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Heterocyclic ring extension of androstenedione: Synthesis and cytotoxicity of fused pyran, pyrimidine and thiazole derivatives

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ABSTRACT

The reaction of androstenedione with either malononitrile or ethyl cyanoacetate and aromatic aldehydes **2a-c** gave the pyran derivatives **4a-f**, respectively. On the other hand, the reaction of androstenedione with thiourea and the aromatic aldehydes **2a-c** gave the pyrimidine derivatives **6a-c**, respectively. Compound **6b** reacted with 2-bromo-1-arylethanone derivatives **7a-d** to give the indeno[2,1-*e*]thiazole derivatives **8a-d**. Some of the produced compounds were used for further heterocyclization reactions. The cytotoxicity of the newly obtained products was evaluated against some cancer cell lines and a normal cell line.

1. Introduction

Steroidal compounds are widely existent in natural world and display a variety of biological activities [1–5]. Some steroidal compounds have been used as traditional medicines, such as, antibacterium and hormone kind medication. Besides the naturally occurring substances, the majority of steroidal drugs are semi-synthetic compounds [6–9]. The introduction of heteroatom, heterocycle or replacement of one or more atoms in the structure of the maternal steroids often results in alterations of its biological properties, for example, enhancing the cytotoxicity against some tumour cell lines [10–15]. Increasing the selectivity and minimizing the side effects are still the priority of the medicinal chemists. Due to their diverse biological properties and wide applications, heterocyclic compounds have gained plenty of attention. These moieties exist not only in naturally occurring compounds, like alkaloids, vitamins, hormones and antibiotics, but also in pharmaceutical synthetic herbicides and dyes [16]. Nitrogen containing heterocyclic systems have prevailed for decades for their various applications [17].

Androstenedione is one of many naturally-occurring steroidal compounds in common use as dietary supplements. These supplements are used for their alleged ability to enhance athletic performance by virtue of their metabolic conversion to testosterone. In vivo, androstenedione is the immediate biosynthetic precursor of both testosterone and estrone supra-physiological levels of androgenic steroids are known to have adverse health consequences in humans including: endocrine disruption (e.g., masculinization in females), hepatotoxicity (peliosis hepatitis, cholestatic jaundice, hepatocellular adenomas), and cardiovascular toxicity (e.g., decreased HDL). Because of these potential health risks, the USFood and Drug Administration mandated that products containing androstenedione could no longer be sold and distributed as dietary supplements (FDA, 2004). Although frank hepatotoxicity in humans is associated with both the therapeutic and illicit use of 17-a-alkylated anabolic–androgenic steroids [18], no clear association with hepatotoxicity has been established for non-alkylated steroids such as androstenedione. To our knowledge there is reported works concerning the ring D extension of androstenedione. Thus, our main aim in this work is to study the heterocyclization of androstenedione together with studying the cytotoxicity of the newly synthesized products against cancer and normal cell lines. Chemical modification of the steroid D-ring provides a way to alter the functional groups, sizes and stereochemistry of the D-ring, and numerous structure–activity relationships have been established by such synthetic alterations [19–21]. Steroids bearing heterocycles fused to the D-ring of the steroid nucleus have been of pharmaceutical interest [22–24]. This study

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was focused on the efficient synthesis of androstenedione possessing pyran, pyrimidine and thiazole ring systems.

2. Experimental

2.1. Synthetic methods, analytical and spectral data

The starting steroid, androstenedione (**1**), 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; ^{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 δ (ppm). Mass spectra were recorded on EIMS (Shimadzu) and ESI-esquire 3000 Bruker Daltonics instrument. Elemental analyses were carried out 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 nomenclature of the newly synthesized compounds were according to the ChemBioDraw Ultra12.

2.2. Chemical syntheses

*2.2.1. (6aR,6bS,8aS,13aS,13bR)-10-amino-6a,8a-dimethyl-4-oxo-12-phenyl-1,2,4,5,6,6a,6b-,7,8,8a,12,13,13a,13b-tetradecahydronaphtho-[2′,1′:4,5]indeno[1,2-b]pyran-11-carbonitrile (**4a**), (6aR,6bS,8aS,13aS,13bR)-10-amino-6a,8a-dimethyl-4-oxo-12-pyridyl-1,2,4,5,6,6a,6b,-7,8,8a,12,13,13a,13b-tetradecahydronaphtho[2′,1′:4,5]indeno[1,2-b]pyran-11-carbonitrile (**4b**), (6aR,6bS,8aS,13aS,13bR)-10amino-6a,8a-dimethyl-4-oxo-12-thienyl-1,2,4,5,6,-6a,6b,7,8,8a,12,13,13a,13b-tetradecahydronaphtho[2′,1′:4,5]indeno[1,2-b]pyran-11-carbonitrile (**4c**), (6aR,6bS,8aS,13aS,13bR)-10-hydroxy-6a,8a-dimethyl-4-oxo-12-phenyl-1,2,4,5,6,6a,6b,7,8,8a,12,13,13a,13b-tetradecahydronaphtho[2′,1′:4,5]indeno[1,2-b]pyran-11-carbonitrile (**4d**), (6aR,6bS,8aS,13aS,13bR)-10-hydroxy-6a,8a-dimethyl-4-oxo-12-pyridyl-1,2,4,5,6,6a,6b,7,8,8a,12,13,13a,13b-tetradecahydron-aphtho[2′,1′:4,5]indeno[1,2-b]pyran-11-carbonitrile (**4e**) and (6aR,6bS,8aS,13aS,13bR)-10-hydroxy-6a,8a-dimethyl-4-oxo-12-thienyl-1,2,4,5,6,6a,6b,7,8,8a,12,13,13a,13b-tetradecahydrona-phtho[2′,1′:4,5]indeno[1,2-b]pyran-11-carbonitrile (**4f**)*

General procedure: To a solution of androstenedione (0.286 g, 1 mmol) in absolute ethanol (40 mL) containing triethylamine (0.025 mL), either of malononitrile (0.066 g, 1 mmol) or ethyl cyanoacetate (0.113 g, 1 mmol) and either of the aromatic aldehydes namely benzaldehyde (106 g, 1 mmol), pyridine-3-aldehyde (0.107 g, 1 mmol) or thiophene-2-aldehyde (0.112 g, 1 mmol) were added. The reaction mixture was heated under reflux for 1 h and the formed solid product produced from the hot solution was collected by filtration. Thin layer chromatography revealed just a single spot which proved the presence of a single product.

Compound ***4a****:* HPLC purity = 90% (C-18 NovaPak column; MeOH:H_2O/70:30), t_r = 20 min; pale yellow crystals from EtOAc: hexane (89%), m.p. 220–224 °C; IR (KBr) cm^{-1}: 3540, 3423, 3057, 2932, 2222, 1667, 1563; ^{1}H-NMR ($CDCl_3$): δ 0.84, 1.01 (2s, 6H), 1.33–2.86 (m, 10H), 2.89–2.95 (m, 4H), 4.23 (s, 2H, NH_2), 5.01, 5.20 (2s, 2H), 5.37 (s, 1H), 6.11 (s, 1H), 6.44 (d, 1H, J = 2.3 Hz), 6.85–7.38 (m, 5H); ^{13}C-NMR ($CDCl_3$): δ 17.0,17.9, 19.8, 23.1, 25.3, 29.2, 30.2, 33.7, 34.4, 34.8, 41.8, 43.8, 44.0, 53.2, 117.8, 127.8, 124.2, 125.9, 128.6, 129.3, 144.3, 146.2, 148.9, 190.8. MS: m/e = 440 (M^+, 28%); *Analysis Calcd* for $C_{29}H_{32}N_2O_2$: C, 79.06; H, 7.32; N, 6.36%. Found: C, 79.22; H, 7.41; N, 6.55%.

Compound ***4b****:* HPLC purity = 92% (C-18 NovaPak column; MeOH:H_2O/80:20), t_r = 22 min; pale yellow crystals from EtOAc: hexane (86%), m.p. 180–182 °C; IR (KBr) cm^{-1}: 3485, 3430, 3054, 2936, 2220, 1683, 1636, 1567; ^{1}H-NMR ($CDCl_3$): δ 0.86, 1.04 (2s, 6H), 1.33–2.84 (m, 10H), 2.90–2.93 (m, 4H), 4.22 (s, 2H, NH_2), 5.11, 5.21 (2s, 2H), 5.38 (s, 1H), 6.11 (s, 1H), 6.41 (d, 1H, J = 2.20 Hz), 6.85–7.38 (m, 4H); ^{13}C-NMR ($CDCl_3$): δ 17.2,17.6, 19.9, 23.1, 25.3, 29.2, 30.4, 33.9, 34.4, 34.8, 41.9, 43.6, 44.1, 53.0, 117.8, 124.5, 134.6, 145.8, 147.3, 150.2, 190.3. MS: m/e = 441 (M^+, 48%); *Analysis Calcd* for $C_{28}H_{31}N_3O_2$: C, 76.16; H, 7.08; N, 9.52%. Found: C, 76.08; H, 6.88; N, 9.36%.

Compound ***4c****:* HPLC purity = 89% (C-18 NovaPak column; MeOH:H_2O/82:18), t_r = 20 min; pale yellow crystals from EtOAc: hexane (84%), m.p. 169–171 °C; IR (KBr) cm^{-1}: 3530, 3443, 3054, 2936, 2222, 1636, 1560; ^{1}H-NMR ($CDCl_3$): δ 0.84, 1.03 (2s, 6H), 1.35–2.87 (m, 10H), 2.89–2.92 (m, 4H), 4.48 (s, 2H, D_2O exchangeable), 5.13, 5.20 (2s, 2H), 5.68 (s, 1H), 6.10 (s, 1H), 6.45 (d, 1H, J = 3.03 Hz), 7.32–7.44 (m, 3H); ^{13}C-NMR ($CDCl_3$): δ 17.2, 17.9 19.5, 22.9, 23.3, 26.9, 27.4, 32.4, 38.8, 40.5, 45.3, 110.8, 128.9,134.2, 136.2, 148.1, 190.4; MS: m/e = 446 (M^+, 28%); *Analysis Calcd* for $C_{27}H_{30}N_2O_2S$: C, 72.61; H, 6.77; N, 7.27; S, 7.18%. Found: C, 72.82; H, 6.59; N, 6.48; S, 7.49%.

Compound ***4d****:* HPLC purity = 88% (C-18 NovaPak column; MeOH:H_2O/70:30), t_r = 21 min; pale yellow crystals from EtOAc: hexane (88%), m.p. 244–247 °C; IR (KBr) cm^{-1}: 3595–3420, 3054, 2936, 2223, 1636, 1562; ^{1}H-NMR ($CDCl_3$): δ 0.83, 1.60 (2s, 6H), 1.30–2.88 (m, 10H), 2.72–2.92 (m, 4H), 5.11, 5.20 (2s, 2H), 5.68 (s, 1H), 6.10 (s, 1H), 6.40 (d, 1H, J = 3.32 Hz), 7.29–7.41 (m, 5H), 8.22 (s, 1H, D_2O exchangeable); ^{13}C-NMR ($CDCl_3$): δ 17.4, 17.8, 19.5, 22.8, 23.2, 26.6, 27.8, 31.6, 38.8, 40.4, 45.4, 112.9, 117.4, 120.7, 123.5, 128.4, 129.3, 137.2, 143.0, 148.3, 190.4. MS: m/e = 441 (M^+, 32%); *Analysis Calcd* for $C_{29}H_{31}NO_3$: C, 78.88; H, 7.08; N, 3.17%. Found: C, 79.03; H, 6.89; N, 3.28%.

Compound ***4e****:* HPLC purity = 80% (C-18 NovaPak column; MeOH:H_2O/90:10), t_r = 18 min; yellow crystals from EtOAc: hexane (86%), m.p. 102–104 °C; IR (KBr) cm^{-1}: 3587–3432, 3056, 2938, 2225, 1638, 1562; ^{1}H-NMR ($CDCl_3$): δ 0.86, 1.7 (2s, 6H), 1.33–2.89 (m, 10H), 2.91–2.89 (m, 4H), 5.09, 5.18 (2s, 2H), 5.65 (s, 1H), 6.12 (s, 1H), 6.48 (d, 1H, J = 2.77 Hz), 7.29–7.41 (m, 4H), 8.21 (s, 1H, D_2O exchangeable); ^{13}C-NMR ($CDCl_3$): δ 17.3, 17.5, 19.5, 23.0, 23.3, 26.6, 27.6, 32.2, 38.4, 40.7, 45.8, 110.7, 128.6,135.7, 138.0, 150.3, 190.6; MS: m/e = 442 (M^+, 33%); *Analysis Calcd* for $C_{28}H_{30}N_2O_3$: C, 75.99; H, 6.83; N, 6.33%. Found: C, 76.03; H, 6.77; N, 6.52%.

Compound ***4f****:* HPLC purity = 84% (C-18 NovaPak column; MeOH:H_2O/88:12), t_r = 23 min; yellow crystals from EtOAc: hexane (77%), m.p. 220–224 °C; IR (KBr) cm^{-1}: 3554–3428, 3053, 2936, 2223, 1635, 1560; ^{1}H-NMR ($CDCl_3$): δ 0.83, 1.03 (2s, 6H), 1.30–2.89 (m, 10H), 2.87–2.91 (m, 4H), 5.11, 5.20 (2s, 2H), 5.64 (s, 1H), 6.11 (s, 1H), 6.49 (d, 1H, J = 3.63 Hz), 7.26–7.43 (m, 3H), 8.23 (s, 1H, D_2O exchangeable); ^{13}C-NMR ($CDCl_3$): δ 17.0, 17.9, 19.8, 23.0, 23.0, 26.6, 27.6, 32.3, 38.6, 40.2, 45.9, 110.8, 117.4, 123.8, 128.5, 129.3, 137.6, 143.0, 148.6, 190.2; MS: m/e = 447 (M^+, 24%); *Analysis Calcd* for $C_{27}H_{29}NO_3S$: C, 72.45; H, 6.53; N, 3.13; S, 7.16%. Found: C, 72.30; H, 6.68; N, 3.44; S, 6.09%.

*2.2.2. (6bS,aS,13aS,13bR)-10-mercapto-8a-methyl-12-phenyl-5,6,6a,6b,7,8,8a,9,12,13,-13a,13b-dodecahydro-1H-naphtho-[2′,1′:4,5]indeno[1,2-d]pyrimidin-4(2H)-one (**6a**), (6bS,aS,13aS,13bR)-10-mercapto-8a-methyl-12-(pyridine-3-yl)-5,6,6a,6b,7,8,8a,9,12,-13,13a,13b-dodecahydro-1H-naphtho-[2′,1′:4,5]indeno[1,2-d]pyrimidin-4(2H)-one (**6b**), and (6bS,aS, 13aS,13bR)-10-mercapto-8a-methyl-12-(thienyl-2-yl)-5,6,6a,6b,7, 8,8a,9,12,13,13a,-13b-dodecahydro-1H-naphtho[2′,1′:4,5]indeno[1,2-d]pyrimidin-4(2H)-one (**6c**)*

General procedure: To a solution of androstenedione (0.286 g, 1 mmol) in absolute ethanol (40 mL) containing triethylamine (0.025 mL) and thiourea (0.76 g, 0.01 mol) either of benzaldehyde

(106 g, 1 mmol), pyridine-3-aldehyde (0.107 g, 1 mmol) or thiophene-2-aldehyde (0.112 g, 1 mmol) were added. The reaction mixture was heated under reflux for 1 h and the formed solid product produced from the hot solution was collected by filtration. Thin layer chromatography revealed just a single spot which proved the presence of a single product.

Compound ***6a****:* HPLC purity = 88% (C-18 NovaPak column; MeOH:H_2O/75:25), t_r = 23 min; yellow crystals from EtOAc: hexane (86%), m.p. 190–192 °C; IR (KBr) cm^{-1}: 3455–3225, 3053, 2930, 1635, 1562; ^{1}H-NMR ($CDCl_3$): δ 0.86, 1.03 (2s, 6H), 1.31–2.88 (m, 10H), 2.90–2.97 (m, 4H), 5.09, 5.22 (2s, 2H), 5.38 (s, 1H), 6.10 (s, 1H), 6.18 (s,1H), 6.47 (d, 1H, J = 2.3 Hz), 7.03–7.39 (m, 5H), 8.30 (s, 1H, D_2O exchangeable); ^{13}C-NMR ($CDCl_3$): δ 17.0,17.9, 19.8, 23.1, 25.3, 29.2, 30.2, 33.7, 34.4, 34.8, 41.8, 43.8, 44.0, 53.2, 126.8, 127.0, 128.6, 129.3, 144.3, 146.2, 148.9, 172.3, 190.3. MS: m/e = 432 (M^+, 39%); *Analysis Calcd* for $C_{27}H_{32}N_2OS$: C, 74.96; H, 7.46; N, 6.48; S, 7.41%. Found: C, 74.88; H, 7.38; N, 6.72; S, 7.63%.

Compound ***6b****:* HPLC purity = 85% (C-18 NovaPak column; MeOH:H_2O/78:22), t_r = 21 min; yellow crystals from EtOAc: hexane (86%), m.p. 144–146 °C; IR (KBr) cm^{-1}: 3455–3232, 3057, 2932, 1675, 1520; ^{1}H-NMR ($CDCl_3$): δ 0.88, 1.04 (2s, 6H), 1.30–2.84 (m, 10H), 2.90–2.95 (m, 4H), 5.12, 5.20 (2s, 2H), 5.34 (s, 1H), 6.11 (s, 1H), 6.18 (s, 1H), 6.40 (d, 1H, J = 3.55 Hz), 7.03–7.39 (m, 4H), 8.30 (s, 1H, D_2O exchangeable); ^{13}C-NMR ($CDCl_3$): δ 17.0,17.9, 19.8, 23.1, 25.3, 29.2, 30.2, 33.7, 34.4, 34.8, 41.8, 43.8, 44.0, 53.2, 126.8, 127.4, 128.7, 129.3, 144.3, 145.2, 147.9, 172.3, 190.1. MS: m/e = 433 (M^+, 24%); *Analysis Calcd* for $C_{26}H_{31}N_3OS$: C, 71.02; H, 7.21; N, 9.69; S, 7.39%. Found: C, 71.17; H, 7.29; N, 9.88; S, 7.44%.

Compound **6c**: HPLC purity = 84% (C-18 NovaPak column; MeOH:H_2O/80:20), t_r = 18 min; yellow crystals from EtOAc: hexane (87%), m.p. 133–135 °C; IR (KBr) cm^{-1}: 3544–3426, 3050, 2962, 1680, 1562; ^{1}H-NMR ($CDCl_3$): δ 0.86, 1.03 (2s, 6H), 1.32–2.89 (m, 10H), 2.83–2.902 (m, 4H), 5.19, 5.22 (2s, 2H), 5.64 (s, 1H), 6.11 (s, 1H), 6.18 (s, 1H), 6.47 (d, 1H, J = 3.22 Hz), 7.31–7.40 (m, 3H), 8.22 (s, 1H, D_2O exchangeable); ^{13}C-NMR ($CDCl_3$): δ 14.6, 19.5, 22.4, 23.5, 26.5, 27.4, 32.4, 38.8, 40.5, 45.0, 110.3, 128.4,135.4, 138.5, 149.6, 172.0, 190.2; MS: m/e = 438 (M^+, 33%); *Analysis Calcd* for $C_{25}H_{30}N_2OS_2$: C, 68.45; H, 6.89; N, 6.39; S, 14.62%. Found: C, 68.79; H, 6.83; N, 6.52; S, 14.87%.

*2.2.3. (6bS,8aS,15aS,15bR)-8a-methyl-10-phenyl-14-(pyridine-3-yl)dodecahydronaphtho-[2′,1′:4,5]indeno[2,1-e]thiazolo[3,2-a]-pyrimidin-4(2H)one (**8a**), (6bS,8aS,15aS,15bR)-8a-methyl-10-phenyl-14-(4-chlorophenyl)dodecahydronaphtho-[2′,1′:4,5]indeno[2,1-e]-thiazolo[3,2-a]pyrimidin-4(2H)one (**8b**), (6bS,8aS,15aS,15bR)-8a-methyl-10-phenyl-14-(4-methylphenyl)dodecahydronaphtho-[2′,1′:4,5]indeno[2,1-e]thiazolo[3,2-a]pyrimidin-4(2H)one (**8c**) and (6bS,8aS,15aS,15bR)-8a-methyl-10-phenyl-14-(4-methoxyphenyl)-dodecahydronaphtho[2′,1′:4,5]indeno[2,1-e]thiazolo[3,2-a]pyrimidin-4(2H)one (**8d**)*

General procedure: To a solution of compound **6b** (0.419 g, 1 mmol) in ethanol (40 mL) either 2-bromo-1-phenylethanone (0.20 g,1 mmol), 2-bromo-1-(4-chlorophenyl)ethanone (0.23 g, 1 mmol), 2-bromo-1-(4-methylphenyl)ethanone (0.21 g, 1 mmol) or 2-bromo-1-(4-methoxyphenyl)ethanone (0.23 g, 1 mmol) was added. The reaction mixture, in each case, was heated under reflux for 1 h then poured onto ice/water 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 = 85% (C-18 NovaPak column; MeOH:H_2O/80:20), t_r = 16 min; yellow crystals from EtOAc: hexane (88%), m.p. 230–233 °C; IR (KBr) cm^{-1}: 3057, 2932, 1638, 1566; ^{1}H-NMR ($CDCl_3$): δ 0.88, 1.04 (2s, 6H), 1.30–2.85 (m, 10H), 2.88–2.95 (m, 4H), 5.13, 5.20 (2s, 2H), 5.39 (s, 1H), 6.09 (s, 1H), 6.28 (s, 1H), 6.43 (d, 1H, J = 3.03 Hz), 7.28–7.41 (m, 9H); ^{13}C-NMR ($CDCl_3$): δ 17.3,17.8, 19.9, 23.4, 25.6, 29.4, 30.1, 33.7, 34.6, 34.8, 41.8, 43.9, 44.2, 53.0, 120.3, 122.8, 126.5, 127.1, 128. 8, 133.4, 144.8, 146.2, 149.2, 172.3, 176.2, 190.1. MS: m/e = 533 (M^+, 44%); *Analysis Calcd* for $C_{34}H_{35}N_3OS$: C, 76.51; H, 6.61; N, 7.87; S, 6.01%. Found: C, 76.38; H, 6.51; N, 6.39; S, 6.08%.

Compound ***8b****:* HPLC purity = 81% (C-18 NovaPak column; MeOH:H_2O/80:20), t_r = 18 min; yellow crystals from EtOAc: hexane (80%), m.p. 266–268 °C; IR (KBr) cm^{-1}: 3053, 2931, 1635, 1567; ^{1}H-NMR ($CDCl_3$): δ 0.89, 1.04 (2s, 6H), 1.32–2.88 (m, 10H), 2.85–2.96 (m, 4H), 5.12, 5.22 (2s, 2H), 5.35 (s, 1H), 6.10 (s, 1H), 6.25 (s,1H), 6.42 (d, 1H, J = 3.03 Hz), 7.28–7.41 (m, 8H); ^{13}C-NMR ($CDCl_3$): δ 17.1,17. 6, 19.9, 23.6, 25.6, 29.4, 30.1, 33.8, 34.6, 34.8, 41.5, 43.9, 44.0, 53.2, 120.4, 122.6, 126.3, 127.2, 128. 8, 133.4, 144.9, 146.3, 149.6, 172.1, 176.3, 190.4. MS: m/e = 568 (M^+, 56%); *Analysis Calcd* for $C_{34}H_{34}ClN_3OS$: C, 71.87; H, 6.03; N, 7.40; S, 5.64%. Found: C, 71.66; H, 5.93; N, 7.44; S, 5.82%.

Compound ***8c****:* HPLC purity = 86% (C-18 NovaPak column; MeOH:H_2O/90:10), t_r = 20 min; Orange from EtOAc: hexane (83%), m.p. 210–212 °C; IR (KBr) cm^{-1}: 3057, 2930, 1637, 1569; ^{1}H-NMR ($CDCl_3$): δ 0.87, 1.04 (2s, 6H), 1.30–2.88 (m, 10H), 2.87–2.98 (m, 4H), 3.18 (s, 3H), 5.10, 5.22 (2s, 2H), 5.35 (s, 1H), 6.10 (s, 1H), 6.28 (s,1H), 6.40 (d, 1H, J = 3.81 Hz), 7.28–7.40 (m, 8H); ^{13}C-NMR ($CDCl_3$): δ 17.0,17.8, 19.6, 23.6, 25.6, 26.8, 29.7, 30.0, 33.8, 34.6, 34.8, 41.6, 43.9, 44.1, 53.0, 120.6, 122.7, 126.2, 127.0, 128. 6, 135.2, 144.9, 148.2, 149.9, 172.4, 176.3, 190.1. MS: m/e = 547 (M^+, 20%); *Analysis Calcd* for $C_{35}H_{37}N_3OS$: C, 76.75; H, 6.81; N, 7.67; S, 5.85%. Found: C, 76.80; H, 6.73; N, 8.03; S, 5.88%.

Compound ***8d****:* HPLC purity = 87% (C-18 NovaPak column; MeOH:H_2O/80:20), t_r = 22 min; Orange from EtOAc: hexane (80%), m.p. 190–193 °C; IR (KBr) cm^{-1}: 3054, 2932, 1637, 1569; ^{1}H-NMR ($CDCl_3$): δ 0.87, 1.03 (2s, 6H), 1.30–2.88 (m, 10H), 2.87–2.98 (m, 4H), 3.11 (s, 3H), 5.11, 5.22 (2s, 2H), 5.36 (s, 1H), 6.11 (s, 1H), 6.41 (d, 1H, J = 3.44 Hz), 6.29 (s,1H), 7.25–7.43 (m, 8H); ^{13}C-NMR ($CDCl_3$): δ 17.0,17.8, 19.6, 23.6, 25.6, 29.7, 30.0, 33.8, 34.6, 34.8, 41.6, 43.9, 44.1, 53.0, 120.6, 122.9, 125.1, 127.0, 128. 6, 135.2, 146.2, 148.2, 149.9, 172.4, 176.3, 190.12. MS: m/e = 563 (M^+, 28%); *Analysis Calcd* for $C_{35}H_{37}N_3O_2S$: C, 74.57; H, 6.62; N, 7.45; S, 5.69%. Found: C, 72.41; H, 6.83; N, 7.44; S, 5.64%.

*2.2.4. (6aR,6bS,8aS,12aS,12bR)-10-amino-6a,8a-dimethyl-4-oxo-2,4,5,6,6a,6b,7,-8,8a,12,12a,12b-dodecahydro-1H-naph-tho[2′,1′:4,5]indeno[2,1-b]thiophene-9-carbonitrile (**10a**) and ethyl (6aR,6bS,-8aS,12aS,12bR)-10-amino-6a,8a-dimethyl-4-oxo-2,4,5,6,6a,-6b,7,8-,8a,12,12a,12b-dodecahydro-1H-naphtho-[2′,1′:4,5]indeno[2,1-b]thiophene-9-carbonitrile (**10b**)*

General procedure: To a solution of androstenedione (0.286 g, 1 mmol) in 1,4-dioxane (20 mL) containing triethylamine (0.50 mL) each of elemental sulphur (0.032 g, 1 mmol) and either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (0.113 g, 0.01 mol) were added. The reaction mixture, in each case, was heated under reflux for 1 h then poured onto ice/water containing few drops of hydrochloric acid 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 ***10a****:* HPLC purity = 82% (C-18 NovaPak column; MeOH:H_2O/75:25), t_r = 22 min; yellow crystals from EtOAc: hexane (88%), m.p. 170–173 °C; IR (KBr) cm^{-1}: 3477, 3326 (NH_2), 3054, 2930, 2220, 1677, 1638, 1566; ^{1}H-NMR ($CDCl_3$): δ 0.86, 1.06 (2s, 6H), 1.34–2.85 (m, 10H), 2.88–2.95 (m, 4H), 4.83 (s, 2H), 5.12, 5.20 (2s, 2H), 5.39 (s, 1H), 6.11 (s, 1H), 6.28 (s, 1H), 6.43 (d, 1H, J = 3.03 Hz); ^{13}C-NMR ($CDCl_3$): δ 17.2,17.6, 19.8, 23.6, 25.2, 29.4, 30.0, 33.7, 34.6, 34.9, 41.8, 43.9, 44.4, 53.0, 118.3, 128.0, 133.4, 144.8, 146.2, 190.2. MS: m/e = 366 (M^+, 30%); *Analysis Calcd* for $C_{22}H_{26}N_2OS$: C, 72.09; H, 7.15; N, 7.64; S, 8.75%. Found: C, 72.22; H, 6.87; N, 7.49; S, 8.59%.

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Compound ***10b***: HPLC purity = 86% (C-18 NovaPak column; MeOH:H_2O/82:18), t_r = 20 min; yellow crystals from EtOAc: hexane (89%), m.p. 230–233 °C; IR (KBr) cm^{-1}: 3480, 3334 (NH_2), 3051, 29330, 1679, 1638, 1560; ^{1}H-NMR ($CDCl_3$): δ 0.88, 1.04 (2s, 6H), 1.16 (t, 3H, J = 7.20 Hz), 1.32–2.87 (m, 10H), 2.86–2.95 (m, 2H), 4.80 (s, 2H), 4.25 (q, 2H, J = 7.20 Hz), 5.11, 5.21 (2s, 2H), 5.39 (s, 1H), 6.11 (s, 1H), 6.28 (s,1H), 6.40 (d, 1H, J = 3.63 Hz); ^{13}C-NMR ($CDCl_3$): δ 17.0, 17.6, 19.8, 20.3, 23.6, 25.2, 29.4, 30.0, 33.9, 34.68, 34.9, 41.8, 43.9, 44.4, 53.0, 56.2, 128.0, 133.4, 144.8, 146.2, 190.2. MS: m/e = 413 (M^+, 20%); *Analysis Calcd* for $C_{24}H_{31}NO_3S$: C, 69.70; H, 7.56; N, 3.39; S, 7.75%. Found: C, 69.62; H, 7.41; N, 3.60; S, 7.80%.

*2.2.5. N-((6aR,6bS,8aS,12aS,12bR)-9-cyano-6a,8a,12b-trimethyl-4-oxo-2,4,5,6,6a,7,8,8a,-12,12a,12b-dodecahydro-1H-naphtho-[2′,1′:4,5]indeno[2,1-b]thiophen-10-yl)-3-oxobutanamide (**12a**), ethyl 3-(((6aR,6bS,8aS,12aS,12bR)-9-cyano-6a,8a,12b-trimethyl-4-oxo-2,4,5,6,6a,7,8,8a,-12,12a,12b-dodecahydro-1H-naphtho-[2′,1′:4,5]indeno[2,1-b]thiophen-10-yl)amino)-3-oxopropanoate (**12b**), (6aR,6bS,8aS,12aS,12bR)-9-cyano-6a,8a,12b-trimethyl-4-oxo-10-(3-oxobutanamido)-2,4,5,6,6a,7,8,8a,-12,12a,12b-dodecahydro-1H-naphtho[2′,1′:4,5]indeno[2,1-b]thiophene-9-carboxylate (**12c**) and (6aR,6bS,8aS,12aS,-12bR)-ethyl 10-(3-ethoxy-3-oxopropanamido)-6a,8a,12b-trimethyl-4-oxo-2,4,5,6,6a,7,8,8a,-12,12a,12b-dodecahydro-1H-naphtho[2′,1′:4,5]indeno[2,1-b]thiophene-9-carboxylate (**12d**)*

General procedure: Equimolecular ratio of either compound **10a** (3.66 g, 0.01 mol) or **10b** (0.413 g, 1 mmol) and either ethyl acetoacetate (0.130 g, 1 mmol) or diethylmalonate (0.160 g, 1 mmol) in 1,4-dioxane (20 mL) was heated under reflux for 2 h. The solid product, formed in each case, upon pouring onto ice/water was collected by filtration. Thin layer chromatography revealed just a single spot which proved the presence of a single product.

Compound ***12a***: HPLC purity = 89% (C-18 NovaPak column; MeOH:H_2O/80:20), t_r = 21 min; yellow crystals from EtOAc: hexane (80%), m.p. 180–183 °C; IR (KBr) cm^{-1}: 3477–3338, 3057, 2930, 2222, 1720, 1689, 1670, 1639, 1563; ^{1}H-NMR ($CDCl_3$): δ 0.85, 1.03 (2s, 6H), 1.32–2.89 (m, 10H), 2.84–2.96 (m, 2H), 3.11 (s, 3H), 4.86 (s, 2H), 5.10, 5.19 (2s, 2H), 5.39 (s, 1H), 6.11 (s, 1H), 6.46 (d, 1H, J = 2.72 Hz), 6.25 (s,1H), 8.30 (s, 1H); ^{13}C-NMR ($CDCl_3$): δ 17.2,17.8, 19.5, 20.3, 23.8, 25.6, 29.4, 30.3, 33.7, 34.6, 34.8, 41.7, 43.9, 44.2, 53.2, 61.8, 127.8, 128.5, 130.6, 149.2, 149.2, 172.3, 188.4, 190.1. MS: m/e = 450 (M^+, 30%); *Analysis Calcd* for $C_{26}H_{30}N_2O_3S$: C, 69.30; H, 6.71; N, 6.22; S, 7.12%. Found: C, 70.19; H, 6.53; N, 6.43; S, 7.13%.

Compound ***12b***: HPLC purity = 83% (C-18 NovaPak column; MeOH:H_2O/88:12), t_r = 23 min; yellow crystals from EtOAc: hexane (84%), m.p. 155–157 °C; IR (KBr) cm^{-1}: 3483–3330, 3054, 2931, 2220, 1722, 1686, 1671, 1636, 1562; ^{1}H-NMR ($CDCl_3$): δ 0.86, 1.03 (2s, 6H), 1.13 (t, 3H, J = 6.94 Hz), 1.30–2.89 (m, 10H), 2.82–2.95 (m, 2H), 4.25 (q, 2H, J = 6.94 Hz), 4.86 (s, 2H), 5.11, 5.19 (2s, 2H), 5.39 (s, 1H), 6.10 (s, 1H), 6.22 (s,1H), 6.48 (d, 1H, J = 3.49 Hz), 8.28 (s, 1H); ^{13}C-NMR ($CDCl_3$): δ 17.1, 17.6, 18.3, 19.9, 20.3, 23.1, 25. 9, 29.4, 30.3, 33.7, 34.6, 34.8, 41.8, 43.9, 44.2, 53.2, 56.8, 61.8, 127.4, 128. 8, 130.2, 149.0, 149. 8, 172.3, 188.6, 190.3. MS: m/e = 480 (M^+, 26%); *Analysis Calcd* for $C_{27}H_{32}N_2O_4S$: C, 67.47; H, 6.71; N, 5.83; S, 6.67%. Found: C, 68.04; H, 6.70; N, 5.83; S, 6.69%.

Compound ***12c***: HPLC purity = 86% (C-18 NovaPak column; MeOH:H_2O/90:10), t_r = 22 min; yellow crystals from EtOAc: hexane (80%), m.p. 180–183 °C; IR (KBr) cm^{-1}: 3456–3333 (NH), 3056, 2930, 1720, 1688, 1670, 1638, 1560; ^{1}H-NMR ($CDCl_3$): δ 0.83, 1.05 (2s, 6H), 1.16 (t, 3H, J = 7.83 Hz), 1.32–2.89 (m, 10H), 2.62 (s, 3H), 2.80–2.95 (m, 2H), 4.22 (q, 2H, J = 7.83 Hz), 4.86 (s, 2H), 5.09, 5.19 (2s, 2H), 5.39 (s, 1H), 6.11 (s, 1H), 6.22 (s, 1H), 6.48 (d, 1H, J = 3.49 Hz), 8.28 (s, 1H); ^{13}C-NMR ($CDCl_3$): δ 17.1, 17.6, 18.3, 19.9, 20.3, 23.1, 25. 9, 26.8, 29.4, 30.3, 33.9, 34.6, 34.6, 41.8, 43.9, 44.2, 53.2, 56.3, 61.2, 127.4, 129. 0, 130.8, 149.3, 149.8, 172.6, 174.6, 188.1, 190.0. MS: m/e = 499 (M^+, 53%); *Analysis Calcd* for $C_{28}H_{35}NO_5S$: C, 67.58; H, 7.09; N, 2.81; S, 6.44%. Found: C, 68.37; H, 7.44; N, 2.94; S, 6.38%.

Compound ***12d***: HPLC purity = 83% (C-18 NovaPak column; MeOH:H_2O/78:22), t_r = 20 min; yellow crystals from EtOAc: hexane (83%), m.p. 267–269 °C; IR (KBr) cm^{-1}: 3477–3320 (NH), 3053, 2932, 1725, 1686, 1668, 1638, 1563; ^{1}H-NMR ($CDCl_3$): δ 0.84, 1.04 (2s, 6H), 1.14, 1.17 (2t, 6H, J = 7.23, 6.53 Hz), 1.32–2.89 (m, 10H), 2.80–2.95 (m, 2H), 4.22,4.26 (2q, 4H, J = 7.23, 6.53 Hz), 4.82 (s, 2H), 5.06, 5.23 (2s, 2H), 5.42 (s, 1H), 6.10 (s, 1H), 6.20 (s,1H), 6.45 (d, 1H, J = 2.69 Hz), 8.28 (s, 1H); ^{13}C-NMR ($CDCl_3$): δ 17.0, 17.8, 18.3, 19.6, 19.9, 20.3, 23.1, 25. 9, 26.8, 29.4, 30.3, 33.9, 34.6, 34.6, 41.8, 43.9, 44.2, 53.2, 56.3, 57.2, 127.4, 129.0, 130.4, 149.3, 150.0, 172.6, 174.1, 188.4, 190.2. MS: m/e = 527 (M^+, 28%); *Analysis Calcd* for $C_{29}H_{37}NO_6S$: C, 66.01; H, 7.07; N, 2.65; S, 6.08%. Found: C, 66.22; H, 7.09; N, 2.64; S, 6.46%.

*2.2.6. N-((6aR,6bS,aS,12aS,12bR)-9-cyano-6a,8a,12b-trimethyl-4-oxo-2,4,5,6,6a,6b,-7,8,8a,12,12a,12b-dodecahydro-1H-naphtho-[2′,1′:4,5]indeno[2,1-b]thiophen-10-yl)-3oxo-2-(2-phenylhydrazono)butanamide (**14a**), N-((6aR,6bS,aS,12aS,12bR)-9-cyano-6a,8a,12b-trimethyl-4-oxo-2,4,5,6,6a,6b,7,8,8a,12,-12a,12b-dodecahydro-1H-naphtho[2′,1′:4,5]-indeno-[2,1-b]thiophen-10-yl)-3oxo-2-(2-p-tolylhydrazono)butanamide (**14b**), N-((6aR,6bS,aS,12aS,12bR)-9-cyano-6a,8a,12b-trimethyl-4-oxo-2,4,5,6,6a,6b,7,8,8a,12,-12a,12b-dodecahydro-1H-naphtho-[2′,1′:4,5]indeno[2,1-b]thiophen-10-yl)-3oxo-2-(2-(4- chlorophenyl) hydrazono)butmide (**14c**), ethyl 3-(((6aR,6bS,8aS,12aS,12bR)-9-cyano-6a,8a,12b-trimethyl-4-oxo-2,4,5,6,6a,6b,7,8,8a,12,-12a,12b-dodecahydro-1H-naphtho-[2′,1′:4,5]indeno[2,1-b]thiophen-10-yl)amino-3-oxo-2-(2-phenylhydrazono)propanoate (**14d**), ethyl 3-(((6aR,6bS,8aS,12aS,12bR)-9-cyano-6a,8a,12b-trimethyl-4-oxo-2,4,5,6,6a,6b,-7,8,8a,12,-12a,12b-dodecahydro-1H-naphtho-[2′,1′:4,5]indeno[2,1-b]thiophen-10-yl)amino-3-oxo-2-(2-p-tolylhydrazono)propanoate (**14e**) and ethyl 3-(((6aR,6bS, 8aS,12aS,12bR)-9-cyano-6a,8a,12b-trimethyl-4-oxo-2,4,5,6,6a,6b,7,8,8a,12,-12a,12b-dodecahydro-1H-naphtho-[2′,1′:4,5]indeno[2,1-b]thiophen-10-yl)amino-3-oxo-2-(2-(4-chlorophenyl)-hydrazono)propanoate (**14f**)*

General procedure: To a solution of either compound **12a** (0464 g, 0.01 mol) or **12b** (0.492 g, 1 mmol) in ethanol (30 mL) containing sodium acetate (2.5 g), either benzenediazonium chloride (0.01 mol), 4-methylbenzenediazonium chloride (1 mmol) or 4-chlorobenzenediazonium 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. Thin layer chromatography revealed just a single spot which proved the presence of a single product.

Compound ***14a***: HPLC purity = 84% (C-18 NovaPak column; MeOH:H_2O/77:23), t_r = 24 min; yellow crystals from EtOAc: hexane (82%), m.p. 130–132 °C; IR (KBr) cm^{-1}: 3465–3332, 3054, 2932, 2227, 1718, 1686, 1670, 1639, 1567; ^{1}H-NMR ($CDCl_3$): δ 0.86, 1.04 (2s, 6H), 1.30–2.87 (m, 10H), 2.86–2.96 (m, 2H), 3.06 (s, 3H), 5.08, 5.17 (2s, 2H), 5.34 (s, 1H), 6.11 (s, 1H), 6.23 (s,1H), 6.48 (d, 1H, J = 3.82 Hz), 7.28–7.38 (m, 5H), 8.27, 8.32 (2s, 2H); ^{13}C-NMR ($CDCl_3$): δ 17.20,17.9, 19.5, 20.3, 23.6, 25.8, 29.4, 30.0, 33.9, 34.4, 34.8, 41.7, 43.9, 44.2, 117.9, 128.0, 128. 3, 130.8, 149.0, 149.6, 172.0, 188.5, 190.2. MS: m/e = 554 (M^+, 21%); *Analysis Calcd* for $C_{32}H_{34}N_4O_3S$: C, 69.29; H, 6.18; N, 10.10; S, 5.78%. Found: C, 69.48; H, 6.39; N, 10.22; S, 5.83%.

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Compound ***14b***: HPLC purity = 84% (C-18 NovaPak column; MeOH:H_2O/80:20), t_r = 22 min; yellow crystals from EtOAc: hexane (82%), m.p. 188–190 °C; IR (KBr) cm^{-1}: 3443–3330, 3056, 2930, 2223, 1716, 1687, 1671, 1639, 1563; ^{1}H-NMR ($CDCl_3$): δ 0.85, 1.05 (2s, 6H), 1.32–2.87 (m, 10H), 2.83–2.99 (m, 2H), 3.08, 3.12 (2s, 6H), 5.18 (s, 2H), 5.14, 5.35 (2s, 2H), 6.20 (s,1H), 6.49 (d, 1H, J = 3.42 Hz), 7.26–7.39 (m, 4H), 8.23, 8.34 (2s, 2H); ^{13}C-NMR ($CDCl_3$): δ 17.22,17.9, 19.5, 20.3, 23.8, 25.8, 29.4,,31.8, 30.0, 33.9, 34.4, 34.8, 41.7, 43.9, 44.0, 117.6, 128.4, 128.8, 130.8, 149.0, 149.8, 172.0, 188.6, 190.3. MS: m/e = 568 (M^+, 40%); *Analysis Calcd* for $C_{33}H_{36}N_4O_3S$: C, 69.69; H, 6.38; N, 9.85; S, 5.50%. Found: C, 70.17; H, 6.70; N, 9.93; S, 5.77%.

Compound ***14c***: HPLC purity = 80% (C-18 NovaPak column; MeOH:H_2O/80:20), t_r = 18 min; yellow crystals from EtOAc: hexane (78%), m.p. 256–259 °C; IR (KBr) cm^{-1}: 3450–3326, 3054, 2931, 2220, 1718, 1684, 1670, 1633, 1563; ^{1}H-NMR ($CDCl_3$): δ 0.85,1.05 (2s, 6H), 1.30–2.87 (m, 10H), 2.84–2.97 (m, 2H), 3.07 (s, 3H), 5.07, 5.15 (2s, 2H), 5.37 (s, 1H), 6.10 (s, 1H), 6.21 (s,1H), 6.46 (d, 1H, J = 3.09 Hz), 7.28–7.37 (m, 4H), 8.27, 8.36 (2s, 2H); ^{13}C-NMR ($CDCl_3$): δ 17.20, 17.9, 19.5, 20.3, 23.8, 25.8, 29.4, 31.8, 30.3, 33.8, 34.4, 34.8, 41.7, 43.9, 44.2, 117.8, 128.6, 128.4, 130.8, 149.2, 149.8, 172.1, 188.7, 190.0. MS: m/e = 5 (M^+, 31%); *Analysis Calcd* for $C_{32}H_{33}ClN_4O_3S$: C, 65.24; H, 5.65; N, 9.51; S, 5.44%. Found: C, 65.53; H, 5.85; N, 9.37; S, 5.48%.

Compound ***14d***: HPLC purity = 83% (C-18 NovaPak column; MeOH:H_2O/85:15), t_r = 17 min; yellow crystals from EtOAc: hexane (77%), m.p. 230–233 °C; IR (KBr) cm^{-1}: 3442–3326, 3053, 2930, 1715, 1683, 1668, 1638, 1562; ^{1}H-NMR ($CDCl_3$): δ 0.80, 1.04 (2s, 6H), 1.18 (t, 3H, J = 6.88 Hz), 1.31–2.90 (m, 10H), 2.81–2.98 (m, 4H), 4.24 (q, 2H, J = 6.88 Hz), 5.15, 5.26 (2s, 2H), 5.36 (s, 1H), 6.10 (s, 1H), 6.20 (s,1H), 6.43 (d, 1H, J = 2.89 Hz), 7.30–7.39 (m, 5H), 8.24, 8.30 (2s, 2H); ^{13}C-NMR ($CDCl_3$): δ 17.2, 17.6, 18.3, 19.8, 20.6, 23.1, 25. 9, 26.8, 29.4, 30.3, 33.9, 34.6, 34.6, 41.8, 43.9, 44.2, 53.4, 56.6, 127.4, 129.3, 132.6, 149.2, 149.6, 172.8, 188.3, 190.1. MS: m/e = 584 (M^+, 39%); *Analysis Calcd* for $C_{34}H_{38}N_4O_4S$: C, 67.78; H, 6.21; N, 9.58; S, 5.48%. Found: C, 68.03; H, 6.29; N, 9.53; S, 5.48%.

Compound ***14e***: HPLC purity = 86% (C-18 NovaPak column; MeOH:H_2O/85:15), t_r = 22 min; yellow crystals from EtOAc: hexane (80%), m.p. 177–179 °C; IR (KBr) cm^{-1}: 3461–3318, 3056, 2930, 1722, 1684, 1668, 1635, 1560; ^{1}H-NMR ($CDCl_3$): δ 0.82, 1.05 (2s, 6H), 1.16 (t, 3H, J = 5.93 Hz), 1.33–2.90 (m, 10H), 2.81–2.96 (m, 2H), 3.11 (s, 3H), 4.23 (q, 2H, J = 5.93 Hz), 5.17, 5.24 (2s, 2H), 5.38 (s, 1H), 6.11 (s, 1H), 6.20 (s, 1H), 6.22 (s,1H), 6.44 (d, 1H, J = 3.47 Hz), 7.27–7.40 (m, 4H), 8.21, 8.30 (2s, 2H); ^{13}C-NMR ($CDCl_3$): δ 17.1, 17.6, 18.3, 19.8, 20.3, 20.6, 23.1, 26.2, 26.6, 29.4, 30.4, 33.9, 34.6, 34.6, 41.8, 43.9, 44.6, 53.4, 56.8, 117.9, 127.6, 129.5, 133.8, 149.2, 149.6, 172.8, 188.6, 190.3. MS: m/e = 611 (M^+, 40%); *Analysis Calcd* for $C_{35}H_{39}N_4O_4S$: C, 68.71; H, 6.43; N, 9.16; S, 5.24%. Found: C, 68.52; H, 6.42; N, 9.36; S, 5.39%.

Compound ***14f***: HPLC purity = 83% (C-18 NovaPak column; MeOH:H_2O/85:15), t_r = 20 min; yellow crystals from EtOAc: hexane (83%), m.p. 103–105 °C; IR (KBr) cm^{-1}: 3453–3312, 3050, 2930, 1705, 1683, 1664, 1638, 1560; ^{1}H-NMR ($CDCl_3$): δ 0.82, 1.02 (2s, 6H), 1.22 (t, 3H, J = 7.30 Hz), 1.33–2.90 (m, 10H), 2.81–2.99 (m, 2H), 4.20 (q, 2H, J = 7.30 Hz), 5.16, 5.26 (2s, 2H), 5.36 (s, 1H), 6.22 (s,1H), 6.41 (d, 1H, J = 3.05 Hz), 7.28–7.39 (m, 4H), 8.25, 8.31 (2s, 2H); ^{13}C-NMR ($CDCl_3$): δ 17.0, 17.6, 18.3, 19.8, 20.6, 23.1, 25. 9, 26.8, 29.4, 30.3, 33.9, 34.6, 34.6, 41.4, 43.9, 44.2, 53.4, 56.8, 117.1, 127.6, 129.7, 132.6, 149.0, 149.6, 172.3, 188.3, 190.2. MS: m/e = 633 (M^+, 22%); *Analysis Calcd* for $C_{33}H_{35}ClN_4O_4S$: C, 64.01; H, 5.70; N, 9.05; S, 5.18%. Found: C, 64.32; H, 5.55; N, 9.03; S, 5.22%.

*2.2.7. (6aR,6bS,8aS,14aS,14bR)-10-Acetyl-9-amino-6a,8a-dimethyl-6,6a,6b,7,8,8a,-12,14,14a,14b-decahydro-1H-naphtho[2″,1″:4′,5′]indeno[1′,2′:4,5]thieno[2,3-b]pyridine-4,11-(2H,5H)-dione (**15a**), (6aR,6bS,8aS,14aS,14bR)-ethyl 9-amino-6a,8a-dimethyl-6,6a,6b,7,8,8a,12,14,-14a,14b-decahydro-1H-naphtho[2″,1″:4′,5′]indeno-[1′,2′:4,5]-thieno[2,3-b]pyridine-10-carboxylate (**15b**), (6aR,6bS,8aS,14aS,14bR)-10-Acetyl-9-hydroxy-6a,8a-dimethyl-6,6a,6b,7,8,8a,12,14,14a,14b-decahydro-1H-naphtho-[2″,1″:4′,5′]-indeno[1′,2′:4,5]-thieno[2,3-b]pyridine-4,11-(2H,5H)-dione (**15c**), (6aR,6bS,8aS,14aS,14bR)-ethyl 9-hydroxy-6a,8a-dimethyl-6,6a,6b,7,8,8a,12,14,14a,14b-decahydro-1H-naphtho-[2″,1″:4′,5′]indeno-[1′,2′:4,5]thieno[2,3-b]pyridine-10-carboxylate (**15d**)*

General procedure: A solution of either **12a** (0464 g, 0.01 mol), **12b** (0.492 g, 1 mmol) in ethanol (30 mL), **12c** (0.511 g, 1 mmol) or **12d** (0.541 g, 1 mmol) in absolute ethanol (40 mL) containing triethylamine (0.50 g) was heated under reflux for 6 h. The mixture was evaporated under vacuum, the remaining product was triturated with diethyl ether 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 ***15a***: HPLC purity = 86% (C-18 NovaPak column; MeOH:H_2O/90:10), t_r = 19 min; yellow crystals from EtOAc: hexane (83%), m.p. 233–235 °C; IR (KBr) cm^{-1}: 3488–3328, 3053, 2931, 1720, 1689, 1672, 1634, 1561; ^{1}H-NMR ($CDCl_3$): δ 0.87, 1.05 (2s, 6H), 1.31–2.86 (m, 10H), 2.82–2.93 (m, 2H), 3.09 (s, 3H), 4.82 (s, 2H), 5.11, 5.20 (2s, 2H), 5.36 (s, 1H), 6.10 (s, 1H), 6.26 (s,1H), 6.41 (d, 1H, J = 4.07 Hz), 8.31 (s, 1H); ^{13}C-NMR ($CDCl_3$): δ 17.0,17.68, 19.5, 20.3, 23.8, 25.6, 29.6, 30.3, 33.7, 34.6, 34.8, 41.7, 43.9, 44.2, 53.2, 123.6, 127.8, 128.7, 130.9, 133.4, 136.0, 149.6, 172.83, 188.3, 190.4. MS: m/e = 450 (M^+, 26%); *Analysis Calcd* for $C_{26}H_{30}N_2O_3S$: C, 69.30; H, 6.71; N, 6.22; S, 7.12%. Found: C, 69.25; H, 6.69; N, 6.04; S, 7.39%.

Compound ***15b***: HPLC purity = 80% (C-18 NovaPak column; MeOH:H_2O/86:14), t_r = 21 min; yellow crystals from EtOAc: hexane (83%), m.p. 196–198 °C; IR (KBr) cm^{-1}: 348o-3312, 3052, 2933, 1720, 1684, 1671, 1636, 1560; ^{1}H-NMR ($CDCl_3$): δ 0.85, 1.02 (2s, 6H), 1.12 (t, 3H, J = 7.09 Hz), 1.30–2.90 (m, 10H), 2.80–2.95 (m, 2H), 4.21 (q, 2H, J = 7.09 Hz), 4.63 (s, 2H), 5.19, 5.24 (2s, 2H), 5.35 (s, 1H), 6.10 (s, 1H), 6.24 (s,1H), 6.45 (d, 1H, J = 3.11 Hz), 8.25 (s, 1H); ^{13}C-NMR ($CDCl_3$): δ 17.4, 17.6, 18.3, 19.9, 20.6, 23.1, 25. 9, 29.4, 30.0, 33.7, 34.6, 34.8, 41.8, 43.9, 44.2, 53.1, 56.5, 127.4, 128. 8, 130.2, 130.9, 133.3, 136.5, 149.3, 149. 8, 172.6, 188.2, 190.2. MS: m/e = 480 (M^+, 36%); *Analysis Calcd* for $C_{27}H_{32}N_2O_4S$: C, 67.47; H, 6.71; N, 5.83; S, 6.67%. Found: C, 67.24; H, 6.66; N, 5.53; S, 6.49%.

Compound ***15c***: HPLC purity = 83% (C-18 NovaPak column; MeOH:H_2O/90:10), t_r = 19 min; yellow crystals from EtOAc: hexane (82%), m.p. 166–168 °C; IR (KBr) cm^{-1}: 3558–3323, 3056, 2930, 1722, 1688, 1671, 1638, 1558; ^{1}H-NMR ($CDCl_3$): δ 0.84, 1.04 (2s, 6H), 1.30–2.89 (m, 10H), 2.82–2.96 (m, 2H), 3.11 (s, 3H), 5.19, 5.26 (2s, 2H), 5.36 (s, 1H), 6.11 (s, 1H), 6.20 (s,1H), 6.43 (d, 1H, J = 3.49 Hz), 8.23 (s, 1H), 10.88 (s, 1H); ^{13}C-NMR ($CDCl_3$): δ 17.3, 17.8, 18.4, 19.9, 20.1, 23.1, 25. 9, 26.8, 29.4, 30.2, 33.9, 34.6, 34.6, 41.8, 43.9, 44.2, 53.2, 56.3, 127.4, 129. 0, 130.8, 130.9, 134.2, 136.7, 149.6, 149. 8, 172.86, 188.10, 190.3. MS: m/e = 451 (M^+, 23%); *Analysis Calcd* for $C_{26}H_{29}NO_4S$: C, 69.15; H, 6.47; N, 3.10; S, 7.10%. Found: C, 68.95; H, 6.69; N, 2.88; S, 7.36%.

Compound ***15d***: HPLC purity = 86% (C-18 NovaPak column; MeOH:H_2O/80:20), t_r = 22 min; yellow crystals from EtOAc: hexane (86%), m.p. 173–176 °C; IR (KBr) cm^{-1}: 3540–3315, 3055, 2932, 1720, 1687, 1668, 1639, 1562; ^{1}H-NMR ($CDCl_3$): δ 0.85, 1.02 (2s, 6H), 1.13 (t, 3H, J = 7.02 Hz), 1.34–2.89 (m, 10H), 2.80–2.97 (m, 4H), 4.23 (q, 2H, J = 7.02 Hz), 5.07, 5.26 (2s, 1H), 5.40 (s, 1H), 6.43 (d, 1H, J = 3.73 Hz), 6.22 (s,1H), 8.26 (s, 1H),10.29 (s,

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Table 1
Cytotoxicity of the novel androstenedione derivatives **4e**, **4f**, **6b**, **6c**, **8b**, **8d**, **10a, 10b, 12d**, **14d**, **15b**, **15c** and **16** with significant activities against a variety of cancer cell lines[a] [IC_{50}[b] (nM)].

Compd no.	Cytotoxocity (IC_{50} in nM)						
	NUGC	DLDI	HA22T	HEPG2	HONE1	MCF	WI38
4e	33	48	120	366	485	128	na
4f	69	190	69	446	277	436	407
6b	180	740	234	837	644	269	na
6c	40	64	82	328	260	173	na
8b	60	220	33	227	63	68	na
8c	181	1464	2155	4235	2663	333	na
8d	35	60	61	2345	82	1189	na
10a	1220	240	820	630	408	254	na
10b	3505	488	1260	1680	2073	1920	na
12d	154	760	275	208	436	2270	na
14d	87	66	238	462	122	2270	na
15b	876	2180	1663	660	2879	2411	na
15c	1294	1549	2739	895	220	2460	na
16	38	36	96	126	64	128	na
CHS 828	25	2315	2067	1245	15	18	na

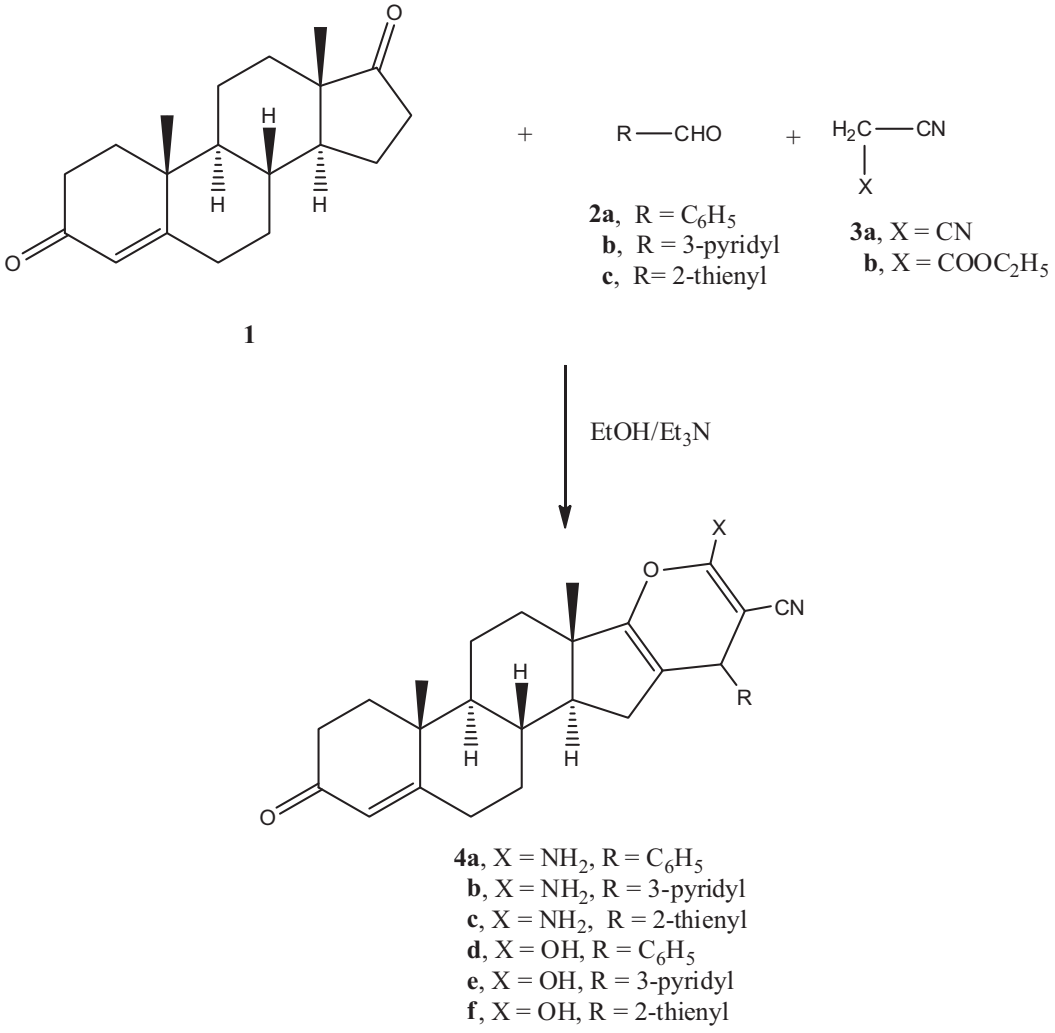
[a] NUGC, gastric cancer, DLDI, colon cancer, HA22T, liver cancer, HEPG2, liver cancer; HONEI, nasopharyngeal carcinoma; HR, gastric cancer; MCF, breast cancer; WI38, normal fibroblast cells.
[b] The sample concentration produces a 50% reduction in cell growth.

1H); ^{13}C-NMR ($CDCl_3$): δ 17.0, 17.8, 18.3, 19.6, 19.9, 20.3, 23.1, 25.9, 26.8, 29.4, 30.3, 33.9, 34.6, 34.6, 41.8, 43.9, 44.2, 53.2, 56.3, 57.2, 127.4, 129.0, 130.4, 133.2, 134.8, 136.9, 149.2, 150.0, 172.4, 188.2, 190.0. MS: m/e = 481 (M^+, 33%); *Analysis Calcd* for $C_{27}H_{31}NO_5S$: C, 67.34; H, 6.49; N, 2.91; S, 6.66%. Found: C, 67.58; H, 6.69; N, 3.16; S, 6.82%.

*2.2.8. (6aR,6bs,8aS,12bR)-6a,8a-dimethyl-9-phenyl-10-thioxo-5,6,6a,6b,7,8,8a,9,10,-12,12a,12b-dodecahydro-1H-napntho-[2′,1′:4,5]indeno[1,2-d]thiazol-4(2H)-one (**16**)*

To a solution of androstenedione (0.286 g, 1 mmol) in 1,4-dioxane (20 mL) containing triethylamine (0.50 mL) each of elemental sulphur (0.032 g, 1 mmol) and phenylisothio-cyanate (0.130 g, 1 mmol) was added. The whole reaction mixture was heated under reflux for 3 h then left to cool overnight. 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 **16**:* HPLC purity = 81% (C-18 NovaPak column; MeOH:H_2O/85:15), t_r = 20 min; yellow crystals from EtOAc: hexane (83%), m.p. 123–125 °C; IR (KBr) cm^{-1}: 3055, 2930, 1718, 1684, 1671, 1632, 1560, 1200–1195; ^{1}H-NMR ($CDCl_3$): δ 0.86, 1.05 (2s, 6H), 1.30–2.88 (m, 10H), 2.82–2.95 (m, 2H), 5.11, 5.22 (2s, 2H), 5.38 (s, 1H), 6.10 (s, 1H), 6.28 (s,1H), 6.38 (d, 1H,



Scheme 1. Synthesis of compounds **4a–f**.

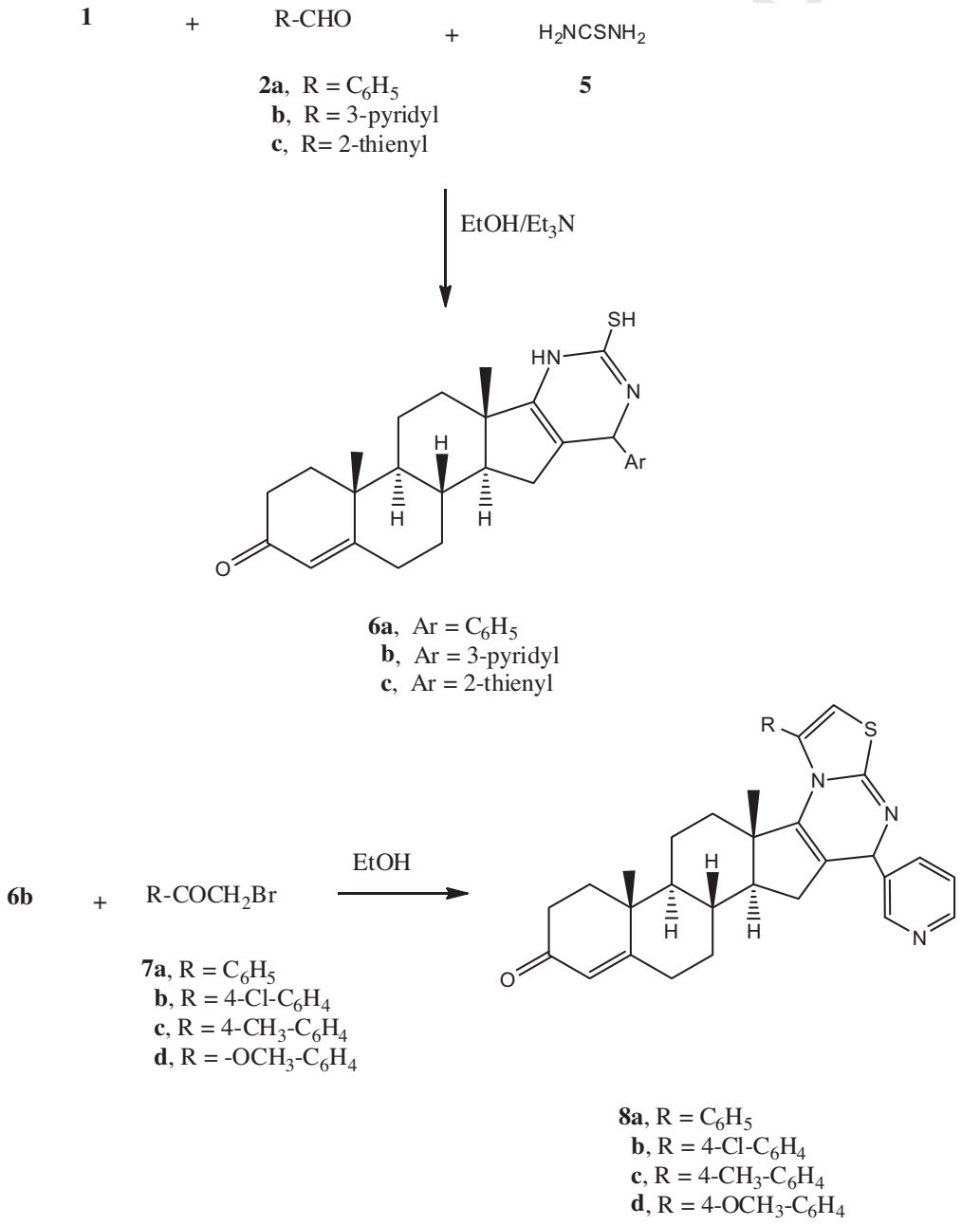
J = 4.22 Hz), 7.28–7.38 (m, 5H, C_6H_5); ^{13}C-NMR ($CDCl_3$): δ 17.0, 17.6, 19.8, 20.4, 23.8, 25.6, 29.6, 30.3, 33.7, 34.6, 34.8, 41.5, 43.9, 44.6, 53.6, 122.3, 123.6, 127.8, 129.6, 130.9, 133.4, 149.8, 188.4, 190.2. MS: m/e = 435 (M^+, 38%); *Analysis Calcd* for $C_{26}H_{29}NOS_2$: C, 71.68; H, 6.71; N, 3.22; S, 14.72%. Found: C, 71.91; H, 6.55; N, 3.08; S, 14.64%.

2.3. Biological evaluation

2.3.1. In vitro cytotoxic assay

2.3.1.1. Chemicals. Fetal bovine serum (FBS) and L-glutamine, were purchased from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was purchased from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were purchased from Sigma Chemical Co. (Saint Louis, USA).

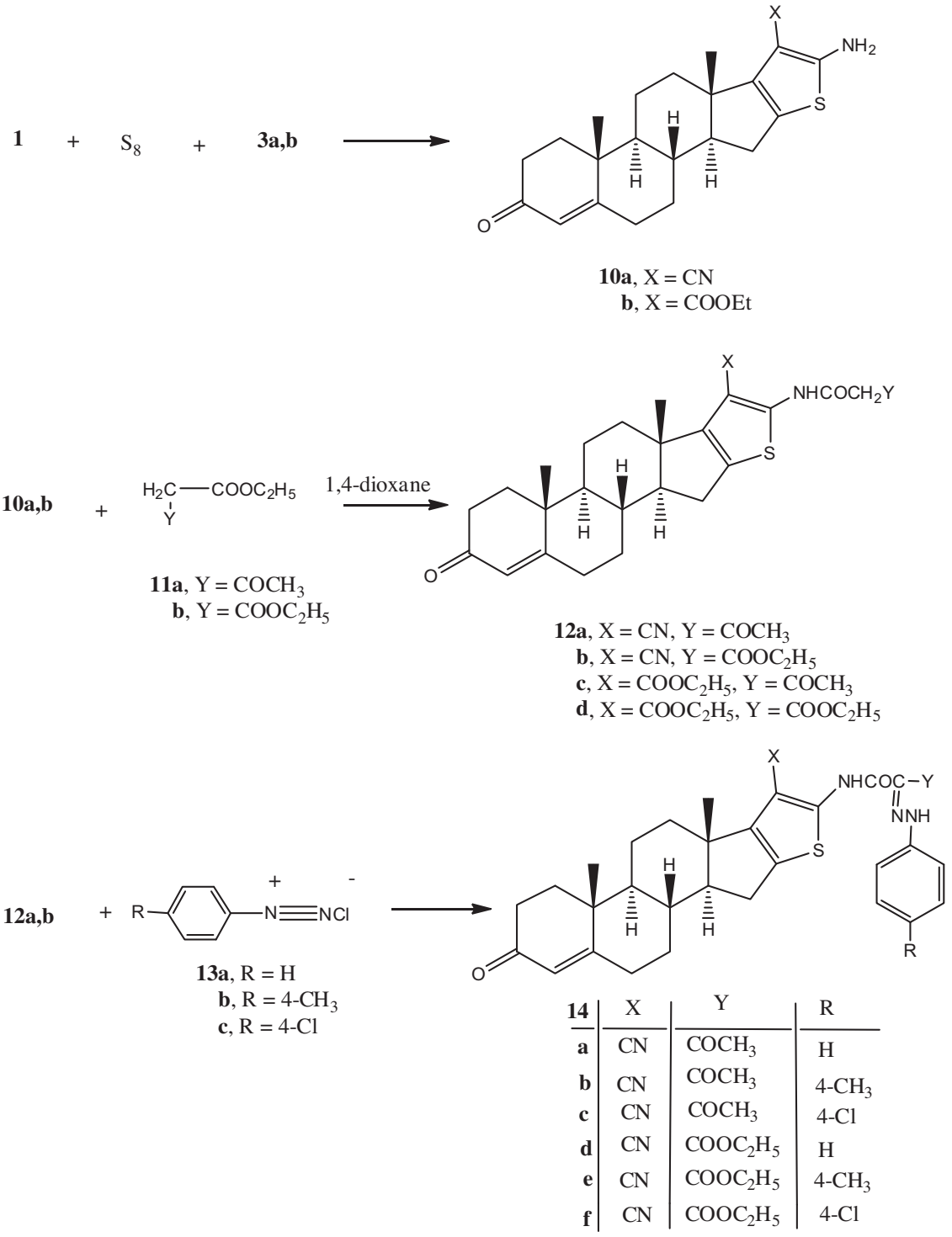
2.3.1.2. Cell cultures. Was obtained from the European Collection of cell Cultures (ECACC, Salisbury, UK) and human gastric cancer (NUGC and HR), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) and normal fibroblast cells (WI38) were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 lg/mL), at 37 °C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5 × 10^5 cells/mL for the



Scheme 2. Synthesis of compounds **6a–c** and **8a–d**.

seven human cancer cell lines including cells derived from 0.75×10^4 cells/mL 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.

The heterocyclic androstenedione derivatives, prepared in this study, were evaluated according to standard protocols for their *in vitro* cytotoxicity against six human cancer cell lines including cells derived from human gastric cancer (NUGC), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) and a normal fibroblast cells (WI38). All of IC_{50} values were listed in Table 1. Some heterocyclic compounds was observed with significant cytotoxicity against most of the cancer cell lines tested (IC_{50} = 10–1000 nM). Normal fibroblasts cells (WI38) were affected to a



14	X	Y	R
a	CN	$COCH_3$	H
b	CN	$COCH_3$	4-CH_3
c	CN	$COCH_3$	4-Cl
d	CN	$COOC_2H_5$	H
e	CN	$COOC_2H_5$	4-CH_3
f	CN	$COOC_2H_5$	4-Cl

Scheme 3. Synthesis of compounds **10a**,**b**; **12a**–**d** and **14a**–**f**.

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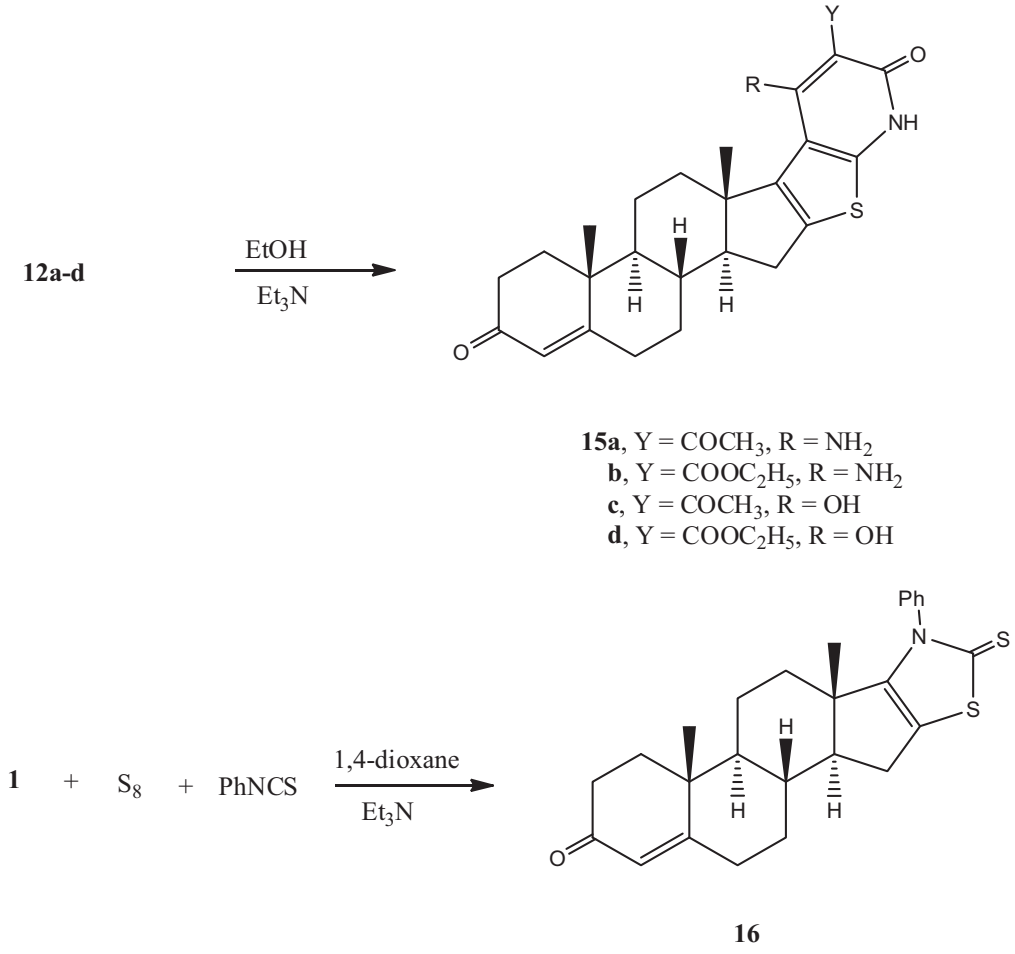

Scheme 4. Synthesis of compounds **15a–d** and **16**.

much lesser extent (IC50 > 10,000 nM). The reference compound used is the CHS-828 which is a pyridyl cyanoguanidine anti-tumor agent.

2.3.1.3. Structure activity relationship. From Table 1 it is clear that the cyclohexene moiety was found to be crucial for the cytotoxic effect of cyclic compounds **4a–d** to **16**. Compounds **4e**, **4f**, **6b**, **6c**, **8b**, **8d**, **10a, 10b, 12d**, **14d**, **15b**, **15c** and **16** exhibited cytotoxic effects against cancer cell lines, with IC_{50}'s in the nM range which were indicated through Table 1, the cytotoxicity of the rest of the synthesized compounds were presented through the Supplementary Section. Comparing the cytotoxicity of the pyran derivatives **4a–f**, it is obvious that the cytotoxicity of **4e** the highest cytotoxicity among the six compounds. The presence of the OH and the thiophene groups are responsible for its high potency. Considering the 4,5,6,7-tetrahydrobenzo[*b*]thiophene derivatives **5a–d**, it is clear that the cytotoxicity of **5c** is higher than that of **5a**, **5b** and **5d**. The high cytotoxicity of **5c** is attributed to the presence of the 4-OCH_3 aryl moiety together with the 3-cyano group of the thiophene moiety. On the other hand, considering the pyrimidine derivatives **6a–c** where compounds **6b** and **6c** showed high cytotoxicity which is attributed to the presence of the pyridyl and thiophenyl groups, respectively. Evaluation of the thiazolo[3,2-*a*]pyrimidine derivatives **8a–d** showed that the presence of the 4-chlorophenyl group in **8b** and the 4-methoxyphenyl group **8d** are responsible for the high potency of these two compounds. However, the 4-chlorophenyl derivative **4b** is more potent that **8d** towards HEPG2 and MCF cell lines.

It is obvious that the high oxygen content of **12d** is responsible for its high cytotoxicity against the five cell lines NUGC, DLDI, HA22T, HEPG2 and HONE1. The arylhydrazonothiophene derivative **12f** with the combination of CN, COOEt and the4-chlorophenyl moieties showed high potency towards the six cancer cell lines among the group of derivatives **14a–f**. The thieno[2,3-*b*]pyridine derivatives **15a–d** showed very low cytotoxicity although compound **15b** showed relatively high cytotoxicity against NUGC and HEPG2 with IC_{50}'s 876 and 660 nM, respectively. Finally the thiazole derivative **16** showed high cytotoxicity towards the six cancer cell lines which is attributed to the high sulphur content of the molecule. It is of great value to notice that the optimal cytotoxicity against the six cancer cell lines were obtained through compounds **4e**, **4f**, **6b**, **6c**, **8b** and **16**.

It is very clear from our present finding that the newly synthesized products with halogen substituted pattern OCH_3, Cl or COOEt show greater cytotoxic property. In every case it was observed that molecules with electronegative substitutions as compounds **4e**, **4f**, **6b**, **6c**, **8b**, **8d**, **10a**, **10b**, **12d**, **14d**, **15b**, **15c** and **16** showed higher cytotoxicity because them were either oxygen or chlorine substituted as well as comprised with similar structural features.

3. Results and discussion

3.1. Chemistry

The present investigation emphasised mainly on two important things, of these one is to the synthesis of heterocyclic compounds

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bearing the androstenedione moiety and the other is to determine their cytotoxicity against cancer and normal cell lines. Throughout our program some of our synthesized products were good candidates as target anticancer agents. The synthetic strategies adopted for the synthesis of the intermediates and target compounds are depicted in Schemes 1–4. Although much attention has been directed to study the biological importance of androstenedione [25–28], no investigations have appeared in the literature to describe its uses in one-pot investigation to form pyran and pyrimidine ring systems. Therefore, the need to create novel androstenedione derivatives for emerging drug targets is an active area of medicinal chemistry. Recently, our research group was involved through a series of heterocyclization of pregnenolone to form thiophene, pyrazole and pyridine derivatives as antitumor agents [29,30]. In the present work, we studied the uses of androstenedione in heterocyclic chemistry through one pot reaction to form novel E-heterocyclic rings of androstenedione as potentially anticancer agents. The obtained products were important in a different strategy, being used to obtain new heterocyclic derivatives of androstenedione together with the comparison of their cytotoxic activities towards human cancer normal cell lines. Thus, the one pot reaction of androstenedione (**1**) with either benzaldehyde (**2a**), pyridine-2-aldehyde (**2b**) and thiophene-2-aldehyde (**2c**) and either malononitrile (**3a**) or ethyl cyanoacetate (**3b**) gave the pyran derivatives **4a**–**f**, respectively (Scheme 1). On the other hand, the reaction of **1** with either benzaldehyde (**2a**), pyridine-2-aldehyde (**2b**) and thiophene-2-aldehyde (**2c**) and thiourea (**5**) gave the pyrimidine derivatives **6a**–**c**, respectively [31,32]. The analytical and spectral data of compounds **6a**–**c** were in agreement with their structures. Compound **6b** with its high yield encouraged us to study its reactivity towards some chemical reagents. Thus, it reacted with either 2-bromo-1-phenylethanone (**7a**) 2-bromo-1-(4-chlorophenyl)ethanone (**7b**), 2-bromo-1-p-tolylethanone (**7c**) or 2-bromo-1-(4-methoxyphenyl)ethanone (**7d**) to give the thiazolo[3,2-*b*]pyrimidine derivatives **8a**–**d**, respectively (Scheme 2). The structures of the latter products were based on analytical and spectral data. On the other hand, the reaction of androstenedione (**1**) with either malononitrile (**3a**) or ethyl cyanoacetate (**3b**) and elemental sulphur gave the thiophene derivatives **10a** and **10b**, respectively. The 2-amino group present in compounds **10a** and **10b** was capable for amide formation. Thus, the reaction of compounds **10a** and **10b** with either ethyl acetoacetate or diethyl malonate gave the amide derivatives **12a**–**d**, respectively. The elemental analyses and spectral data were the basis of their structural elucidations. Additionally, we focused on the azo coupling reactions of the 2-amido derivatives **12a** and **12b**. Thus, the coupling reaction of **12a**,**b** with either benzenediazonium chloride (**13a**), 4-methylbenzenediazonium chloride (**13b**) or 4-chlorobenzenediazonium chloride (**13c**) gave the arylhydrazo derivatives **14a**–**f**, respectively (Scheme 3).

Compounds **12a**–**d** underwent ready cyclization when heated in ethanol containing a catalytic amount of triethylamine to give the thieno[2,3-*b*]pyridine derivatives **15a**–**d**, respectively. All the micro-analysis and spectroscopic data were in accordance with the suggested structures of **15a**–**d**. Furthermore, this study was extended to include the behaviour of androstenedione (**1**) towards thiazole formation. Thus, the reaction of compound **1** with elemental sulphur and phenylisothiocyanate in ethanol containing triethylamine gave the thiazole derivative **16** (Scheme 4).

4. Conclusion

In summary, we have shown herein that our strategy is compatible with the synthesis of a wide range of androstenedione derivatives and particularly when being corporate to heterocyclic and fused derivatives. The cytotoxicity of the newly synthesized products were evaluated against human gastric cancer (NUGC and HR), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) and normal fibroblast cells (WI38). The results showed that compounds **4e**, **4f**, **6b**, **6c**, **8b** and **16** exhibited the optimal cytotoxic effect against the six cancer cell lines, with IC_{50}'s in the nM range.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2014.04.011.

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