134.8 (C_6H_5), 55.9, 106.9, 122.4, 154.9 (pyran C), 168.6 (C-5), 189.9 (C-3); MS: *m/e* = 493 (M^+); Anal. Calcd. for $C_{32}H_{35}N_3O_2$: C, 77.86; H, 7.15; N, 8.51. Found C, 77.93; H, 7.42; N, 8.37.

*2-Amino-4-(4-chlorophenyl)-6-((8S,9S,10R,13S,14S)-10,13-dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)-4H-pyran-3,5-dicarbonitrile (**15b**)* HPLC purity = 95 % (C-18 NovaPak column; MeOH:H_2O/85:15), t_r = 19 min;

yellow crystals from ethanol (82 %), m.p. 111–113 °C; IR (KBr) cm^{-1}: 3476, 3321 (NH_2), 3057 (CH aromatic), 2926 (CH_2), 2224, 2220 (2CN), 1686 (CO), 1632 (C=C); ^1H NMR (DMSO-d_6): δ 0.80 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.60–1.86 (m, 6H, $3CH_2$), 2.20–2.36 (m, 3H, 3CH), 2.80 (t, $J = 7.03$ Hz, 1H, CH), 3.12–3.50 (m, 10H, $5CH_2$), 4.62 (s, 2H, D_2O exchangeable, NH_2), 5.80 (s, 1H CH=C), 6.20 (s, 1H, pyran H-4), 7.26–7.42 (m, 4H, C_6H_4); ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.0, 21.6 (C-18, C-19), 32.5 (C-16), 37.8 (C-15), 43.6 (C-11), 44.9 (C-12), 49.2 (C-1), 49.7 (C-6), 50.7 (C-2), 57.0 (C-7), 71.2, 77.9, 83.5, 84.6 (C-9, C-8, C-14, C-17), 55.3, 106.9, 122.4, 154.2 (pyran C), 117.4, 119.1 (2CN), 122.9, 124.9, 127.5, 129.0, 138.4 (C_6H_5), 189.8 (C-3); MS: $m/e = 527$ (M^+); Anal. Calcd. for $C_{32}H_{34}ClN_3O_2$: C, 72.78; H, 6.49; Cl, 6.71; N, 7.96. Found C, 72.42; H, 6.29; C, 7.06; N, 7.66.

*2-Amino-6-((8S,9S,10R,13S,14S)-10,13-dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14, 15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)-4-(4-methoxyphenyl)-4H-pyran-3,5-dicarbonitrile (**15c**)* HPLC purity = 96 % (C-18 NovaPak column; MeOH:H_2O/90:10), $t_r = 22$ min; yellow crystals from ethanol (73 %), m.p. 190–193 °C; IR (KBr) cm^{-1}: 3445, 3315 (NH_2), 3054 (CH aromatic), 2928, 2889 (CH_3, CH_2), 2222, 2218 (2CN), 1688 (CO), 1635 (C=C); ^1H NMR (DMSO-d_6): δ 0.82 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.60–1.88 (m, 6H, $3CH_2$), 2.20–2.36 (m, 3H, 3CH), 2.81 (t, $J = 6.95$ Hz, 1H, CH), 3.04 (s, 3H, CH_3), 3.12–3.52 (m, 10H, $5CH_2$), 4.62 (s, 2H, D_2O exchangeable, NH_2), 5.83 (s, 1H, CH=C), 6.23 (s, 1H, pyran H-4), 7.28–7.40 (m, 4H, C_6H_4); ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.0, 21.6 (C-18, C-19), 57.0 (OCH_3), 31.5 (C-16), 37.8 (C-15), 43.6 (C-11), 44.9 (C-12), 49.3 (C-1), 49.8 (C-6), 50.7 (C-2), 57.3 (C-7) 71.0, 77.5, 83.5, 84.7 (C-9, C-8, C-14, C-17), 117.5, 119.0 (2CN), 55.0, 106.9, 122.6, 150.3 (pyran C), 122.9 (C-4), 124.9, 126.3, 128.8, 136.8 (C_6H_4), 168.7 (C-5), 189.6 (C-3); MS: $m/e = 523$ (M^+); Anal. Calcd. for $C_{33}H_{37}N_3O_3$: C, 75.69; H, 7.12; N, 8.02. Found C, 75.83; H, 7.36; N, 7.99.

*Ethyl 2-amino-5-cyano-6-((8S,9S,10R,13S,14S)-10,13-dimethyl-3-oxo-2,3,6,7,8,9, 10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)-4-phenyl-4H-pyran-3-carboxylate (**15d**)* HPLC purity = 92 % (C-18 NovaPak column; MeOH:H_2O/90:10), $t_r = 22$ min; yellow crystals from ethanol (78 %), m.p. 222–224 °C; IR (KBr) cm^{-1}: 3458, 3322 (NH_2), 3058 (CH aromatic), 2926, 28886 (CH_3, CH_2), 2228, 2220 (CN), 1684 (CO), 1632 (C–C); ^1H NMR (DMSO-d_6): δ 0.80 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.12 (t, $J = 7.55$ Hz, 3H, 3CH), 1.60–1.88 (m, 6H. $3CH_2$), 2.20–2.36 (m, 3H, 3CH), 2.83 (t, $J = 7.29$ Hz, 1H, CH), 3.04 (s, 3H, CH_3), 3.12–3.48 (m, 10H, $5CH_2$), 4.22 (q, $J = 7.29$ Hz, 2H, CH_2), 4.62 (s, 2H, D_2O exchangeable, NH_2), 5.84 (s, 1H, CH=C), 6.28 (s, 1H, pyran H-4), 7.30–7.44 (m, 5H, C_6H_5); ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.1, 21.6, (C-18, C-19), 16.2 (ester CH_3), 32.5 (C-16, 37.8 (C-15), 43.6 (C-11), 44.9 (C-12), 49.8 (C-1), 50.7 (C-6), 57.2 (C-7), 60.3 (ester CH_2), 71.0, 77.7, 83.5, 84.9 (C-9, C-8, C-14, C-17), 116.9, 119.0 (2CN), 55.2, 106.9, 122.6, 150.4 (pyran C), 122.3 (C-4), 124.4, 126.3, 129.3, 134.2, 139.2, 168.3 (C-5) 172.3, 189.8 (ester CO, C-3); MS: $m/e = 540$ (M^+); Anal. Calcd. for $C_{34}H_{40}N_2O_4$: C, 75.53; H, 7.46; N, 5.18. Found C, 75.69; H, 7.21; N, 4.83.

*Ethyl 2-amino-4-(4-chlorophenyl)-5-cyano-6-((8S,9S,10R,13S,14S)-10,13-dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)-4H-pyran-3-carboxylate (**15e**)* HPLC purity = 95 % (C-18 NovaPak column; MeOH:H_2O/85:15), $t_r = 19$ min; yellow crystals from ethanol (72 %), m.p. 172–175 °C; IR (KBr) cm^{-1}: 3473, 3342 (NH_2), 3060 (CH aromatic), 2929, 2897 (CH_3, CH_2), 2222 (CN), 1689, 1686 (2CO), 1634 (C=C); ^1H NMR (DMSO-d_6): δ 0.80 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.13 (t, $J = 7.29$ Hz, 3H, ester CH_3), 1.60–1.88 (m, 6H, $3CH_2$), 2.20–2.36 (m, 3H, 3CH), 2.83 (t, $J = 7.29$ Hz, 1H, CH), 3.12–3.50 (m, 10H, $5CH_2$), 4.19 (q, $J = 7.29$ Hz, 2H, ester CH_2), 4.58 (s, 2H, D_2O exchangeable NH_2), 5.86 (s, 1H, CH=C), 6.25 (s, 1H, pyran H-4), 7.23-7.42 (2d, 4H, C_6H_4); ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.0, 21.6 (C-18, C-19), 16.9 (ester CH_3), 31.5 (C-16), 37.8 (C-15), 43.6 (C-11), 44.9 (C-12), 49.8 (C-1), 49.7 (C-6), 50.7 (C-2), 57.4 (C-7), 71.4, 77.8, 83.5, 84.6 (C-9, C-8, C-14, C-17), 57.0 (ester CH_2), 116.8, 119.0 (2 CN), 55.6, 106.9, 122.2, 150.4 (pyran C), 122.6, 125.9, 126.3, 130.6 (C_6H_4), 168.3 (C-5), 172.0, 189.6 (ester CO, C-3); MS: $m/e = 574$ (M^+); Anal. Calcd. for $C_{34}H_{39}ClN_2O_4$: C, 71.00; H, 6.83; Cl, 6.16; N, 4.87. Found C, 70.83; H, 6.62; Cl, 5.93; N, 5.26.

*Ethyl 2-amino-4-(4-methoxyphenyl)-5-cyano-6-((8S,9S,10R,13S,14S)-10,13-dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)-4H-pyran-3-carboxylate (**15f**)* HPLC purity = 95 % (C-18 NovaPak column; MeOH:H_2O/85:15), $t_r = 22$ min; yellow crystals from ethanol (67 %), m.p. 113–115 °C; IR (KBr) cm^{-1}: 3469, 3322 (NH_2), 3054 (CH aromatic), 2945, 2922 (CH_3, CH_2), 2224 (CN), 1688, 1687 (2CO), 1638 (C=C); ^1H NMR (DMSO-d_6): δ 0.80 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.11 (t, $J = 7.22$ Hz, 3H, ester CH_3), 1.60–1.88 (m, 6H, $3CH_2$), 2.20–2.36 (m, 3H, 3CH), 2.83 (t, $J = 7.29$ Hz, 1H, CH), 3.04 (s, 3H, OCH_3), 3.09–3.53 (m, 10H, $5CH_2$), 4.19 (q, $J = 7.22$ Hz, 2H, ester CH_2), 4.61 (s, 2H, D_2O exchangeable, NH_2), 5.87 (s, 1H, CH=C), 6.28 (s, 1H, pyran H-4), 7.25–7.39 (m, 4H, C_6H_4); ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.0, 21.6 (C-1, C-19), 16.7 (ester CH_3), 57.7 (OCH_3), 31.5 (C-16), 37.8 (C-15), 43.6

(C-11), 44.9 (C-12), 49.2 (C-1), 49.8 (C-6), 50.7 (C-2), 57.4 (C-7), 71.6, 77.8, 83.0, 84.3 (C-9, C-, C-14, C-17), 57. 4 (ester CH_2), 116.9 (CN), 119.0 (C-4),), 55.9, 106.3, 122.2, 150.8 (pyran C), 123.8, 125.9, 132.6, 134.2 (C_6H_4), 168.3 (C-5), 172.1, 190.2 (ester CO, C-3); MS: $m/e = 570$ (M^+); Anal. Calcd. for $C_{35}H_{42}N_2O_5$: C, 73.66; H, 7.42; N, 4.91. Found C, 73.81; H, 7.36; N, 5.19.

Conclusion

The objective of the present study was to synthesize a series of heterocyclic derivatives of progesterone. The antibacterial activity (MIC) of the newly synthesized compounds against the Streptococcus pneumonia strains showed that compounds **3a**, **4a**, **6b**, **6d**, **8**, **10b**, **13b**, **15b**, and **15c** are the most active compounds toward the four Streptococcus pneumonia serotypes. The presence of either an OH, Cl, or CN groups in any of such compounds is responsible for such reactivity. Compounds, **3a** and **4a** showed high activity against AV3 (serotype 3) and AV14 (serotype 14). On the other hand, compound **6b**, **13b**, and **15b** showed high activity toward AV3, AV6 (serotype 6B), and AV23 (serotype 23F).

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