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#### Research Article

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# Design, synthesis, and biological activities of novel thiophene, pyrimidine, pyrazole, pyridine, coumarin and isoxazole: Dydrogesterone derivatives as antitumor agents

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**Abstract:** On the basis of our consideration to design and to develop antitumor activities of heterocyclic compound derivatives, especially in fused ring system, we refer to the possibility of the heterocyclic extension of one of the most important steroid compounds used as a medicinal drug. The reaction of dydrogesterone with each of the malononitrile or ethylcyanoacetate containing elemental sulfur afforded thiophene derivatives 1a,b. Also, dydrogesterone was reacted with a mixture of ethylcvanoacetate-hydrazine, ethylcyanoacetae-urea, or ethylcyanoacetate-thiourea to produce pyrazole derivative 4 and pyrimidine derivatives 5a,b. Thienopyrimidine derivatives 2a-d were introduced from the reaction of thiophene derivatives **1a,b** with either phenylisothiocyanate or benzoylisothioyanate. Furthermore, compounds 1a,b were directed toward the reaction with ethylcyanoacetate to produce compounds 6a,b, and the last compounds **6a,b** were directed toward cyclization to obtain thienopyridine derivatives 7a,b. In addition, compounds 6a,b were subjected to react with different carbonyl compounds, such as salicylaldehyde, cyclopentanone-elemental sulfur, malonaldehyde, and acetylacetone to produce coumarin derivatives 8a,b, fused thiophene derivatives 9a,b, and pyridine derivatives **10a-d**. Isooxazole derivatives **12a,b** were afforded through the reaction of compounds 6a,b with

Hassan A. Alhazmi: Pharmaceutical Chemistry Department, Pharmacy College, Jazan University, P. O. Box 114, Postal Code 45142, Jazan, Saudi Arabia; Substance Abuse Research Centre, Jazan University, P. O. Box 114, Postal Code 45142, Jazan, Saudi Arabia hydroxylamine hydrochloride. Finally, 2-pyridone derivatives **14a,b** were obtained through the reaction of compounds **6a,b** with benzoylacetonitrile. Conformation structure of the synthesized compounds was established by applying IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR, and mass spectrometry, and their antitumor activity was examined. Some compounds showed promising growth inhibitory effects on the three different cell lines.

**Keywords:** dydrogesterone, heterocyclic extension, antitumor activity

# 1 Introduction

Dydrogesterone is one of the most important nonacetylated pregnane [1] derivatives (Figure 1); many medical uses of dydrogestrone were introduced in the treatment of either miscarriage threats or miscarriage prevention [2,3]. The oral treatment of dydrogesterone versus vaginal progesterone gel was applied in the luteal phase support [4]. Oral estradiol and dydrogesterone combination therapy in postmenopausal women was applied [5]. Dydrogesterone was used in the treatment of endometriosis, adenomyosis [6], and recurrent pregnancy loss, and it was also used for modulation of cytokine production [7]. Furthermore, it was used in the treatment of premenstrual syndrome [8] and for the prevention of myometrial contraction [9]. Also, dydrogesterone and its derivative act as selective enzyme regulators for breast cancer cell lines [10] and used for the treatment of dysmenorrhea [11]. Moreover, dydrogesterone has an effective role against abortion [12]. However, the combination of pregnane derivative with heterocyclic rings afforded biologically active properties; thus, pregnane containing imidazole, triazole rings, glycoside moiety, and piperazine ring has anticancer activity, cytotoxic activity [13], antioxidant activity [14], and antileukemic properties [15], respectively.

In this study, the synthesis of some heterocyclic extensions of dydrogesterone 1a,b, 2a-d, 4, 5a,b, 6a,b, 7a,b, 8a,b, 9a,b, 10a-d, 12a,b, and 14a,b was presented, and the antitumor activity test results were presented in Table 1.

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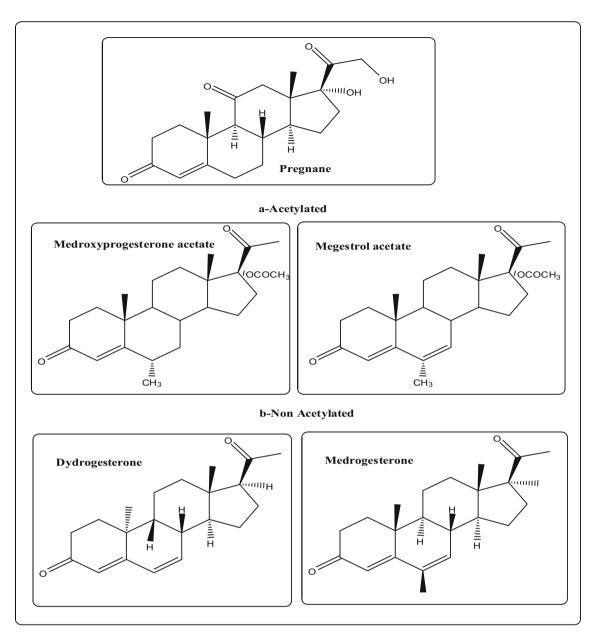


Figure 1: Pregnane and its derivatives.

# 2 Experimental

# 2.1 Materials and methods

# 2.1.1 Chemicals and reagents

All the chemicals and reagents in this study were obtained from Sigma Aldrich (United Kingdom).

#### 2.1.2 Instruments

The melting points of the synthesized dydrogesterone derivatives were measured in open capillaries, and they are uncorrected. Elemental analyses were performed on a Yanaco CHNS Corder elemental analyzer (Japan). IR spectra were estimated using KBr discs on a Pye Unicam SP-1000 spectrophotometer. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were

evaluated on a Varian EM 390-200 MHz instrument with  $CD_3SOCD_3$  as a solvent and TMS as an internal standard reference material, and chemical shifts were expressed as  $\delta$  ppm. Mass spectra were recorded on Kratos (75 eV) MS equipment (Germany).

# 2.2 Synthesis

# 2.2.1 General procedures for the synthesis of compounds 1a,b

Either malononitrile (0.66 g, 0.01 mol) or ethylcyanoacetate (1.131 g, 0.01 mol) together with elemental sulfur (0.32 g, 0.01 mol) was added to a solution of dydrogesterone (3.124 g, 0.01 mol) in 1,4 dioxane (100 mL) containing catalytic amount of triethylamine (1.0 mL). The reaction mixture in each case was heated under reflux for 5 h and then poured onto ice/water mixture containing few drops of hydrochloric acid (HCl). The solid product formed was collected by filtration and recrystallized from absolute ethanol.

# 2.2.1.1 2-Amino-4-((8*S*,9*R*,10*S*,13*S*,14*S*,17*S*)-10,13-dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17-dodecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)thiophene-3 carbonitrile (1a)

Off-white crystals; yield: 63%, 2.473 g; m.p. 218–221°C; IR (KBr):  $\nu_{\text{max}}$  3,368–3,223 (NH<sub>2</sub>), 2,987, 2,956 (2CH<sub>3</sub>), 2,883 (CH<sub>2</sub>), 2,234 (CN), 1,668 (C=O), 1,645 (C=C) cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$  0.95, 1.12 (2s, 6H, 2CH<sub>3</sub>), 1.68, 1.94 (2m, 12H, 6CH<sub>2</sub>), 4.12 (s, 2H, D<sub>2</sub>O-exchangeable, NH<sub>2</sub>), 4.86, 4.97 (2s, 2H), 5.23 (s, 1H), 5.33, 5.48 (2s, 2H), 5.76, 5.89 (d.d, 2H, J = 2.48 Hz), 6.14 (s, 1H, thiophene ring); EI-MS: m/z 392 (M<sup>+</sup>, 32.2%). Ana. Calcd for C<sub>24</sub>H<sub>28</sub>N<sub>2</sub>OS (392.56): C, 73.43; H, 7.19; N, 7.14; S, 8.17%. Found: C, 73.69; H, 7.39; N, 7.35; S, 8.45%.

# 2.2.1.2 Ethyl-2-amino-4-((8*S*,9*R*,10*S*,13*S*,14*S*,17*S*)-10,13-dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17-dodecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)thiophene-3-carboxylate (1b)

Creamy white crystals; yield: 58%, 2.55 g; m.p. 254–256°C; IR (KBr):  $\nu_{\text{max}}$  3,354–3,187 (NH<sub>2</sub>), 2,956, 2,943 (2CH<sub>3</sub>), 2,876

Table 1: Effect of compounds 1a,b-14a,b on the growth of three human tumor cell lines

		Gl <sub>50</sub> (μmol L <sup>-1</sup> )		
Comp. No	SF-268	MCF-7	NCI-H460	WI-38
1a	33.2 ± 7.5	21.8 ± 4.6	27.5 ± 3.7	>100
1b	$19.3 \pm 5.3$	$15.5 \pm 6.1$	$27.9 \pm 4.4$	>100
2a	$4.3 \pm 0.07$	$3.1 \pm 0.04$	$6.5 \pm 1.1$	$77.5 \pm 10.2$
2b	$2.9\pm0.05$	$4.8 \pm 0.04$	$3.4 \pm 0.05$	na
2c	$1.12\pm2.1$	$0.09 \pm 0.03$	$1.6 \pm 3.7$	>100
2d	$0.05 \pm 0.02$	$0.03 \pm 0.02$	$0.06 \pm 0.03$	>100
4	$41.0 \pm 8.9$	$37.2 \pm 9.3$	$31.7 \pm 6.5$	>100
5a	$4.8 \pm 0.9$	$3.7 \pm 0.7$	$2.9 \pm 2.3$	na
5b	$2.7 \pm 0.8$	$2.2 \pm 0.6$	$5.3 \pm 0.7$	>100
6a	$3.7 \pm 0.4$	$6.2 \pm 0.6$	$7.6 \pm 0.8$	>100
6b	5.5 ± 1.3	$9.3 \pm 1.8$	$6.3 \pm 1.2$	na
7a	$39.1 \pm 9.2$	37.7 ± 8.5	$42.8 \pm 9.1$	57.1 ± 8.2
7b	$40.3 \pm 8.4$	$41.9 \pm 7.2$	$33.8 \pm 8.5$	55.4 ± 7.4
8a	$33.2 \pm 8.5$	$27.3 \pm 6.4$	$29.1 \pm 8.9$	>100
8b	$38.3 \pm 7.8$	$36.3 \pm 8.2$	$34.9 \pm 10.2$	>100
9a	$25.2 \pm 8.8$	$33.2 \pm 8.1$	$35.1 \pm 8.5$	>100
9b	26.5 ± 7.9	$40.9 \pm 5.3$	43.7 ± 6.5	>100
10a	$1.7 \pm 0.06$	$1.4 \pm 0.9$	$1.1 \pm 0.5$	>100
10b	$8.7 \pm 2.5$	$4.4 \pm 1.9$	$3.8 \pm 8.5$	na
10c	$3.9 \pm 0.9$	$2.8 \pm 1.2$	$4.9 \pm 0.9$	>100
10d	$7.1 \pm 1.2$	4.5 ± 1.7	$3.6 \pm 0.7$	na
12a	$28.5 \pm 6.2$	$23.8 \pm 4.7$	$30.9 \pm 5.8$	>100
12b	$40.1 \pm 12.2$	$42.4 \pm 10.4$	$38.8 \pm 7.9$	na
14a	$26.7 \pm 7.5$	$42.4 \pm 8.4$	$30.6 \pm 12.8$	na
14b	41.7 ± 8.5	$43.4 \pm 6.5$	29.8 ± 10.5	na
Doxorubicin	$0.05 \pm 0.007$	$0.07 \pm 0.007$	$0.07 \pm 0.008$	>100

 $(CH_2)$ , 1,775, 1,674 (2C=0), 1,642 (C=C) cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$  0.82, 1.26 (2s, 6H, 2CH<sub>3</sub>), 1.84, 1.99 (2m, 12H, 6CH<sub>2</sub>), 2.67 (t, 3H, J = 3.5 Hz, CH<sub>3</sub>), 3.95 (q, 2H, J = 3.5 Hz, CH<sub>2</sub>), 4.34 (s, 2H, D<sub>2</sub>O-exchangeable, NH<sub>2</sub>), 4.75, 4.88 (2s, 2H), 5.16 (s, 1H), 5.28, 5.36 (2s, 2H), 5.52, 5.73 (d.d, 2H,  $J = 2.31 \,\text{Hz}$ ), 6.24 (s, 1H, thiophene ring); EI-MS: m/z 439 (M<sup>+</sup>, 19.3%). Ana. Calcd for C<sub>26</sub>H<sub>33</sub>NO<sub>3</sub>S (439.61): C, 71.04; H, 7.57; N, 3.19; S, 7.29%. Found: C, 70.79; H, 7.28; N, 3.31; S, 7.57%.

### 2.2.2 General procedure for the synthesis of compounds 2a-d

Either phenylisothiocyanate (0.406 g, 0.003 mol) or benzoyl isothiocyanate (0.49 g, 0.003 mol) was added to each of the solution of compound 1a (1.177 g, 0.003 mol) or the solution of compound 1b (1.319 g, 0.003 mol) in 1,4dioxane (50 mL) containing 0.5 mL of triethylamine. The reaction mixture was refluxed for 7 h, cooled, and poured onto ice/water mixture containing few drops of HCl. The formed solid product in each case was collected by filtration and recrystallized from absolute ethanol.

2.2.2.1 (85,9R,105,135,145,175)-17-(4-Imino-2mercapto-3-phenyl-3,4-dihydrothieno-[2,3-d] pyrimidin-5-yl)-10,13-dimethyl-8,9,10,11,12,13,14,15,16,17-decahydro-1Hcyclopenta[a]phenanthren-3(2H)-one (2a)

Off-white crystals; yield: 52%; 0.823 g; m.p. 141–143°C; IR (KBr):  $v_{\text{max}}$  3,391 (NH), 3,062 (CH aromatic), 2,881 (CH<sub>2</sub>),  $2,652 \text{ (SH)}, 1,665 \text{ (C=O)}, 1,651 \text{ (C=N)}, 1,642 \text{ (C=C)} \text{ cm}^{-1},$ <sup>1</sup>H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$  0.91, 1.12 (2s, 6H, 2CH<sub>3</sub>), 1.76, 1.86 (2m, 12H, 6CH<sub>2</sub>), 4.63, 4.74 (2s, 2H), 5.11 (s, 1H), 5.34, 5.56 (2s, 2H), 6.58, 6.73 (d.d, 2H, J =3.38 Hz), 6.78 (s, 1H, thiophene ring), 7.33-7.54 (m, 5H,  $C_6H_5$ ), 8.68 (bs, 1H, SH), 9.11 (s, 1H,  $D_2O$ -exchangeable, NH); EI-MS: m/z 527 (M<sup>+</sup>, 15.3%). Ana. Calcd for  $C_{31}H_{33}N_3OS_2$ (527.74): C, 70.55; H, 6.30; N, 7.96; S, 12.15%. Found: C, 70.83; H, 6.06; N, 7.69; S, 12.38%.

2.2.2.2 (85,9R,105,135,145,175)-17-(3-Benzoyl-4imino-2-mercapto-3,4-dihydrothieno-[2,3-d] pyrimidin-5-yl)-10,13-dimethyl-8,9,10,11,12,13,14,15,16,17-decahydro-1Hcyclopenta[a]phenanthren-3(2H)-one (2b)

Off-white crystals; yield: 61%; 1.017 g; m.p. 122–124°C; IR (KBr):  $v_{\text{max}}$  3,288 (NH), 3,037 (CH aromatic), 2,827 (CH<sub>2</sub>),

2,689 (SH), 1,714, 1,678 (2C=O), 1,618 (C=N), 1,533 (C=C) cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$  0.82, 1.06 (2s, 6H, 2CH<sub>3</sub>), 1.61, 1.72 (2m, 12H, 6CH<sub>2</sub>), 4.33, 4.58 (2s, 2H), 4.89 (s, 1H), 5.17, 5.41 (2s, 2H), 6.34, 6.52 (d.d, 2H,  $I = 5.32 \,\mathrm{Hz}$ ), 6.44 (s, 1H, thiophene ring), 7.45–7.68 (m, 5H, C<sub>6</sub>H<sub>5</sub>), 7.88 (bs, 1H, SH), 9.52 (s, 1H, D<sub>2</sub>O-exchangeable, NH); EI-MS: m/z 555 (M<sup>+</sup>, 23.7%). Ana. Calcd for  $C_{32}H_{33}N_3OS_2$  (555.75): C, 69.16; H, 5.99; N, 7.56; S, 11.54%. Found: C, 69.42; H, 6.08; N, 7.62; S, 11.31%.

2.2.2.3 ((85,9R,105,135,145,175)-10,13-Dimethyl-3oxo-2,3,8,9,10,11,12,13,14,15,16,17dodecahydro-1H-cyclopenta[a]phenanthren-17yl)-2-mercapto-3-phenylthieno-[2,3-d] pyrimidin-4(3H)-one (2c)

White crystals; yield: 48%; 0.761 g; m.p. 179-181°C; IR (KBr):  $v_{\text{max}}$  3,052 (CH aromatic), 2,887 (CH<sub>2</sub>), 2,711 (SH), 1,668, 1,653 (2C=0), 1,648 (C=N), 1,641 (C=C) cm $^{-1}$ ,  $^{1}$ H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$  0.84, 1.23 (2s, 6H, 2CH<sub>3</sub>), 1.55, 1.67 (2m, 12H, 6CH<sub>2</sub>), 4.56, 4.64 (2s, 2H), 5.22 (s, 1H), 5.41, 5.52 (2s, 2H), 6.47, 6.68 (d.d, 2H, J =3.38 Hz), 6.79 (s, 1H, thiophene ring), 7.12-7.42 (m, 5H,  $C_6H_5$ ), 8.47 (bs, 1H, SH); EI-MS: m/z 528 (M<sup>+</sup>, 31.3%). Ana. Calcd for C<sub>31</sub>H<sub>32</sub>N<sub>2</sub>O<sub>2</sub>S<sub>2</sub> (528.73): C, 70.42; H, 6.10; N, 5.30; S, 12.13%. Found: C, 70.71; H, 6.01; N, 5.59; S, 12.37%.

2.2.2.4 3-Benzoyl-5-((8S,9R,10S,13S,14S,17S)-10,13dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17dodecahydro-1H-cyclopenta[a]phenanthren-17yl)-2-mercaptothieno[2,3-d]pyrimidin-4(3H)one (2d)

White crystals; vield: 59%; 0.985 g; m.p. 159–160°C; IR (KBr): 3,068 (CH aromatic), 2,873 (CH<sub>2</sub>), 2,684 (SH), 1,668, 1,660, 1,653 (3C=0), 1,646 (C=N), 1,640 (C=C) cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$  1.17, 1.29 (2s, 6H, 2CH<sub>3</sub>), 1.64, 1.78 (2m, 12H, 6CH<sub>2</sub>), 4.26, 4.45 (2s, 2H), 5.11 (s, 1H), 5.36, 5.48 (2s, 2H), 6.18, 6.44 (d.d, 2H, J =4.67 Hz), 6.69 (s, 1H, thiophene ring), 7.28-7.47 (m, 5H,  $C_6H_5$ ), 8.31 (bs, 1H, SH); EI-MS: m/z 556 (M<sup>+</sup>, 27.1%). Ana. Calcd for C<sub>31</sub>H<sub>32</sub>N<sub>2</sub>O<sub>3</sub>S<sub>2</sub> (556.74): C, 69.03; H, 5.79; N, 5.03; S, 11.52%. Found: C, 69.21; H, 6.04; N, 5.22; S, 11.35%.

# 2.2.3 General procedure for the synthesis of compounds 4,5a,b

Ethylcyanoacetate (0.262 g, 0.002 mol) together with hydrazine (0.1 g, 0.002 mol), urea (0.12 g, 0.002 mol), or thiourea (0.152 g, 0.002 mol) was added to a solution of dydrogesterone (0.625 g, 0.002 mol) in 1,4 dioxane (50 mL) containing catalytic amount of triethylamine (1.0 mL). The reaction mixture in each case was heated under reflux for 8 h and then poured onto ice/water containing few drops of HCl. The precipitated product formed was collected by filtration and recrystallized from ethanol-water.

# 2.2.3.1 (85,9R,105,135,145,175)-17-((E/Z)-1-(3-amino-5hydroxy-4H-pyrazol-4-ylidene)ethyl)-10,13dimethyl-8,9,10,11,12,13,14,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-3(2H)-one (4)

Off-white crystals; yield: 57%, 0.448 g; m.p. 263-265°C. IR (KBr):  $v_{\text{max}}$  3,394–3,169 (OH, NH<sub>2</sub>), 2,955 (CH<sub>3</sub>), 2,843  $(CH_2)$ , 1,653 (C=0), 1,643 (C=C) cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$  1.18, 1.34, 1.47 (3s, 9H, 3CH<sub>3</sub>), 1.77, 2.12 (2m, 12H, 6CH<sub>2</sub>), 4.38 (s, 2H, D<sub>2</sub>O-exchangeable, NH<sub>2</sub>), 4.56, 4.63 (2s, 2H), 5.38 (s, 1H), 5.55, 5.67 (2s, 2H), 6.79, 6.89 (d.d, 2H,  $J = 3.12 \,\text{Hz}$ ), 8.13 (s, 1H, D<sub>2</sub>O-exchangeable, OH); EI-MS: m/z 393 (M<sup>+</sup>, 21.2%). Ana. Calcd for  $C_{24}H_{31}N_3O_2$  (393.52): C, 73.25; H, 7.94; N, 10.68%. Found: C, 73.61; H, 7.69; N, 10.39%.

# 2.2.3.2 (E/Z)-4-Amino-5-(1-((8S,9R,10S,13S,14S,17S)-10,13-dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17-dodecahydro-1Hcyclopenta[a]phenanthren-17-yl)ethylidene)-6hydroxypyrimidin-2(5H)-one (5a)

Creamy white crystals; yield 63%, 0.53 g; m.p. 212-214°C. IR (KBr):  $v_{\text{max}}$  3,331–3,125 (OH, NH<sub>2</sub>), 2,961 (CH<sub>3</sub>), 2,855  $(CH_2)$ , 1,653, 1,646 (2C=0), 1,640 (C=C) cm<sup>-1</sup>, <sup>1</sup>H NMR  $(390-200 \text{ MHz}, \text{DMSO-}d_6)$ :  $\delta$  0.87, 1.21, 1.37 (3s, 9H, 3CH<sub>3</sub>), 1.68, 1.89 (2m, 12H, 6CH<sub>2</sub>), 4.12 (s, 2H, D<sub>2</sub>O-exchangeable, NH<sub>2</sub>), 4.27, 4.78 (2s, 2H), 5.16 (s, 1H), 5.34, 5.71 (2s, 2H), 6.73, 6.85 (d.d, 2H, J = 2.68 Hz), 7.45 (s, 1H,  $D_2O_2$ exchangeable, OH);  $^{13}$ C NMR (300 MHz, DMSO- $d_6$ ):  $\delta$ 16.6, 19.2, 21.1, 24.8, 25.9, 26.7, 29.2, 32.3, 43.6, 48.5, 49.6, 119.8, 121.6, 122.9, 124.7, 126.2, 127.2, 130.8, 132.5, 139.2, 140.1, 142.3, 157.6, 159.4; EI-MS: m/z 421 (M<sup>+</sup>, 27.3%). Ana. Calcd for C<sub>25</sub>H<sub>31</sub>N<sub>3</sub>O<sub>3</sub> (421.53): C, 71.23; H, 7.41; N, 9.97%. Found: C, 70.91; H, 7.68; N, 10.22%.

# 2.2.3.3 (8S,9R,10S,13S,14S,17S)-17-((E/Z)-1-(4-amino-6-hydroxy-2-thioxopyrimidin-5(2H)-ylidene) ethyl)-10,13-dimethyl-8,9,10,11,12,13,14,15,16,17-decahydro-1*H*-cyclopenta[a]phenanthren-3(2H)-one (5b)

White crystals; yield 57%, 0.499 g; m.p. 190-192°C. IR (KBr):  $v_{\text{max}}$  3,378–3,212 (OH, NH<sub>2</sub>), 2,955 (CH<sub>3</sub>), 2,848  $(CH_2)$ , 1,657, (C=0), 1,642 (C=C) cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$  0.91, 1.18, 1.43 (3s, 9H, 3CH<sub>3</sub>), 1.78, 1.98 (2m, 12H, 6CH<sub>2</sub>), 4.34 (s, 2H, D<sub>2</sub>O-exchangeable, NH<sub>2</sub>), 4.45, 4.73 (2s, 2H), 5.28 (s, 1H), 5.44, 5.77 (2s, 2H), 6.54, 6.75 (d.d, 2H, J = 2.14 Hz), 8.24 (s, 1H, D<sub>2</sub>Oexchangeable, OH);  $^{13}$ C NMR (300 MHz, DMSO- $d_6$ ):  $\delta$ 14.5, 17.8, 20.2, 23.1, 24.8, 26.9, 28.4, 30.5, 43.2, 46.4, 48.1, 113.5, 119.2, 120.3, 124.6, 125.1, 126.3, 129.2, 131.7, 134.7, 139.2, 142.8, 154.5, 158.3; EI-MS: m/z 437 (M<sup>+</sup>, 20.7%). Ana. Calcd for C<sub>25</sub>H<sub>31</sub>N<sub>3</sub>O<sub>2</sub>S (437.6): C, 68.62; H, 7.14; N, 9.60: S, 7.33%. Found: C, 68.91; H, 7.45; N, 9.29; S, 7.61%.

### 2.2.4 General procedure for the synthesis of compounds 6a,b

The solution dydrogesterone thiophene derivative 1a (1.177 g, 0.003 mol) or **1b** (1.319 g, 0.003 mol) in absolute ethanol (70 mL) containing triethylamine (1 mL) and ethylcyanoacetate (0.339 g, 0.003 mol) was added. The reaction mixture was heated under reflux for 6 h, and the reaction was observed by TLC control, and then, the mixture poured onto ice/water containing few drops of HCl. The formed solid product in each case was filtered, dried, and recrystallized from the absolute ethanol.

#### 2.2.4.1 2-Cyano-N-(3-cyano-4-

((85,9R,105,135,145,175)-10,13-dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17-dodecahydro-1Hcyclopenta[a]phenanthren-17-yl)thiophen-2-yl) acetamide (6a)

Yellowish-white crystals; yield 64%, 0.882 g; m.p. 112-114°C. IR (KBr):  $v_{\text{max}}$  3,176 (NH), 2,956, 2,934 (2CH<sub>3</sub>), 2,871 (CH<sub>2</sub>), 2,242, 2,221 (2CN), 1,669, 1,663 (2C=O), 1,647 (C=C), cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$  1.15, 1.23 (2s, 6H, 2CH<sub>3</sub>), 1.88, 1.98, 3.12 (2m, s, 14H, 7CH<sub>2</sub>), 4.92, 5.11 (2s, 2H), 5.31 (s, 1H), 5.44, 5.62 (2s, 2H), 6.53, 6.89 (d.d, 2H, J =

3.12 Hz), 6.94 (s, 1H, thiophene ring), 9.23 (s, 1H, D<sub>2</sub>Oexchangeable, NH); EI-MS: m/z 459 (M<sup>+</sup>, 18.2%). Ana. Calcd for C<sub>27</sub>H<sub>29</sub>N<sub>3</sub>O<sub>2</sub>S (459.60): C, 70.56; H, 6.36; N, 9.14; S, 6.98%. Found: C, 70.29; H, 6.39; N, 9.35; S, 6.65%.

# 2.2.4.2 Ethyl-2-(2-cyanoacetamido)-4-((85,9R,105,135,145,175)-10,13-dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17-dodecahydro-1Hcyclopenta[a]phenanthren-17-yl)thiophene-3carboxylate (6b)

Faint brown crystals; yield 51%, 0.775 g; m.p. 226-228°C. IR (KBr):  $v_{\text{max}}$  3,216 (NH), 2,985–2,930 (3CH<sub>3</sub>), 2,841–2,815 (CH<sub>2</sub>), 2,239 (CN), 1,667, 1,660, 1,649 (3C=0), 1,640 (C=C), cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$  1.12, 1.21, 1.42 (2s, t 9H, 3CH<sub>3</sub>), 2.12, 2.33, 3.12, 3.38 (2m, s, q 16H, 8CH<sub>2</sub>), 4.77, 4.89 (2s, 2H), 5.27 (s, 1H), 5.33, 5.56 (2s, 2H), 6.35, 6.47 (d.d, 2H, J = 2.82 Hz), 6.76 (s, 1H, thiophene ring), 9.14 (s, 1H,  $D_2O$ -exchangeable, NH); EI-MS: m/z 506  $(M^+, 13.3\%)$ . Ana. Calcd for  $C_{29}H_{34}N_2O_4S$  (506.66): C, 68.75; H, 6.76; N, 5.53; S, 6.33%. Found: C, 68.51; H, 6.46; N, 5.25; S, 6.66%.

# 2.2.5 General procedure for the synthesis of compounds 7a,b

#### 2.2.5.1 Method A

The solution of either compound 6a (0.46 g, 0.001 mol) or **6b** (0.507 g, 0.001 mol) in absolute ethanol (50 mL) containing triethylamine (1 mL) was added. The reaction was heated under reflux for 4 h, and then it was monitored under TLC control and poured onto ice/water containing few drops of HCl. The formed solid product for each case was filtered, dried, and recrystallized from 1,4-dioxane.

#### 2.2.5.2 Method B

Ethylcyanoacetate (0.113 g, 0.001 mol) was added to a suspension of either 1a (0.392 g, 0.001 mol) or 1b (0.439 g, 0.001 mol) in sodium ethoxide [(0.001 mol; prepared by dissolving sodium metal (0.023 g, 0.001 mol) in absolute ethanol (30 mL)]. The reaction mixture was heated in a boiling water bath for 6 h and then left to cool. The formed solid product was poured onto ice/water containing few drops of HCL, and it was collected by filtration and dried.

# 2.2.5.3 4-Amino-3-((85,9R,105,135,145,175)-10,13dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17dodeca-hydro-1H-cyclopenta[a]phenanthren-17yl)-6-oxo-6,7-dihydrothieno[2,3-b]pyridine-5carbonitrile (7a)

Faint brown crystals; yield 57%, 0.262 g; m.p. 187-189°C. IR (KBr):  $v_{\text{max}}$  3,338–3,154 (NH<sub>2</sub>,NH), 2,944, 2,927 (2CH<sub>3</sub>), 2,843 (CH<sub>2</sub>), 2,239 (CN), 1,665, 1,656 (2C=0), 1,642 (C=C) cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$  1.28, 1.43 (2s, 6H, 2CH<sub>3</sub>), 1.81, 2.27 (2m, 12H, 6CH<sub>2</sub>), 4.34 (s, 2H, D<sub>2</sub>Oexchangeable, NH<sub>2</sub>), 4.86, 5.18 (2s, 2H), 5.22 (s, 1H), 5.47, 5.68 (2s, 2H), 6.63, 6.78 (d.d, 2H, J = 2.54 Hz), 6.85 (s, 1H, thiophene ring), 9.45 (s, 1H, D<sub>2</sub>O-exchangeable, NH); <sup>13</sup>C NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  12.5, 17.2, 19.2, 21.2, 23.4, 25.1, 27.3, 29.8, 38.2, 43.7, 48.9, 114.1, 117.4, 125.5, 127.8, 128.2, 129.4, 130.2, 132.7, 134.5, 136.5, 138.5, 140.2, 141.7, 155.6, 160.3; EI-MS: m/z 459 (M<sup>+</sup>, 13.6%). Ana. Calcd for C<sub>27</sub>H<sub>29</sub>N<sub>3</sub>O<sub>2</sub>S (459.60): C, 70.56; H, 6.36; N, 9.14; S, 6.98%. Found: C, 70.87; H, 6.65; N, 9.43; S, 6.72%.

# 2.2.5.4 3-((8S,9R,10S,13S,14S,17S)-10,13-Dimethyl-3oxo-2,3,8,9,10,11,12,13,14,15,16,17dodecahydro-1H-cyclopenta[a]phenanthren-17yl)-4-hydroxy-6-oxo-6,7-dihydrothieno[2,3-b] pyridine-5-carbonitrile (7b)

Yellowish white crystals; yield 61%, 0.281 g; m.p. 221-223°C. IR (KBr):  $v_{\text{max}}$  3,254–3,123 (OH,NH), 2,982, 2,945 (2CH<sub>3</sub>), 2,851 (CH<sub>2</sub>), 2,223 (CN), 1,663, 1,651 (2C=0), 1,644 (C=C) cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$  1.15, 1.32 (2s, 6H, 2CH<sub>3</sub>), 1.57, 1.86 (2m, 12H, 6CH<sub>2</sub>), 4.52, 4.68 (2s, 2H), 5.13 (s, 1H), 5.59, 5.65 (2s, 2H), 6.47, 6.63 (d.d, 2H, J =2.78 Hz), 6.77 (s, 1H, thiophene ring), 7.82 (s, 1H, D<sub>2</sub>Oexchangeable, NH), 9.23 (s, 1H, D<sub>2</sub>O-exchangeable, OH); <sup>13</sup>C NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  14.2, 16.7, 20.1, 21.8, 23.8, 25.7, 28.1, 29.9, 36.3, 40.6, 46.7, 113.4, 116.5, 122.5, 125.7, 128.9, 129.6, 131.3, 132.9, 134.3, 136.3, 137.6, 141.1, 142.8, 153.7, 158.9; EI-MS: m/z 460 (M<sup>+</sup>, 17.4%). Ana. Calcd for C<sub>27</sub>H<sub>28</sub>N<sub>2</sub>O<sub>3</sub>S (460.59): C, 70.41; H, 6.13; N, 6.08; S, 6.96%. Found: C, 70.16; H, 6.44; N, 6.33; S, 6.67%.

# 2.2.6 General procedure for the synthesis of compounds 8a,b

Salicylaldehyde (0.122 g, 0.001 mol) was added to a solution of either dydrogesterone thiophene derivative 6a (0.46 g, 0.001 mol) or **6b** (0.507 g, 0.001 mol) in ethanol (50 mL) containing triethylamine (1 mL). The mixture of the reaction was refluxed under heating for 7 h and then evaporated under vacuum. The solid product for each compound was triturated with ethanol and collected by filtration, dried, and recrystallized from 1,4-dioxane.

2.2.6.1 N-(3-Cyano-4-((85,9R,105,135,145,175)-10,13dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17dodeca-hydro-1H-cyclopenta[a]phenanthren-17yl)thiophen-2-yl)-2-oxo-2H-chromene-3carboxamide (8a)

Off-white crystals; yield 55%, 0.31 g; m.p. 149-151°C. IR (KBr):  $v_{\text{max}}$  3,212 (NH), 3,048 (CH arom.), 2,973, 2,928 (2CH<sub>3</sub>), 2,852 (CH<sub>2</sub>), 2,228 (CN), 1,668, 1,661, 1,657 (3C=0), 1,642 (C=C) cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz), DMSO- $d_6$ ):  $\delta$  1.23, 1.35 (2s, 6H, 2CH<sub>3</sub>), 1.51, 1.77 (2m, 12H, 6CH<sub>2</sub>), 4.53, 4.79 (2s, 2H), 5.22 (s, 1H), 5.31, 5.73 (2s, 2H), 6.33, 6.45 (d.d, 2H, J = 2.87 Hz), 6.57 (s, 1H, coumarin H-4), 6.73 (s, 1H, thiophene ring), 7.28-7.41 (m, 4H,  $C_6H_4$ ), 9.45 (s, 1H,  $D_2O$ -exchangeable, NH); <sup>13</sup>C NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  13.8, 15.5, 18.8, 20.7, 23.4, 24.9, 26.6, 28.5, 33.6, 38.6, 44.2, 111.5, 115.8, 121.7, 123.8, 126.5, 128.2, 130.1, 132.7, 133.5, 135.3, 136.9, 138.7, 139.5, 141.7, 142.6, 151.6, 154.5, 157.8, 160.2, 173.2; EI-MS: m/z 564 (M<sup>+</sup>, 33.4%). Ana. Calcd for C<sub>34</sub>H<sub>32</sub>N<sub>2</sub>O<sub>4</sub>S (564.69): C, 72.32; H, 5.71; N, 4.96; S, 5.86%. Found: C, 72.05; H, 5.44; N, 4.65; S, 5.95%.

2.2.6.2 Ethyl-4-((8S,9R,10S,13S,14S,17S)-10,13dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17dodecahydro-1H-cyclopenta[a]phenanthren-17vl)-2-(2-oxo-2*H*-chromene-3-carboxamido) thiophene-3-carboxylate (8b)

Off-white crystals; yield 60%, 0.367 g; m.p. 168–170°C. IR (KBr):  $v_{\text{max}}$  3,182 (NH), 3,062 (CH arom.), 2,984, 2,943, 2,924 (3CH<sub>3</sub>), 2,844 (CH<sub>2</sub>), 2,228 (CN), 1,668, 1,661, 1,657 (3C=0), 1,642 (C=C) cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz)DMSO- $d_6$ ):  $\delta$  1.11, 1.23, 1.35 (2s, t, 9H, 3CH<sub>3</sub>), 1.51, 1.77, 3.62 (2m, q, 14H, 7CH<sub>2</sub>), 4.33, 4.79 (2s, 2H), 5.18 (s, 1H), 5.21, 5.53 (2s, 2H), 6.33, 6.48 (d.d, 2H, J = 2.87 Hz), 6.52 (s, 1H, coumarin H-4), 6.63 (s, 1H, thiophene ring), 7.12-7.38 (m, 4H,  $C_6H_4$ ), 10.17 (s, 1H,  $D_2O$ -exchangeable, NH);  $^{13}C$ NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  11.2, 13.9, 14.7, 19.8, 20.5, 24.4, 25.8, 26.7, 27.3, 29.2, 31.6, 37.5, 44.9, 112.2, 115.2, 120.9, 122.6, 125.7, 126.8, 129.7, 131.4, 133.7, 136.8, 137.9, 138.9, 139.4, 141.3, 143.7, 150.9, 155.8, 157.6, 161.5, 166.3, 173.2; EI-MS: m/z 611 (M<sup>+</sup>, 21.2%). Ana. Calcd for

C<sub>36</sub>H<sub>37</sub>NO<sub>6</sub>S (611.75): C, 70.68; H, 6.10; N, 2.29; S, 5.24%. Found: C, 70.99; H, 5.94; N, 2.61; S, 5.05%.

#### 2.2.7 General procedure for the synthesis of compounds 9a,b

Cyclopentanone (0.084 g, 0.001 mol) together with elemental sulfur (0.032 g, 0.001) was added to a solution of either compounds 6a (0.46 g, 0.001 mol) or 6b (0.507 g, 0.001 mol) in 50 mL absolute ethanol containing triethylamine (1.0 mL). The reaction mixture was heated under reflux for 4 h and then poured onto ice/water mixture containing few drops of HCL. The solid product formed in each case was collected by filtration, dried, and recrystallized from 1,4-dioxane.

#### 2.2.7.1 2-Amino-N-(3-cyano-4-

((85,9R,105,135,145,175)-10,13-dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17dodecahydro-1Hcyclopenta[a]phenanthren-17-yl)thiophen-2-yl)-5,6-dihydro-4H-cyclopenta[b] thiophene-3carboxamide (9a)

Brown crystals; yield 71%, 0.395 g; m.p. 200-202°C; IR (KBr):  $\nu_{\text{max}}$  3,328–3,130 (NH<sub>2</sub>, NH), 2,973, 2,912 (2CH<sub>3</sub>), 2,855 (CH<sub>2</sub>), 2,233 (CN), 1,664, 1,652 (2C=O), 1,641 (C=C) cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$  1.05, 1.18 (2s, 6H, 2CH<sub>3</sub>), 1.76, 2.23, 2.85 (3 m, 18H, 9CH<sub>2</sub>), 4.12 (s, 2H, D<sub>2</sub>Oexchangeable, NH<sub>2</sub>), 4.45, 4.67 (2s, 2H), 5.15 (s, 1H), 5.37, 5.43 (2s, 2H), 6.23, 6.44 (d.d, 2H, J = 2.49 Hz), 6.63 (s, 1H, thiophene ring), 9.45 (s, 1H, D<sub>2</sub>O-exchangeable, NH); EI-MS: m/z 557 (M<sup>+</sup>, 27.2%). Ana. Calcd for  $C_{32}H_{35}N_3O_2S_2$ (557.77): C, 68.91; H, 6.32; N, 7.53; S, 11.50%. Found: C, 68.62; H, 6.03; N, 7.84; S, 11.80%.

2.2.7.2 Ethyl-2-(2-amino-5,6-dihydro-4*H*-cyclopenta[*b*] thiophene-3-carboxamido)-4-((8s,9 R,10s,13s,14s, 17s)-10,13-dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17-dodecahydro-1Hcyclopenta[a] phenanthren-17-yl)thiophene-3carboxylate (9b)

Light brown crystals; yield 63%, 0.381 g; m.p. 231-233°C. IR (KBr):  $v_{\text{max}}$  3,367–3,182 (NH<sub>2</sub>, NH), 2,951, 2,933, 2,921  $(3CH_3)$ , 2,834  $(CH_2)$ , 1,728, 1,660, 1,647 (3C=0), 1,635 (C=C) cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$  1.11, 1.23, 1.63 (2s, t, 9H, 3CH<sub>3</sub>), 1.91, 2.17, 2.38, 2.72 (3 m, q, 20H, 10CH<sub>2</sub>), 4.26 (s, 2H, D<sub>2</sub>O-exchangeable, NH<sub>2</sub>), 4.39,

4.62 (2s, 2H), 5.21 (s, 1H), 5.34, 5.48 (2s, 2H), 6.31, 6.49 (d.d, 2H,  $I = 2.32 \,\text{Hz}$ ), 6.72 (s, 1H, thiophene ring), 10.15 (s, 1H, D<sub>2</sub>O-exchangeable, NH); EI-MS: m/z 604 (M<sup>+</sup>, 31.9%). Ana. Calcd for C<sub>34</sub>H<sub>40</sub>N<sub>2</sub>O<sub>4</sub>S<sub>2</sub> (604.82): C, 67.52; H, 6.67; N, 4.63; S, 10.60%. Found: C, 67.17; H, 6.36; N, 4.34; S, 10.91%.

### 2.2.8 General procedure for the synthesis of compounds 10a-d

Either malonaldehyde (0.072 g, 0.001 mol) or acetylacetone (0.1 g, 0.001 mol) was added to a solution of either compound **6a** (0.46 g, 0.001 mol) or **6b** (0.507 g, 0.001 mol) in absolute ethanol (50 mL) containing piperidine (1 mL). The reaction mixture in each case was heated under reflux for 7 h and then poured onto ice/ water containing few drops of HCL. The formed solid product was collected, filtered, dried, and recrystallized from ethanol-water.

2.2.8.1 1-(3-Cyano-4-((85,9R,105,135,145,175)-10,13dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17dodeca-hydro-1H-cyclopenta[a]phenanthren-17yl)thiophen-2-yl)-2-oxo-1,2-dihydropyridine-3carbonitrile (10a)

Creamy white crystals; yield 63%, 0.312 g; m.p. 131–133°C. IR (KBr):  $v_{\text{max}}$  2,993, 2,972 (2CH<sub>3</sub>), 2,856 (CH<sub>2</sub>), 2,233, 2,212 (2CN), 1,665, 1,655 (2C=O), 1,640 (C=C) cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$  1.33, 1.42 (2s, 6H, 2CH<sub>3</sub>), 1.76, 1.92 (2m, 12H, 6CH<sub>2</sub>), 4.22, 4.54 (2s, 2H), 5.12 (s, 1H), 5.37, 5.65 (2s, 2H), 6.18, 6.33 (d.d, 2H, J = 2.24 Hz), 6.48 (m, 3H, pyridine ring), 6.56 (s, 1H, thiophene ring); <sup>13</sup>C NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  14.2, 15.7, 23.7, 24.5, 26.9, 27.8, 31.7, 36.4, 44.3, 111.2, 114.6, 120.8, 122.9, 127.7, 128.9, 130.3, 132.4, 135.6, 136.7, 137.8, 138.2, 139.9, 140.5, 142.1, 143.7, 147.5, 151.9, 159.4, 161.2, 166.2; EI-MS: m/z 495 (M<sup>+</sup>, 21.7%). Ana. Calcd for C<sub>30</sub>H<sub>29</sub>N<sub>3</sub>O<sub>2</sub>S (495.64): C, 72.70; H, 5.90; N, 8.48; S, 6.47%. Found: C, 72.36; H, 5.64; N, 8.75; S, 6.81%.

2.2.8.2 1-(3-Cyano-4-((8S,9R,10S,13S,14S,17S)-10,13dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17dodeca-hydro-1H-cyclopenta[a]phenanthren-17yl)thiophen-2-yl)-4,6-dimethyl-2-oxo-1,2dihydro-pyridine-3-carbonitrile (10b)

Off-white crystals; yield 51%, 0.267 g; m.p. 138-140°C. IR (KBr):  $\nu_{\text{max}}$  2,963–2,941 (CH<sub>3</sub>), 2,848 (CH<sub>2</sub>), 2,254, 2,235 (2CN), 1,669, 1,653 (2C=0), 1,641 (C=C) cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$  1.17, 1.29, 1.34 (2s, m, 12H, 4CH<sub>3</sub>), 1.55, 1.82 (2m, 12H, 6CH<sub>2</sub>), 4.11, 4.43 (2s, 2H), 5.23 (s, 1H), 5.36, 5.61 (2s, 2H), 6.21, 6.35 (d.d, 2H, J = 2.19 Hz),6.76 (s, 1H, pyridine ring), 6.83 (s, 1H, thiophene ring); <sup>13</sup>C NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  13.4, 15.9, 19.5, 20.2, 22.6, 23.5, 25.5, 27.4, 30.4, 33.7, 42.4, 110.6, 113.5, 121.2, 122.7, 125.6, 127.7, 130.8, 131.3, 134.5, 136.9, 137.6, 138.5, 139.6, 141.3, 142.4, 143.8, 145.3, 150.9, 153.7, 160.4, 167.5; EI-MS: m/z 523 (M<sup>+</sup>, 17.7%). Ana. Calcd for  $C_{32}H_{33}N_3O_2S$  (523.69): C, 73.39; H, 6.35; N, 8.02; S, 6.12%. Found: C, 73.65; H, 6.64; N, 8.34; S, 6.41%.

2.2.8.3 Ethyl2-(3-cyano-2-oxopyridin-1(2H)-yl)-4-((85,9R,105,135,145,175)-10,13-dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17-dodecahydro-1Hcyclopenta[a]phenanthren-17-yl)thiophene-3carboxylate (10c)

Buff crystals; yield 64%, 0.347 g; m.p. 157-159°C. IR (KBr):  $v_{\text{max}}$  2,988–2,928 (CH<sub>3</sub>), 2,873 (CH<sub>2</sub>), 2,244 (CN), 1,662, 1,652 (2C=0), 1,643 (C=C) cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$  1.21, 1.37, 1.56 (2s, t, 9H, 3CH<sub>3</sub>), 1.61, 1.82, 3.34 (2m, q, 14H, 7CH<sub>2</sub>), 4.14, 4.32 (2s, 2H), 5.19 (s, 1H), 5.27, 5.52 (2s, 2H), 6.12, 6.27 (d.d, 2H, J =2.54 Hz), 6.52 (m, 3H, pyridine ring), 6.61 (s, 1H, thiophene ring);  ${}^{13}$ C NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  16.2, 17.3, 19.4, 23.3, 25.5, 27.2, 28.7, 29.3, 31.5, 36.2, 43.2, 113.1, 114.8, 121.6, 123.8, 125.8, 128.6, 131.2, 133.5, 135.7, 136.4, 137.9, 139.3, 140.3, 141.4, 143.6, 145.3, 150.2, 155.5, 160.3, 164.1; EI-MS: m/z 542 (M<sup>+</sup>, 33.5%). Ana. Calcd for  $C_{32}H_{34}N_2O_4S$ (542.69): C, 70.82; H, 6.31; N, 5.16; S, 5.91%. Found: C, 70.54; H, 6.62; N, 5.45; S, 5.62%.

2.2.8.4 Ethyl-2-(3-cyano-4,6-dimethyl-2-oxopyridin-1(2H)-vl)-4-((8S,9R,10S,13S,14S,17S)-10,13dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17dodecahydro-1H-cyclopenta[a]phenanthren-17vl)thiophene-3-carboxylate (10d)

Buff crystals; yield 48%, 0.228 g; m.p. 237-239°C. IR (KBr):  $v_{\text{max}}$  2,976–2,912 (CH<sub>3</sub>), 2,878–2,824 (CH<sub>2</sub>), 2,220 (CN), 1,668, 1,652 (2C=0), 1,647 (C=C) cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$  1.13, 1.25, 1.43, 1.68 (2s, t, m, 15H, 5CH<sub>3</sub>), 1.76, 1.93, 3.14 (2m, q, 14H, 7CH<sub>2</sub>), 4.26, 4.36 (2s, 2H), 5.06 (s, 1H), 5.22, 5.33 (2s, 2H), 6.14, 6.39 (d.d, 2H, J = 3.45 Hz), 6.47 (s, 1H, pyridine ring), 6.58 (s, 1H, pyridi1H, thiophene ring);  $^{13}$ C NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  13.7, 15.9, 16.7, 17.8, 19.2, 21.4, 23.6, 26.1, 28.3, 29.7, 30.8, 35.4, 42.7, 114.2, 115.9, 120.6, 121.7, 124.7, 126.9, 130.4, 132.1, 135.5, 136.5, 137.8, 139.2, 140.6, 141.6, 142.7, 146.4, 152.3, 156.3, 158.4, 163.2; EI-MS: m/z 570 (M<sup>+</sup>, 23.8%). Ana. Calcd for C<sub>34</sub>H<sub>38</sub>N<sub>2</sub>O<sub>4</sub>S (570.74): C, 71.55; H, 6.71; N, 4.91; S, 5.62%. Found: C, 71.83; H, 6.42; N, 5.25; S, 5.42%.

# 2.2.9 General procedure for the synthesis of compounds 12a,b

Hydroxyl amine hydrochloride (0.069 g, 0.001 mol) was added to a solution of either compounds 6a (0.46 g, 0.001 mol) or **6b** (0.507 g, 0.001 mol) in absolute ethanol (50 mL) containing sodium acetate (0.082 g, 0.001 mol). The reaction mixture was heated under reflux for 5 h, and now a nonsoluble intermediate 11a,b and the latter final product 12a,b were formed. After cooling the reaction mixture, it was poured onto ice/water containing few drops of HCl. The formed solid product was collected, filtered, dried, and recrystallized from 1,4-dioxane.

# 2.2.9.1 2-(5-Aminoisoxazol-3-ylamino)-4-((85,9R,105,135,145,175)-10,13-dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17-dodecahydro-1Hcyclopenta[a]phenanthren-17-yl)thiophene-3carbonitrile (12a)

Creamy white crystals; yield 66%, 0.313 g; m.p. 218–220°C. IR (KBr):  $v_{\text{max}}$  3,375–3,238 (NH<sub>2</sub>, NH), 2,965–2,944 (2CH<sub>3</sub>), 2,841 (CH<sub>2</sub>), 2,242 (CN), 1,658 (C=O), 1,643 (C=C) cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$  1.23, 1.37 (2s, 6H, 2CH<sub>3</sub>), 2.21, 2.37 (2m, 12H, 6CH<sub>2</sub>), 4.27 (s, 2H, D<sub>2</sub>O-exchangeable, NH<sub>2</sub>), 4.53, 4.69 (2s, 2H), 5.06 (s, 1H), 5.27, 5.52 (2s, 2H), 6.18, 6.34 (d.d, 2H, J = 2.16 Hz), 6.56, 6.74 (s, s, 2H, thiophene, isoxazole rings), 10.38 (s, 1H, D<sub>2</sub>O-exchangeable,

NH); EI-MS: m/z 474 (M<sup>+</sup>, 31.5%). Ana. Calcd for  $C_{27}H_{30}N_4O_2S$ (474.62): C, 68.33; H, 6.37; N, 11.80; S, 6.76%. Found: C, 68.02; H, 6.68; N, 11.55; S, 6.47%.

# 2.2.9.2 Ethyl-2-(5-aminoisoxazol-3-ylamino)-4-((85,9R,105,135,145,175)-10,13-dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17-dodecahydro-1Hcyclopenta[a]phenanthren-17-yl)thiophene-3carboxylate (12b)

Faint brown crystals; yield 58%, 0.302 g; m.p. 237–239°C; IR (KBr):  $v_{\text{max}}$  3,321–3,188 (NH<sub>2</sub>, NH), 2,976–2,930 (2CH<sub>3</sub>), 2,822 (CH<sub>2</sub>), 1,753, 1,653 (2C=0), 1,644 (C=C) cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$  0.93, 1.12, 1.33 (2s, t, 9H, 3CH<sub>3</sub>), 1.86, 2.11, 3.16 (2m, q, 14H, 7CH<sub>2</sub>), 4.41 (s, 2H, D<sub>2</sub>Oexchangeable, NH<sub>2</sub>), 4.56, 4.65 (2s, 2H), 5.11 (s, 1H), 5.21, 5.42 (2s, 2H), 6.22, 6.37 (d.d, 2H, J = 2.28 Hz), 6.63, 6.78 (s, s, 2H, thiophene, isoxazole rings), 10.54 (s, 1H, D<sub>2</sub>Oexchangeable, NH); EI-MS: m/z 521 (M<sup>+</sup>, 28.2%). Ana. Calcd for C<sub>29</sub>H<sub>35</sub>N<sub>3</sub>O<sub>4</sub>S (521.67): C, 66.77; H, 6.76; N, 8.05; S, 6.15%. Found: C, 67.05; H, 6.48; N, 8.32; S, 6.43%.

# 2.2.10 General procedure for the synthesis of compounds 14a,b

Benzoyl acetonitrile (0.145 g, 0.001 mol) was added to the solution of either compound 6a (0.46 g, 0.001 mol) or 6b (0.507 g, 0.001 mol) in sodium ethoxide (0.001 mol) [prepared by dissolving sodium metal (0.023 g, 0.001 mol) in absolute ethanol (50 mL)]. The mixture of the reaction was heated under reflux for 6 h, and the solvent was then evaporated under vacuum. The product was triturated with ethanol, and the formed product was collected by filtration, dried, and recrystallized from 1,4-dioxane.

# 2.2.10.1 6-Amino-1-(3-cyano-4-((85,9R,105,135,145,175)-10,13-dimethyl-3oxo-2,3,8,9,10,11,12,13,14,15,16,17dodecahydro-1H-cyclopenta[a]phenanthren-17yl)thiophen-2-yl)-2-oxo-4-phenyl-1,2dihydropyridine-3-carbonitrile (14a)

Buff crystals; yield 58%, 0.340 g; m.p. 250-251°C. IR (KBr):  $v_{\text{max}}$  3,338 (NH<sub>2</sub>), 3,065 (CH arom.), 2,951, 2,937 (2CH<sub>3</sub>), 2,843 (CH<sub>2</sub>), 2,242, 2,230 (2CN), 1,670, 1,658 (2C=0), 1,643 (C=C) cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$  1.22, 1.35 (2s, 6H, 2CH<sub>3</sub>), 1.56, 1.89 (2m, 12H, 6CH<sub>2</sub>), 3.98, 4.16 (2s, 2H), 4.43 (s, 2H, D<sub>2</sub>O-exchangeable, NH<sub>2</sub>), 4.87 (s, 1H), 5.12,

5.41 (2s, 2H), 5.83, 5.94 (d.d, 2H, J = 2.53 Hz), 6.68, 6.84 (s, s, 2H, thiophene, pyridine rings), 7.23–7.41 (m, 5H,  $C_6H_5$ ); <sup>13</sup>C NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  13.1, 14.8, 24.6, 25.3, 26.3, 27.9, 30.4, 35.3, 43.7, 112.2, 114.3, 120.7, 121.6, 125.8, 126.4, 128.4, 130.9, 131.8, 133.9, 135.3, 136.4, 137.2, 138.6, 139.5, 140.8, 141.7, 142.5, 143.8, 145.9, 148.3, 151.4, 156.1, 158.1, 164.8, 166.2; EI-MS: m/z 586 (M<sup>+</sup>, 16.5%). Ana. Calcd for  $C_{36}H_{34}N_4O_2S$ (586.75): C, 73.69; H, 5.84; N, 9.55; S, 5.46%. Found: C, 73.96; H, 5.60; N, 9.25; S, 5.14%.

2.2.10.2 Ethyl-2-(6-amino-3-cvano-2-oxo-4phenylpyridin-1(2H)-yl)-4-((85,9R,105,135,145,175)-10,13-dimethyl-3oxo-2,3,8,9,10,11,12,13,14,15,16,17dodecahydro-1H-cyclopenta[a]phenanthren-17yl) thiophene-3-carboxylate (14b)

Faint brown crystals; yield 66%, 0.418 g; m.p. 272-274°C. IR (KBr):  $v_{\text{max}}$  3,340 (NH<sub>2</sub>), 3,054 (CH arom.), 2,963–2,942 (CH<sub>3</sub>), 2,856 (CH<sub>2</sub>), 2,234 (CN), 1,778, 1,668, 1,654 (3C=0), 1,641 (C=C) cm<sup>-1</sup>, <sup>1</sup>H NMR (390-200 MHz, DMSO- $d_6$ ):  $\delta$ 0.87, 1.19, 1.97 (2s, t, 9H, 3CH<sub>3</sub>), 1.69, 1.93, 3.25 (2m, q, 14H, 7CH<sub>2</sub>), 3.87, 4.05 (2s, 2H), 4.35 (s, 2H, D<sub>2</sub>O-exchangeable, NH<sub>2</sub>), 4.64 (s, 1H), 5.23, 5.47 (2s, 2H), 5.76, 5.91 (d.d, 2H, J = 2.84 Hz), 6.53, 6.73 (s, s, 2H, thiophene,pyridine rings), 7.18-7.39 (m, 5H,  $C_6H_5$ );  $^{13}C$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  13.7, 14.9, 17.2, 23.5, 24.8, 26.7, 28.6, 30.9, 34.7, 41.8, 111.9, 114.8, 119.6, 122.7, 124.4, 126.5, 127.8, 129.6, 131.5, 132.7, 134.6, 136.3, 137.5, 138.9, 139.8, 141.6, 142.4, 143.6, 144.7, 145.8, 147.3, 150.6, 155.3, 157.9, 163.5, 165.5; EI-MS: m/z 633 (M<sup>+</sup>, 14.8%). Ana. Calcd for C<sub>38</sub>H<sub>39</sub>N<sub>3</sub>O<sub>4</sub>S (633.80): C, 72.01; H, 6.20; N, 6.63; S, 5.06%. Found: C, 71.70; H, 5.92; N, 6.35; S, 5.38%.

# 2.3 Antitumor activity tests

All the reagents and chemicals, penicillin, streptomycin, doxorubicin, sulforhodamine B (SRB), and dimethyl sulfoxide (DMSO), used for antitumor activity tests were obtained from Sigma Chemical Co. (USA). Fetal bovine serum (FBS) and L-glutamine were purchased from Gibco Invitrogen Co. (UK). RPMI-1640 medium was obtained from Cambrex (USA).

The three human tumor cell lines that are used for the evaluation are as follows: SF-268 (CNS cancer), MCF-7 (breast adenocarcinoma), and NCI-H460 (non-small lung cancer cell). The SF-268, NCI-H460, and normal fibroblast cells (WI-38) were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt), and MCF-7 was obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK).

The cell lines were grown as monolayers and were routinely maintained in RPMI-1640 medium supplemented with 5% heat-inactivated FBS, antibiotics (penicillin  $100 \text{ U mL}^{-1}$  and streptomycin  $100 \text{ µg mL}^{-1}$ ), and  $2 \text{ mmol L}^{-1}$ glutamine at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. Exponentially growing cells were obtained by plating 1.5  $\times$  105 cell mL<sup>-1</sup> for SF-268 and MCF-7 and  $0.75 \times 104 \text{ cell mL}^{-1}$  for NCI-H460, followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.

Tumor cell growth assay: The effects on the in vitro growth of human tumor cell lines were evaluated on the newly synthesized compounds 1a,b, 2a-d, 4-10a-d, 12a,b, and 14a,b according to the specified procedure by the National Cancer Institute (NCI, USA) [16] that uses the protein-binding dye sulforhodamine B to consider the cell growth. Exponentially growing cells in 96-well plates were exposed for 48 h to five serial concentrations of each synthesized compound starting from the maximum concentration of 150  $\mu$ mol L<sup>-1</sup>. After this exposure period, the adherent cells were fixed, washed, and stained. The bound stain was dissolved in dimethylsulfoxide, and then, the absorbance was measured at 492 nm in a plate reader (Power wave XS, Bio-Tec Instruments, USA). For each test compound and cell line, a dose-response curve was obtained and the growth inhibition of 50% (GI<sub>50</sub>; corresponding to the concentration of each compound that inhibited 50% of the net cell growth) was calculated as described earlier [17]. Doxorubicin was used as a positive control test, and it was tested under the same condition.

**Ethical approval:** The conducted research is not related to either human or animal use.

# **Results and discussion**

#### 3.1 Chemistry

In the framework of our ongoing attempts of our team to try to develop and synthesize some of the heterocyclic compound derivatives with interesting antitumor activities [18,19], here we designed the newly dydrogesterone

Scheme 1: Synthesis of compounds 1a,b, 2a-d, 4 and 5a,b.

heterocyclic extension compounds, such as thiophene, thienopyrimidine, pyrimidine, pyrazole, thienopyridine, coumarin, and isoxazole derivatives, Schemes 1–4. The reaction of dydrogesterone with either malononitrile or ethylcyanoacetate together with elemental sulfur to form 2-aminothiophene derivatives 1a,b, the structure of such compounds 1a,b was established using analytical and spectral data. Fused thiophene derivatives were afforded through the reaction of the latter compounds 1a,b with

either isothiocyanate derivatives or ethylcyanoacetate to afford thienopyrimidine derivatives  $2\mathbf{a}-\mathbf{d}$  and thienopyridine derivatives  $7\mathbf{a}$ ,  $\mathbf{b}$ , respectively. The structure of compounds  $2\mathbf{a}-\mathbf{d}$  and  $7\mathbf{a}$ ,  $\mathbf{b}$  was established by applying analytical and spectral data; the compound  $2\mathbf{a}^{-1}\mathbf{H}$  NMR spectrum indicated the presence one singlet at  $\delta=6.78$  ppm because of the presence of 1H of thiophene ring, and multiplet at  $\delta=7.33-7.54$  ppm indicates the presence of 5H of phenyl group, broad signal at  $\delta=8.68$  ppm

$$1a,b + NC$$

$$C = \frac{1}{10}$$

$$C = \frac{1$$

Scheme 2: Synthesis of compounds 6a,b, 7a,b, 8a,b and 9a,b.

corresponding to 1H of SH group and one singlet,  $D_2O$ -exchangeable at  $\delta = 9.11$  ppm because of the presence of 1H of NH group. In addition, the mass spectrum revealed m/z at 527  $[M]^+$  and m/z at 77  $[C_6H_5]^+$  for the phenyl moiety. In addition, compounds **7a,b** were obtained through the reaction of ethylcyanoacetate with compounds **1a,b** to produce compounds **6a,b**, which underwent cyclization afforded fused thiophene derivatives **7a,b**. The structures of these compounds are confirmed

using analytical and spectral data. Also, the reaction of ethylcyanoacetate with dydrogesterone in the presence of hydrazine, urea, or thiourea afforded either pyrazole derivative **4** or pyrimidine derivatives **5a,b**. The structure of compound **5a** was proved using analytical and spectral data; <sup>1</sup>H NMR spectrum showed singlet,  $D_2O$ -exchangeable at  $\delta = 4.12$  ppm because of the presence of 2H of NH<sub>2</sub> group, singlet at  $\delta = 7.45$  ppm,  $D_2O$ -exchangeable because of the presence of 1H of OH group. Furthermore,

Scheme 3: Synthesis of compounds 10a-d and 12a,b.

the  $^{13}$ C NMR spectrum revealed two signals at  $\delta=119.8$  and 121.6 ppm because of the presence of C=C group and three signals at  $\delta=130.8$ , 132.5, 139.2 ppm corresponding to the pyrimidine ring. A series of chemical reaction were acceptable of compounds 6a,b; thus, cyanoacetamidothiophene derivatives 6a,b were reacted with either salicylaldehyde or cyclopentanone and elemental sulfur to form coumarin derivatives 8a,b and cyclopenta[b]thiophene derivatives 9a,b, respectively. The structure of such compounds was confirmed according to the analytical and spectral data. Compound 8a was proved using the analytical and spectral data;  $^{1}$ H NMR spectrum showed the presence of singlet at  $\delta=6.57$  ppm because

of the presence of 1H of coumarin ring, multiplet at  $\delta$  = 7.28–7.41 ppm corresponding to 4H of benzene ring. In addition, IR spectrum of compound **8a** detected the presence of stretching vibration bands of the CN group at  $v_{\rm max}$  = 2,228 cm<sup>-1</sup> (Schemes 1 and 2).

However, compounds **6a,b** were directed toward the reaction with either 1,3-dicarbonyl compounds or hydroxylaminehydrochloride to produce pyridine derivatives **10a-d** or isoxazole derivatives **12a,b** through intermediate formation **11a,b**, respectively (Scheme 3). The structure of compounds **10a-d** and **12a,b** was confirmed by the analytical and spectral data. The <sup>1</sup>H NMR spectrum of compound **10a** showed the presence of multiplet at

Scheme 4: Synthesis of compounds 14a,b.

 $\delta$  = 6.48 ppm, which indicated 3H of pyridine ring, singlet at  $\delta$  = 6.56 ppm because of the presence of 1H of thiophene ring. Also, the <sup>13</sup>C NMR spectrum revealed eight signals at  $\delta$  = 138.2, 139.9, 140.5, 142.1, 143.7, 147.5, 151.9, and 159.4 ppm because of the presence of thiophene ring and pyridine ring. Furthermore, the mass spectrum revealed m/z at 495 [M]<sup>+</sup>. Finally, the reaction of compounds **6a,b** with benzoyl acetonitrile afforded phenyl-pyridine derivatives **14a,b** through intermediate formation **13a,b**. The isomeric structures **15a,b** were excluded (Scheme 4). The <sup>1</sup>H NMR spectral data in compounds **14a,b** showed that the final products contained two singlets at  $\delta$  = 4.43 and 4.35 ppm, respectively, which represented the presence of NH<sub>2</sub> groups. However, both OH and NH groups of compounds **15a,b** were absent.

# 3.2 Effect on the growth of human tumor cell lines and OSAR

The inhibitory effect of the newly synthesized compounds **1a,b–14a,b** was evaluated on the *in vitro* growth of the three human tumor cell lines representing different tumor types, namely, breast adenocarcinoma (MCF-7), nonsmall cell lung cancer (NCI-H460), and CNS cancer (SF-268) after a continuous exposure for 48 h. All of the synthesized products **1a,b–14a,b** were able to inhibit the growth of the tested human tumor cell lines in a dose-dependent manner. The results (Table 1) showed that benzoyl thienopyrimidine derivative **2d** is the highest one effective against all the three different tumor cell lines, (SF-268), (MCF-7), and (NCI-H460), with respect to the

reference standard material (doxorubicin). In addition, phenyl thienopyrimidine derivative **2c** showed the highest inhibitory effect against all the three tumor cell lines, (SF-268), (MCF-7), and (NCI-H460), corresponding to the remaining synthesized compounds. However, imino-thienopyrimidine derivatives **2a,b**, hydroxypyrimidine derivatives **5a,b**, cyanoacetamido thiophene derivatives **6a,b**, and thienopyridine derivatives **10a**–**d** showed good inhibitory effects against three cancer cell lines, (SF-268), (MCF-7), and (NCI-H460). Regarding the remaining, compounds **1a,b**, **4**, **7a,b**, **8a,b**, **9a,b**, **12a,b**, and **14a,b** showed a moderate growth in the inhibitory effect.

Comparing thienopyrimidine derivatives **2a-d**, it was found that compound 2d acts as the most effective one, that is may be because of the presence of benzoylpyrimidinone moiety. Also, comparing thienopyridine compound derivatives 10a-d, one can say that compound 10a is the most effective one in this group. It may be because of the presence of cyano group with the absence of the methyl group. Furthermore, on comparing compounds hydroxypyrimidine derivatives **5a,b** and cyanoacetamido thiophene derivatives **6a,b**, although these compounds nearly has the same good inhibitory effect against three different cell lines, (SF-268), (MCF-7), and (NCI-H460), it may be that they are containing hydroxypyrimidine and cyanoacetamidothiophene moieties, respectively, but it was clear that compound **5b** is slightly more effective than the remaining compounds 5a,6a,b on three different tumor cell lines, (SF-268), (MCF-7), and (NCI-H460). It may be because of containing thiopyrimidine moiety. In addition, comparing compounds 1a,b, 4, 7a,b, 8a,b, 9a,b, 12a,b, and 14a,b, it was clear that they have a moderate inhibitory effect, but compound 1b showed that it is the most effective one in this groups. It may be because of the presence of both amino and ethylcarboxylate groups of thiophene moiety.

The results are presented in concentrations that were able to cause 50% of cell growth inhibition ( $GI_{50}$ ) after a continuous exposure for 48 h and show means  $\pm$  SEM of three independent experiments performed in duplicate.

# 4 Conclusion

In this article, the synthesized compounds of dydrogesterone heterocyclic extension such as thiophene, thienopyrimidine, pyrimidine, pyrazole, thienopyridine, coumarin, and isoxazole derivatives were investigated to detect the antitumor activity of such compounds against three different cell lines. Among the synthesized compounds, benzoyl thienopyrimidine derivative **2d** showed the highest one

effective against all the three different tumor cell lines, (SF-268), (MCF-7), and (NCI-H460), with respect to the reference standard material (doxorubicin). In addition, phenyl thienopyrimidine derivative **2c** showed the highest inhibitory effect against all the three tumor cell lines, (SF-268), (MCF-7), and (NCI-H460), corresponding to the remaining synthesized compounds.

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**Author contributions:** K. A. and H. A.: conceived the research formulations and information of the synthesized compounds and wrote the paper; M. M.: performed and established the structures by applying different spectral data; M. M.: performed to integrate and maintain the obtained research data; K. A. and A. N.: prepared, write, and revised the manuscript for publication.

**Conflict of interest:** The authors declare no conflict of interest.

**Data availability statement:** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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