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# The Knoevenagel reaction of cyanoacetylhydrazine with pregnenolone: Synthesis of thiophene, thieno[2,3-*d*]pyrimidine, 1,2,4-triazole, pyran and pyridine derivatives with anti-inflammatory and anti-ulcer activities

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ABSTRACT

The reaction of pregnenolone with cyanoacetylhydrazine and ammonium acetate at 120 °C gave the Knoevenagel condensation product **3**. The latter reacted with different reagents to give thiophene, thieno[2,3-*d*]pyrimidine, 1,2,4-triazole and pyran derivatives. The anti-inflammatory and anti-ulcer evaluations of the newly synthesized products were evaluated and the results showed that compounds **4**, **8c**, **10**, **11**, **13c**, **15a**, **15c**, **17a**, **17b**, **17e**, **18a** and **18f** possessed higher activity compared to the rest of the compounds. In addition to this, the toxicity of these active compounds was studied against shrimp larvae where compounds **15a**, **15c** and **18a** showed non-toxicity against the tested organisms.



## 1. Introduction

Steroid-based chemotherapeutic drugs possess various advantages such as low toxicity, less vulnerability to multi-drug resistance (MDR) and high bioavailability. This may be attributed to their ability to penetrate the cell wall and to undergo a predictable and controllable one- or two-step transformations *in vivo* to the inactive metabolites *via* known process of enzymatic deactivation [1]. In the past decade, an extensive focus of research was directed towards the rational modifications of steroidal molecules [2–7]. Moreover, several studies have revealed that steroidal molecules incorporating heteroatom (N, O or S) into the A- and D-ring of steroids showed a wide range of biological activities such as antimicrobial, anti-inflammatory, anti-tumor, hypocholesterolemic and diuretic activities [8–10]. As a result, a variety of heterocyclic units such as pyrazoles, pyrazolines, isoxazoles, isoxazolines, thiazoles, and thiadiazoles were introduced into the steroidal backbone [11–14]. For example, nitrogen heterocycles with triazole ring have recently been well studied due to their less toxic effects and numerous pharmacological properties such as antitumor [15,16], anti-inflammatory [17], anti-parasitic [18], anticonvulsant [19] and antimicrobial effects [20]. In view of the biological importance of these steroidal heterocycles which coupled with our interest in utilization of pregnenolone to prepare new biologically active compounds [21]. As continuation of our goals [22–26], in the present paper, we report on the efficient synthesis of androstane derivatives possessing thiophene, thieno[2,3-*d*]pyrimidine, 1,2,4-triazole, pyran and pyridine derivatives. This study focused on the synthesis and biochemical evaluation of the newly synthesized steroidal heterocyclic compounds which was then subjected through anti-inflammatory and anti-ulcer evaluations.

## 2. Experimental

### 2.1. Synthetic methods, analytical and spectral data

The starting steroid, pregnenolone, was purchased from Sigma Company, USA. All solvents were dried by distillation prior to using. Melting points were recorded on Buchi melting point apparatus D-545; UV spectra were measured on a Varian Cary 50 UV–vis Spectrophotometer. IR spectra (KBr discs) were recorded on

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Bruker Vector 22 instrument. $^{13}C$ NMR and $^{1}H$ NMR spectra were recorded on 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 mentioned in $\delta$ (ppm). Mass spectra were recorded on EIMS (Shimadzu) and ESI-esquire 3000 Bruker Daltonics instrument. Elemental analyses were carried by the Microanalytical Data Unit Ludwig-Maximilians-Universitaet-Muenchen, Germany. The progress of all reactions was monitored by TLC on 2 × 5 cm pre-coated silica gel 60 F254 plates of thickness of 0.25 mm (Merck). The purity of all compounds was determined through used for peak purity determination in high performance liquid chromatography (HPLC). Where in this work the purity of compounds was improved through changing the eluents being used and the solvent introduced through the data was that which minimize the peak distortion and consequently indicating the best purity of the described compounds [27,28]. All described compounds showed the characteristic spectral data of cyclopentanoperhydrophenanthrene nuclei of pregnene and androstane series was similar to those reported in literature [29,30]. 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 [31,32].

### 2.2. Chemical synthesis

#### 2.2.1. 2-Cyano-3-((8S,9S,10R,13S,14S,17S)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)but-2-enehydrazide (**3**)

To a dry solid of pregnenolone (**1**) (0.316 g, 1 mmol), 2-cyanoacetylhydrazine (0.100 g, 1 mmol) and ammonium acetate (0.077 g, 1 mmol) were added. The whole reaction mixture was heated in an oil bath at 120 °C for 40 min then left to cool. The solid product formed upon trituration with ethanol was collected by filtration then was subjected to TLC to assure its purity. HPLC purity = 90% (C-18 NovaPak column; MeOH:$H_2O$/90:10), RT = 20 min; pale yellow crystals from ethanol, yield 80%, m.p. 205–208 °C; UV–vis (MeOH) $\lambda_{max}$ 288 nm (IR (KBr) $cm^{-1}$: 3644–3427, 2948, 2260, 1689, 1634, 1563; $^{1}H$ NMR (CDCl3): $\delta$ 0.68 (s, 3H), 1.72 (s, 3H), 1.60–1.92 (m, 12H), 2.20–2.38 (m, 3H), 2.68 (s, 3H), 2.83 (t, $J$ = 7.8 Hz, 1H), 3.20 (s, 2H), 3.53 (m, 2H), 4.45 (s, 2H, $D_2O$ exchangeable), 5.33 (s, 1H), 6.78 (d, $J$ = 12.5 Hz, 1H), 8.28 (s, 1H), 8.38 (s, 1H, $D_2O$ exchangeable); $^{13}C$ NMR ($CDCl_3$): $\delta$ 13.4, 19.6, 21.9, 22.3, 24.7, 31.0, 31.8, 31.9, 37.2, 38.9, 43.9, 45.0, 48.6, 48.9, 49.3, 49.8, 50.0, 52.8, 57.2, 61.7, 98.3, 104.3, 116.8, 167.3; MS: m/e = 397 ($M^+$, 28%); Anal. Calcd. for $C_{24}H_{35}N_3O_2$: C, 72.51; H, 8.87; N, 10.57. Found: C, 72.80; H, 8.66; N, 10.63.

#### 2.2.2. 2-Amino-4-((8S,9S,10R,13S,14S,17S)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12, 13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)thiophene-3-carbohydrazide (**4**)

To a solution of compound **3** (0.397 g, 1 mmol) in 1,4-dioxane (20 mL) containing triethylamine (0.50 mL) elemental sulfur (0.032 g, 1 mmol) was added. The reaction mixture was heated under reflux for 1 h and the formed solid product was collected by filtration, dried and monitored through TLC for the purity. Thin layer chromatography revealed just a single spot which proved the presence of a single product.

HPLC purity = 90% (C-18 NovaPak column; MeOH:$H_2O$/85:15), RT = 23 min; yellow crystals from 1,4-dioxane, yield 88%, m.p. 198–200 °C; UV–vis (MeOH) $\lambda_{max}$ 267 nm (IR (KBr) $cm^{-1}$: 3625–3439, 2953, 1632, 1560; $^{1}H$ NMR ($CDCl_3$): $\delta$ 0.64 (s, 3H), 1.68 (s, 3H), 1.62–1.90 (m, 12H), 2.24–2.35 (m, 3H), 2.80 (t, $J$ = 7.4 Hz, 1H), 3.23 (s, 2H), 3.51 (m, 2H), 4.48, (s, 2H, $D_2O$ exchangeable), 5.21 (s, 2H, $D_2O$ exchangeable), 5.28 (s, 1H), 6.68 (d, $J$ = 10.8 Hz, 1H), 6.11 (s, 1H), 8.24 (s, 1H), 8.47 (s, 1H, $D_2O$ exchangeable); $^{13}C$ NMR ($CDCl_3$): $\delta$ 13.7, 19.3, 21.5, 22.8, 24.7, 31.3, 31.9, 37.0, 38.9, 43.9, 45.3, 48.6, 48.8, 49.3, 49.9, 50.2, 53.2, 57.2, 61.9, 116.4, 123.6, 128.4, 134.6, 156.6, 167.8; MS: m/e = 429 ($M^+$, 36%); Anal. Calcd. for $C_{24}H_{35}N_3O_2S$: C, 67.10; H, 8.21; N, 9.78; S, 7.46. Found: C, 67.29; H, 8.40; N, 9.83; S, 7.62.

#### 2.2.3. 3-Amino-5-((8S,9S,10R,13S,14S,17S)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12, 13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)thieno[2,3-d]pyrimidin-4(3H)-one (**6**)

To a solution of compound **4** (0.429 g, 1 mmol) in 1,4-dioxane (30 mL) formaldehyde (0.030 g, 1 mmol) was added. The reaction mixture was heated under reflux for 4 h then evaporated under vacuum. The remaining product was triturated with diethylether and the formed solid product was collected by filtration. Thin layer chromatography revealed just a single spot which proved the presence of a single product.

HPLC purity = 89% (C-18 NovaPak column; MeOH:$H_2O$/85:15), RT = 23 min; yellow crystals from ethanol, yield 88%, m.p. 166–168 °C; UV–vis (MeOH) $\lambda_{max}$ 293 nm (IR (KBr) $cm^{-1}$: 3625–3458, 2959, 1688, 1638, 1545; $^{1}H$ NMR ($CDCl_3$): $\delta$ 0.62 (s, 3H), 1.63 (s, 3H), 1.60–1.88 (m, 12H), 2.22–2.38 (m, 3H), 2.81 (t, $J$ = 6.32 Hz, 1H), 3.26 (s, 2H), 3.54 (m, 2H), 5.28 (s, 2H, $D_2O$ exchangeable), 5.26 (s, 1H), 6.12 (s, 1H), 6.78 (s, 1H), 6.73 (d, $J$ = 12.6 Hz, 1H), 8.28 (s, 1H); $^{13}C$ NMR ($CDCl_3$): $\delta$ 13.4, 21.5, 22.8, 24.7, 28.4, 31.3, 31.9, 37.0, 38.9, 43.9, 45.3, 48.2, 48.4, 49.3, 49.9, 50.8, 53.2, 57.2, 62.4, 122.9, 126.3, 135.8, 155.4, 164.8, 170.4; MS: m/e = 439 ($M^+$, 22%); Anal. Calcd. for $C_{25}H_{33}N_3O_2S$: C, 68.30; H, 7.57; N, 9.56; S, 7.29. Found: C, 68.21; H, 7.29; N, 9.72; S, 7.37.

#### 2.2.4. 3-Amino-5-((8S,9S,10R,13S,14S,17S)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,-11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta [a]phenanthren-17-yl)-2-phenylthieno[2,3-d]pyrimidin-4(3H)-one (**8a**), 3-amino-2-(4-chlorophenyl)-5-((8S,9S,10R,13S,14S,17S)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)thieno[2,3-d]pyrimidin-4(3H)-one (**8b**) and 3-amino-2-(4-methoxyphenyl)-5-((8S,9S,10R,13S,14S,17S)-3-hydroxy-10,13-dimethyl-2,3,4,7,8, 9,10,11,12,13,14,15,-16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)thieno[2,3-d] pyrimidin-4(3H)-one (**8c**)

General procedure: To a solution of compound **4** (0.429 g, 1 mmol) in 1,4-dioxane (30 mL) any of benzaldehyde (0.107 g, 1 mmol), 4-chlorobenzaldehyde (0.140 g, 1 mmol) or 4-methoxybenzaldehyde (0.136 g, 1 mmol) was added. The reaction mixture in each case was heated under reflux for 2 h then evaporated under vacuum. The remaining product in each case was triturated with diethylether and the formed solid product was collected by filtration. Thin layer chromatography revealed just a single spot which proved the presence of a single product.

Compound **8a**: HPLC purity = 91% (C-18 NovaPak column; MeOH:$H_2O$/80:20), RT = 20 min; yellow crystals from 1,4-dioxane, yield 85%, m.p. 188–190 °C; UV–vis (MeOH) $\lambda_{max}$ 279 nm (IR (KBr) $cm^{-1}$: 3588–3418, 2953, 1687, 1636, 1548; $^{1}H$ NMR ($CDCl_3$): $\delta$ 0.60 (s, 3H), 1.62 (s, 3H), 1.61–1.86 (m, 12H), 2.23–2.38 (m, 3H), 2.83 (t, $J$ = 7.14 Hz, 1H), 3.23 (s, 2H), 3.52 (m, 1H), 5.31 (s, 2H, $D_2O$ exchangeable), 5.28 (s, 1H), 6.91 (s, 1H), 6.12 (s, 1H), 6.69 (s, 1H), 6.72 (d, $J$ = 10.3 Hz, 1H), 7.28–7.41 (m, 5H), 8.30 (s, 1H); $^{13}C$ NMR ($CDCl_3$): $\delta$ 13.2, 21.5, 22.8, 24.3, 28.2, 31.2, 31.9, 37.2, 38.7, 43.9, 45.3, 48.2, 48.8, 48.4, 49.2, 49.9, 50.8, 53.5, 57.2, 62.4, 122.6, 122.3, 124.0, 128.4, 129.2, 137.2, 153.8, 164.3, 166.3, 170.3; MS: m/e = 515 ($M^+$, 39%); Anal. Calcd. for $C_{31}H_{37}N_3O_2S$: C, 72.20; H, 7.23; N, 8.15; S, 6.22. Found: C, 72.41; H, 6.99; N, 8.05; S, 6.39.

Compound **8b**: HPLC purity = 90% (C-18 NovaPak column; MeOH:$H_2O$/87:13), RT = 24 min; orange crystals from 1,4-dioxane, yield 83%, m.p. 244–246 °C; UV–vis (MeOH) $\lambda_{max}$ 272 nm (IR (KBr) $cm^{-1}$: 3594–3431, 2956, 1690, 1633, 1543; $^{1}H$ NMR ($CDCl_3$): $\delta$ 0.63