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

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ABSTRACT

The one pot reaction of estrone with the aromatic aldehydes **2a–c** and either of malononitrile or ethyl cyanoacetate afforded the fused pyran derivatives **4a–f**. On the other hand, carrying the same reaction using thiourea instead of the cyanomethylene reagent gave the fused pyrimidine derivatives **6a–c**. The latter compounds reacted with phenacyl bromide to give the thiazolo[3,2-*a*]pyrimidine derivatives **8a–c**. The reaction of the title compound with bromine gave the monobromo derivative **13** which in turn reacted with either thiourea or cyanothioacetamide to give the thiazole derivatives **14** and **16**, respectively. The cytotoxicity of the newly synthesized products was evaluated against six human cancer and normal cell lines where the results showed that compounds **4c**, **4f**, **6b**, **8b**, **8c**, **10**, **13**, **16**, **18c** and **19c** exhibited optimal cytotoxic effect against the cancer cell lines, with IC_{50}'s in the nM range.

1. Introduction

Several D-ring alkylated estrone analogues display exceptionally high affinity for estrogen receptors [1–3]. In particular, compounds in which an E-ring is formed are known to be involved in the inhibition of steroidogenic enzymes [4,5]. Such compounds also have an effect on steroid dehydrogenase activity and the ability to inhibit the detrimental action of the steroid sulfatase enzyme [6–8]. Generally, E-ring extended steroids have been accessed by modification of the C17-ketone in the D-ring by either arylimine or oximino formation [9–11], addition of a carbon nucleophile [12] or hydrazone formation [13–15]. Other approaches have included ketone reduction, silyl enol ether formation or ring-closing metathesis (giving five- or six membered E-rings) [16,17]. 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. Steroids bearing heterocycles fused to the D-ring of the steroid nucleus have been of pharmaceutical interest [18–21]. In the present paper, we report an efficient synthesis of estrone possessing pyran, pyrimidine and thiazole ring systems. This study focused on the synthesis of heterocyclic estrone derivatives together with their cytotoxic evaluations towards human cancer and normal cell lines.

2. Experimental

2.1. Synthetic methods, analytical and spectral data

The starting steroid, estrone, 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 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-Universitat-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).

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*2.1.1. (6bS, 8aS,13aS,13bR)-10-amino-4-hydroxy-8a-methyl-12-phen yl-1,2,6b,7,8,8a-12,13,13a,13b-decahydronaphtho[2′,1′:4,5]indeno[1,2-b]pyran-11-carbonitrile (**4a**) (6bS, 8aS,13aS,13bR)-4,10-dihydroxy-8a-methyl-12-phenyl-1,2,6b,7,8,8a-12,13,13a,13b-decahydronaphtho[2′, 1′: 4,5] indeno[1,2-b]pyran-11-carbonitrile (**4b**), (6bS, 8aS,13aS,13bR)-10-amino-8a-methyl-12-(4-chlorophenyl)-4-hydroxy-8a-methyl-1,2, 6b,7,8,8a-12,13,13a,13b-decahydronaphtho[2′,1′:4,5] indeno[1,2-b] pyran-11-carbonitrile (**4c**), (6bS, 8aS,13aS,13bR)-4,10-dihydroxy-8a-methyl-12-(4-chlorophenyl)-8a-methyl-1,2,6b,7,8,8a-12,13,13a,13b-decahydronaphtho [2′,1′:4,5] indeno[1,2-b]pyran-11-carbonitrile (**4d**) (6bS, 8aS,13aS,13bR)-10-amino-8a-methyl-12-(4-methoxyphenyl)-4-hydroxy-8a-methyl-1,2,6b,7,8,8a-12,13,13a,13b-decahydronaphtho [2′, 1′:4,5] indeno[1,2-b]pyran-11-carbonitrile (**4e**), (6bS, 8aS,13aS,13bR) -4,10-dihydroxy-8a-methyl-12-(4-methoxyphenyl)-8a-methyl-1,2,6b, 7, 8,8a-12,13,13a,13b-decahydronaphtho [2′,1′:4,5] indeno[1,2-b]pyran-11-carbonitrile (**4f**)*

General procedure: To a solution of estrone (0.270 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), 4-chlorobenzaldehyde (0.140 g, 1 mmol) or 4-methoxybenzaldehyde (0.136 g, 1 mmol) were added. The reaction mixture, in each case, 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 = 24 min; pale yellow crystals from EtOAc:hexane (88%), m.p. 199–202 °C; IR (KBr) cm^{-1}: 3580–3403, 3055, 2938, 2220, 1638, 1560; ^{1}H-NMR (DMSO): δ 0.89 (s, 3H), 1.30–1.60 (m, 5H), 1.69 (dt, 3H, J = 7.0, 2.8 Hz), 1.72–1.83 (m, 2H), 1.88–2.02 (m, 1H), 2.90–2.97 (m, 2H), 2.99–3.12 (m, 1H), 4.44 (s, 2H, D_2O exchangeable), 5.77 (s, 1H), 6.44 (d, 1H, J = 2.3 Hz,), 6.76 (dd, 1H, J = 8.2 Hz and J = 2.3 Hz), 6.88–7.36 (m, 5H), 9.02 (s, 1H, D_2O exchangeable); ^{13}C-NMR (DMSO): δ 13.8, 22.8, 23.3, 26.9, 31.6, 38.6, 45.6, 49.6, 112.6, 117.8, 120.3, 124.2, 126.3, 128.6, 129.0, 135.4, 136.9, 140.3, 142.6, 146.8, 148.5, 149.5, 154.3, 155.9, 160.3, 196.6. MS: m/e = 424 (M^+, 29%); *Analysis Calcd* for $C_{28}H_{28}N_2O_2$: C, 79.22; H, 6.65; N, 6.60%. Found: C, 79.41; H, 6.83; N, 6.49%.

Compound **4b**: HPLC purity = 92% (C-18 NovaPak column; MeOH:H_2O/70:30), t_r = 22 min; pale yellow crystals from EtOAc:hexane (90%), m.p. 168–169 °C; IR (KBr) cm^{-1}: 3540–3423, 3057, 2932, 2222, 1637, 1563; ^{1}H-NMR (DMSO): δ 0.84 (s, 3H), 1.33–1.84 (m, 5H), 1.70 (dt, 3H, J = 6.89, 3.0 Hz), 1.72–1.85 (m, 2H), 1.88–1.22 (m, 1H), 2.88–2.94 (m, 2H), 2.96–3.11 (m, 1H), 5.73 (s, 1H), 6.44 (d, 1H, J = 2.3 Hz), 6.78 (dd, 1H, J = 7.8 Hz and J = 2.3 Hz), 6.85–7.38 (m, 5H), 9.03 (s, 1H, D_2O exchangeable); ^{13}C-NMR (DMSO): δ 14.6, 19.8, 22.6, 23.3, 26.9, 27.3, 31.5, 38.8, 40.2, 45.6, 49.8, 112.4, 116.9, 120.5, 125.9, 129.3, 135.4, 137.2, 140.3, 142.6, 146.8, 148.8, 155.9, 159.4, 160.1, 196.8. MS: m/e = 425 (M^+, 28%); *Analysis Calcd* for $C_{28}H_{27}NO_3$: C, 79.03; H, 6.40; N, 3.29%. Found: C, 79.18; H, 6.64; N, 3.33%.

Compound **4c**: HPLC purity = 93% (C-18 NovaPak column; MeOH:H_2O/70:30), t_r = 18 min; pale yellow crystals from EtOAc:hexane (88%), m.p. 180–182 °C; IR (KBr) cm^{-1}: 3535–3433, 3056, 2936, 2224, 1639, 1562; ^{1}H-NMR (DMSO): δ 0.82 (s, 3H), 1.37–1.58 (m, 5H), 1.67 (dt, 3H, J = 7.0, 2.8 Hz,), 1.72–1.82 (m, 2H), 1.86–2.03 (m, 1H), 2.90–2.94 (m, 2H), 2.96–3.09 (m, 1H), 4.60 (s, 2H, D_2O exchangeable), 5.68 (s, 1H), 6.45 (d, 1H, J = 2.2 Hz), 6.60 (dd, 1H, J = 6.89 Hz, J = 2.2 Hz), 6.88–7.41 (m, 4H), 9.06 (s, 1H, D_2O exchangeable); ^{13}C-NMR (DMSO): δ 14.4, 19.7, 22.8, 23.3, 26.9, 27.3, 31.8, 38.6, 40.5, 45.3, 49.3, 110.6, 117.2, 120.6,123.9, 126.4, 130.6, 135.6, 141.3, 143.8, 148.0, 154.8, 155.4, 159.3, 160.6. MS: m/e = 458 (M^+, 33%); *Analysis Calcd* for $C_{28}H_{27}ClN_2O_2$: C, 73.27; H, 5,93; N, 6.10; Cl, 7.72%. Found: C, 73,22; H, 6.05; N, 6.37; Cl, 7.99%.

Compound **4d**: HPLC purity = 90% (C-18 NovaPak column; MeOH:H_2O/85:15), t_r = 23 min; pale yellow crystals from EtOAc:hexane (86%), m.p. 140–142 °C; IR (KBr) cm^{-1}: 3544–3426, 3053, 2939, 2220, 1634, 1560; ^{1}H-NMR (DMSO): δ 0.82 (s, 3H), 1.32–1.56 (m, 5H), 1.67 (dt, 3H, J = 7.0, 2.8 Hz), 1.70–1.84 (m, 2H), 1.88–2.03 (m, 1H), 2.91–2.97 (m, 2H), 2.98–3.11 (m, 1H), 5.70 (s, 1H), 6.42 (d, 1H, J = 3.0 Hz), 6.67 (dd, 1H, J = 8.0 Hz, J = 3.0 Hz), 6.86–7.40 (m, 4H), 8.06, 9.05 (2s, 2H, D_2O exchangeable); ^{13}C-NMR (DMSO): δ 14.3, 19.8, 22.7, 23.0, 26.9, 27.8, 31.5, 38.8, 40.4, 45.2, 112.6, 117.2, 120.8, 123.8, 125.2, 129.3, 135.4, 137.2, 140.8, 143.0, 146.8, 154.3, 155.9, 159.7, 160.8, 196.3. MS: m/e = 459 (M^+, 36%); *Analysis Calcd* for $C_{28}H_{26}ClNO_3$: C, 73.11; H, 5,70; N, 3.05; Cl, 7.71%. Found: C, 73.38; H, 5.44; N, 6.94; Cl, 7.93%.

Compound **4e**: HPLC purity = 93% (C-18 NovaPak column; MeOH:H_2O/90:10), t_r = 20 min; pale yellow crystals from EtOAc:hexane (84%), m.p. 188–190 °C; (IR (KBr) cm^{-1}: 3532–3422, 3058, 2932, 2223, 1636, 1563; ^{1}H-NMR (DMSO): δ 0.86 (s, 3H), 1.31–1.59 (m, 5H), 1.66 (dt, 3H, J = 7.0, 2.8 Hz,), 1.71–1.83 (m, 2H), 1.87–2.02 (m, 1H), 2.90–2.96 (m, 2H), 2.98–3.11 (m, 1H), 3.18 (s, 3H), 4.43 (s, 2H, D_2O exchangeable), 5.71 (s, 1H), 6.44 (d, 1H, J = 3.8 Hz), 6.62 (dd, 1H, J = 6.8 Hz, J = 3.8 Hz), 6.83–7.43 (m, 4H), 9.02, 9.32 (2s, 2H, D_2O exchangeable); ^{13}C-NMR (DMSO): δ 14.6, 19.4, 22.7, 23.5, 26.9, 27.9, 31.3, 38.8, 40.6, 45.4, 49.7, 54.6, 112.2, 116.4, 121.4, 124.2, 125.6, 128.4, 130.4, 133.2, 136.4, 140.4, 142.9, 146.8, 148.6, 154.6, 159.9, 160.8. MS: m/e = 454 (M^+, 16%); *Analysis Calcd* for $C_{29}H_{30}N_2O_3$: C, 76.63.11; H, 6.65; N, 6.16%. Found: C, 76.82; H, 6.73; N, 6.29%.

Compound **4f**: HPLC purity = 88% (C-18 NovaPak column; MeOH:H_2O/90:10), t_r = 24 min; pale yellow crystals from EtOAc:hexane (73%), m.p. 233–235 °C; IR (KBr) cm^{-1}: 3548–3432, 3053, 2935, 2220, 1638, 1570; ^{1}H-NMR (DMSO): δ 0.83 (s, 3H), 1.30–1.62 (m, 5H), 1.67 (dt, 3H, J = 7.0, 2.8 Hz), 1.72–1.85 (m, 2H), 1.86–2.02 (m, 1H), 2.90–2.96 (m, 2H), 2.98–3.11 (m, 1H), 3.14 (s, 3H), 5.71 (s, 1H), 6.48 (d, 1H, J = 3.3 Hz), 6.70 (dd, 1H, J = 8.2 Hz and J = 3.3 Hz), 6.88–7.45 (m, 4H), 8.16 (s, 1H, D_2O exchangeable); ^{13}C-NMR (DMSO): δ 14.2, 19.3, 22.9, 23.5, 26.9, 27.9, 31.5, 38.4, 40.8, 44.6, 49.5, 54.6, 112.3, 116.8, 122.6, 124.5, 125.8, 129.1, 130.6, 132.8, 134.2, 141.2, 143.4, 146.8, 148.6, 153.2, 155.9, 160.2. MS: m/e = 455 (M^+, 32%); *Analysis Calcd* for $C_{29}H_{29}NO_4$: C, 76.46.11; H, 6.42; N, 3.07%. Found: C, 76.51; H, 6.53; N, 2.99%.

*2.1.2. 6bS,8aS,13aS,13bR)-10-Mercapto-8a-methyl-12-phenyl-2,6b,7, 8,8a,9,12,13,13a,13b-decahydro-1H-naphtho[2′,1′:4,5]indeno[1,2-d] pyrimidin-4-ol (**6a**), 6bS,8aS,13aS,13bR)-10-Mercapto-8a-methyl-12-(4-chlorophenyl)-2,6b,7,8,8a,9,12,13,13a,13b-decahydro-1H-naphtho [2′,1′:4,5] indeno[1,2-d]pyrimidin-4-ol (**6b**) and 6bS,8aS,13aS,13bR)-10-Mercapto-8a-methyl-12-(4-methoxyphenyl)-2,6b,7,8,8a,9,12,13, 13a,13b-decahydro-1H-naphtho [2′,1′:4,5] indeno[1,2-d]pyrimidin-4-ol (**6c**)*

General procedure: To a solution of estrone (0.270 g, 1 mmol) in absolute ethanol (40 mL) containing triethylamine (0.025 mL), either of the aromatic aldehydes namely benzaldehyde (106 g, 1 mmol), 4-chlorobenzaldehyde (0.140 g, 1 mmol) or 4-methoxybenzaldehyde (0.136 g, 1 mmol) together with thiourea (0.76 g,1 mmol) were added. The reaction mixture, in each case, was heated under reflux for 4 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 = 91% (C-18 NovaPak column; MeOH:H_2O/90:10), t_r = 20 min; white crystals from EtOAc: hexane (83%), m.p. 222–225 °C; IR (KBr) cm^{-1}: 3532–3421, 3058, 2938, 1622, 1560; ^{1}H-NMR (DMSO): δ 0.83 (s, 3H), 1.03 (s, 1H),

1.32–1.57 (m, 5H), 1.68 (dt, *J* = 7.2, 2.9 Hz, 3H), 1.70–1.85 (m, 2H), 1.83–2.02 (m, 1H), 2.92–2.97 (m, 2H), 2.98–3.11 (m, 1H), 5.73 (s, 1H), 6.42 (d, 1H, *J* = 2.5 Hz), 6.60 (dd, 1H, *J* = 7.23 Hz and *J* = 2.5 Hz), 6.93–7.42 (m, 5H), 8.22, 9.03 (2s, 2H, D_2O exchangeable); ^{13}C-NMR (DMSO): δ 13.9, 19.4, 22.8, 23.3, 26.9, 27.3, 31.6, 38.6, 40.2, 45.8, 60.3, 112.6, 113.8, 121.9, 124.8, 126.4, 127.2, 129.5, 135.4, 146.8, 153.0 154.8, 162.9. MS: *m*/*e* = 416 (M^+, 42%); *Analysis Calcd* for $C_{26}H_{28}N_2OS$: C, 74.96; H, 6.77; N, 6.72; S, 7.70%. Found: C, 75.72; H, 6.89; N, 6.66; S, 7.93%.

Compound **6b**: HPLC purity = 83% (C-18 NovaPak column; MeOH:H_2O/80:20), t_r = 21 min; white crystals from EtOAc: hexane (86%), m.p. 183–186 °C; IR (KBr) cm^{-1}: 3548–3433, 3054, 2933, 1631, 1562; ^{1}H-NMR (DMSO): δ 0.85 (s, 3H), 1.04 (s, 1H), 1.30–1.59 (m, 5H), 1.67 (dt, *J* = 7.1, 2.7 Hz, 3H), 1.71–1.87 (m, 2H), 1.85–2.04 (m, 1H), 2.91–2.96 (m, 2H), 2.98–3.09 (m, 1H), 5.70 (s, 1H), 6.44 (d, 1H, *J* = 2.8 Hz), 6.66 (dd, 1H, *J* = 6.94 Hz and *J* = 2.8 Hz), 6.82–7.40 (m, 4H), 8.06, 9.05 (2s, 2H, D_2O exchangeable); ^{13}C-NMR (DMSO): δ 14.2, 19.6, 22.8, 23.3, 26.9, 27.6, 31.6, 38.6, 40.3, 45.8, 60.5, 112.8, 114.0, 124.8, 126.8, 129.5, 135.4, 138.8, 146.9, 147.3, 152.3, 154.3, 162.9. MS: *m*/*e* = 451 (M^+, 40%); *Analysis Calcd* for $C_{26}H_{27}ClN_2OS$: C, 69.24; H, 6.03; Cl, 7.86; N, 6.21; S, 7.11%. Found: C, 69.03; H, 6.33; Cl, 9.05; N, 6.39; S, 7.30%.

Compound **6c**: HPLC purity = 89% (C-18 NovaPak column; MeOH:H_2O/87:13), t_r = 18 min; white crystals from EtOAc: hexane (88%), m.p. 203–205 °C; IR (KBr) cm^{-1}: 3528–3412, 3052, 2930, 1622, 1560; ^{1}H-NMR (DMSO): δ 0.86 (s, 3H), 1.04 (s, 1H), 1.32–1.61 (m, 5H), 1.66 (dt, *J* = 7.0, 2.7 Hz, 3H), 1.71–1.85 (m, 2H), 1.83–2.01 (m, 1H), 2.90–2.96 (m, 2H), 2.96–3.11 (m, 1H), 3.11 (s, 3H), 5.69 (s, 1H), 6.40 (d, 1H, *J* = 3.4 Hz), 6.60 (dd, 1H, *J* = 8.31 Hz and *J* = 3.4 Hz), 6.77–7.38 (m, 4H), 8.04, 9.05 (2s, 2H, D_2O exchangeable); ^{13}C-NMR (DMSO): δ 14.0, 19.4, 22.6, 23.0, 26.9, 27.8, 31.6, 38.6, 40.6, 45.7, 56.4, 60.2, 112.8, 113.8, 122.3, 126.8, 129.7, 138.6, 142.8, 146.4, 148.3, 151.8, 152.6, 154.3, 163.0. MS: *m*/*e* = 446 (M^+, 24%); *Analysis Calcd* for $C_{27}H_{30}N_2O_2S$: C, 72.61; H, 6.77; N, 6.27; S, 7.18%. Found: C, 72.88; H, 6.83; N, 6.18; S, 7.44%.

*2.1.3. (6bS,8aS,15aS,15R)-8a-Methyl-10,14-diphenyl-1,2,6b,7,8,8a,14,15,15a,15b-decahydronaphtho[2′,1′:4,5]indeno[2,1-e]thiazolo[3,2-a]pyrimidin-4-ol (**8a**), (6bS,8aS,15aS,15R)-10-(4-chlorophenyl)-8a-methyl-14-phenyl-1,2,6b,7,8,8a,14,15,15a,15b-decahydronaphtho [2′,1′:4,5] indeno[2,1-e]thiazolo[3,2-a]pyrimidin-4-ol (**8b**), (6bS,8aS,15aS,15R)-10-(4-methylphenyl)-8a-Methyl-14-phenyl–1,2,6b,7,8,8a,14,15,15a,15b-decahydronaphtho [2′,1′:4,5] indeno[2,1-e]thiazolo[3,2-a]pyrimidin-4-ol (**8c**), (6bS,8aS,15aS,15R)-10-(4-methoxyphenyl)-8a-Methyl-14-phenyl–1,2,6b,7,8,8a,14,15,15a,15b-decahydronaphtho [2′,1′:4,5] indeno[2,1-e]thiazolo[3,2-a]pyrimidin-4-ol (**8d**)*

General procedure: To a solution of compound **6a** (0.416 g, 1 mmol) in ethanol (40 mL), either 2-bromo-1-phenylethanone (0.20 g, 1 mmol), 2-bromo-1-(4-chlorophenyl)ethanone (0.235 g, 1 mmol), 2-bromo-1-(4-methylphenyl)ethanone (0.215 g, 1 mmol) or 2-bromo-1-(4-methoxyphenyl)ethanone (0.247 g, 1 mmol) was added. The reaction mixture, in each case was heated under reflux for 3 h then evaporated under vacuum. The remaining product was triturated with diethyl ether and the formed solid product was collected by filtration.

Compound **8a**: HPLC purity = 87% (C-18 NovaPak column; MeOH:H_2O/90:10), t_r = 18 min; white crystals from EtOAc:hexane (80%), m.p. 130–133 °C; IR (KBr) cm^{-1}: 3562–3441, 3054, 2936, 1631, 1562; ^{1}H-NMR (DMSO): δ 0.82 (s, 3H), 1.31–1.60 (m, 5H), 1.68 (dt, *J* = 7.1, 2.9 Hz, 3H), 1.71–1.87 (m, 2H), 1.81–2.01 (m, 1H), 2.90–2.94 (m, 2H), 2.96–3.03 (m, 2H), 6.44 (d, 1H, *J* = 3.2 Hz), 6.64 (dd, 1H, *J* = 8.2 Hz and *J* = 3.2 Hz), 6.51 (s, 1H), 7.29–7.42 (m, 10H), 9.05 (s, 1H, D_2O exchangeable); ^{13}C-NMR (DMSO): δ 13.4, 19.2, 22.8, 23.6, 26.9, 27.3, 31.6, 38.6, 40.4, 45.2, 57.3, 60.2, 104.8, 12.3, 122.4, 123.6, 124.4, 124.9, 125.9, 126.8, 128.5, 129.5, 135.4, 141.8, 142.6, 143.1, 149.4, 154.3, 154.8, 162.7. MS: *m*/*e* = 516 (M^+, 37%); *Analysis Calcd* for $C_{34}H_{32}N_2OS$: C, 79.03; H, 6.24; N, 5.42; S, 6.21%. Found: C, 78.88; H, 6.37; N, 5.28; S, 6.07%.

Compound **8b**: HPLC purity = 73% (C-18 NovaPak column; MeOH:H_2O/80:20), t_r = 19 min; white crystals from EtOAc:hexane (82%), m.p. 222–225 °C; IR (KBr) cm^{-1}: 3544–3423, 3052, 2935, 1630, 1563; ^{1}H-NMR (DMSO): δ 0.83 (s, 3H), 1.32–1.58 (m, 5H), 1.67 (dt, *J* = 6.9, 2.5 Hz, 3H), 1.70–1.88 (m, 2H), 1.80–2.03 (m, 1H), 2.90–2.93 (m, 2H), 2.96–3.01 (m, 2H), 6.45 (d, 1H, *J* = 3.9 Hz), 6.63 (dd, 1H, *J* = 6.99 Hz, *J* = 3.9 Hz), 6.50 (s, 1H), 6.92–7.40 (m, 9H), 9.04 (s, 1H, D_2O exchangeable); ^{13}C-NMR (DMSO): δ 14.0, 19.8, 22.8, 23.7, 26.9, 27.8, 31.6, 38.6, 40.1, 45.8, 57.3, 60.8, 104.2, 122.9, 123.8, 124.0, 125.9, 126.8, 128.5, 129.5, 130.3, 135.6, 142.0, 142.6, 143.1, 146.2, 149.6, 154.0, 154.8, 162.9. MS: *m*/*e* = 551 (M^+, 42%); *Analysis Cald* for $C_{34}H_{31}ClN_2OS$: C, 74.09; H, 5.67; N, 6.43; S, 5.82%. Found: C, 73.87; H, 5.72; N, 4.88; S, 6.08%.

Compound **8c**: HPLC purity = 88% (C-18 NovaPak column; MeOH:H_2O/87:13), t_r = 20 min; pale yellow crystals from EtOAc: hexane (80%), m.p. 188–190 °C; IR (KBr) cm^{-1}: 3538–3453, 3058, 2928, 1625, 1562; ^{1}H-NMR (DMSO): δ 0.83 (s, 3H), 1.35–1.59 (m, 5H), 1.68 (dt, *J* = 6.8, 2.7 Hz, 3H), 1.71–1.86 (m, 2H), 1.80–2.01 (m, 1H), 2.90–2.96 (m, 2H), 2.97–3.01 (m, 2H), 3.11 (s, 3H), 6.42 (d, 1H, *J* = 3.8 Hz), 6.52 (s, 1H), 6.64 (dd, 1H, *J* = 7.38 Hz and *J* = 3.8 Hz), 6.84–7.42 (m, 9H), 9.05 (s, 1H, D_2O exchangeable); ^{13}C-NMR (DMSO): δ 14.2, 19.6, 21.8, 22.6, 23.3, 25.4, 26.6, 27.9, 31.6, 38.8, 40.2, 45.7, 50.9, 60.3, 112.6, 119.3, 122.6, 123.6, 124.8, 126.2, 126.8, 129.9, 133.6, 138.6, 141.8, 142.3, 143.0, 146.6, 151.9, 153.3, 163.2. MS: *m*/*e* = 530 (M^+, 38%); *Analysis Cald* for $C_{35}H_{34}N_2OS$: C, 79.21; H, 6.46; N, 5.28; S, 6.04%. Found: C, 79.03; H, 6.55; N, 6.37; S, 5.93%.

Compound **8d**: HPLC purity = 88% (C-18 NovaPak column; MeOH:H_2O/87:13), t_r = 17 min; pale yellow crystals from EtOAc: hexane (77%), m.p. 122–124 °C; IR (KBr) cm^{-1}: 3538–3453, 3058, 2928, 1625, 1562; ^{1}H-NMR (DMSO): δ 0.83 (s, 3H), 1.35–1.59 (m, 5H), 1.67 (dt, *J* = 6.9, 2.5 Hz, 3H), 1.70–1.88 (m, 2H), 1.80–2.03 (m, 1H), 2.90–2.96 (m, 2H), 2.85–3.01 (m, 2H), 3.21(s, 3H), 6.40 (d, 1H, *J* = 2.6 Hz), 6.53 (s, 1H), 6.63 (dd, 1H, *J* = 8.72 Hz and *J* = 2.6 Hz), 6.81–7.40 (m, 9H), 9.06 (s, 1H, D_2O exchangeable); ^{13}C-NMR (DMSO): δ 14.4, 19.8, 22.6, 23.3, 26.8, 27.9, 30.5, 31.6, 38.8, 40.6, 44.6, 50.5, 55.3, 60.8, 112.8, 120.8, 122.8, 123.4, 124.2, 126.8, 127.3, 129.9, 133.8, 134.2, 138.9, 141.4, 142.3, 143.0, 147.3, 152.3, 154.6, 163.1. MS: *m*/*e* = 546 (M^+, 24%); *Analysis Calcd* for $C_{35}H_{34}N_2O_2S$: C, 76.89; H, 6.27; N, 5.12; S, 5.86%. Found: C, 76.93; H, 6.38; N, 5.06; S, 5.66%.

*2.1.4. (6bS,8aS,12aS,12bR)-4-hydroxy,8a-methyl-9-phenyl-6b,7,8,8a,9,12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[1,2-d]thiazole-10(2H)-thione (**10**)*

To a solution of estrone (0.270 g, 1 mmol) in absolute ethanol (40 mL) containing triethylamine (0.025 mL) elemental sulphur (0.32 g, 1 mmol) and phenylisothiocyanate (1.30 g, 1 mmol) were added. The reaction mixture was heated under reflux for 2 h till a solid product being formed and the latter was collected by filtration.

Compound **10**: HPLC purity = 75% (C-18 NovaPak column; MeOH:H_2O/80:20), t_r = 18 min; pale yellow crystals from EtOAc: hexane (80%), m.p. 193–195 °C; IR (KBr) cm^{-1}: 3520–3432, 3056, 2930, 1640, 1566, 1200–1195; ^{1}H-NMR ($CDCl_3$): δ 0.80 (s, 3H), 1.34–1.61 (m, 5H), 1.68 (dt, *J* = 7.0, 2.9 Hz, 3H), 1.70–1.86 (m, 2H), 1.83–2.02 (m, 1H), 2.86–2.90 (m, 2H), 2.92–3.08 (m, 2H), 6.63 (dd, 1H, *J* = 7.5 Hz and *J* = 3.2 Hz), 7.31–7.42 (m, 5H), 9.04 (s, 1H); ^{13}C-NMR ($CDCl_3$): δ 14.0, 19.6, 21.6, 22.5, 23.9, 25.4, 31.4, 38.3, 41.3, 44.3, 50.7, 54.0, 112.3, 118.7, 120.8, 129.2, 130.8, 133.2, 142.3, 143.0, 149.2, 152.3, 154.4, 183.2. MS: *m*/*e* = 419 (M^+, 32%); *Analysis Calcd* for $C_{25}H_{25}NOS_2$: C, 71.56; H, 6.01; N, 3.34; S, 15.28%. Found: C, 71.49; H, 5.87; N, 3.44; S, 15.06%.

*2.1.5. (6bS,8aS,12aS,12bR)-10-hydrazono-8a-methyl-9-phenyl-2,6b,7,8,8a,9,10,12,12a,12b-decahydro-1H-naphtho[2′,1′:4,5]indeno[1,2-d]-thiazol-4-ol (**12a**) and (6bS,8aS,12aS,12bR)- 8a-methyl-9-phenyl-10 (2-phenylhydrazono)-2,6b,7,8,8a,9,10,12,12a,12b-decahydro-1H-naphtho[2′,1′:4,5]indeno[1,2-d]thiazol-4-ol (**12a**)*

General procedure: To a solution of compound **10** (0.419 g, 1 mmol) in 1,4-dioxan (40 mL), either hydrazine hydrate (0.50 mL, 1 mmol) or phenylhydrazine (0.109 g, 1 mmol) was added. The reaction mixture was heated under reflux for 3 h then evaporated under vacuum. The remaining product was triturated with diethyl ether and the formed solid product was collected by filtration.

Compound **12a**: HPLC purity = 80% (C-18 NovaPak column; MeOH:H_2O/85:15), t_r = 23 min; pale yellow crystals from EtOAc: hexane (84%), m.p. 204–207 °C; IR (KBr) cm^{-1}: 3568–3412, 3053, 2935, 1660, 1638, 1562; ^{1}H-NMR ($CDCl_3$): δ 0.82 (s, 3H), 1.32–1.58 (m, 5H), 1.69 (dt, J = 6.8, 3.2 Hz, 3H), 1.68–1.82 (m, 2H), 2.84–2.92 (m, 2H), 2.95–3.06 (m, 2H), 4.88 (s, 2H, D_2Oexchangeable), 6.37 (d, 1H, J = 2.7 Hz), 6.62 (dd, 1H, J = 8.4 and J = 2.7 Hz), 7.31–7.46 (m, 5H), 9.03 (s, 1H); ^{13}C-NMR ($CDCl_3$): δ 13.8, 19.8, 21.6, 22.7, 23.9, 25.6, 31.6, 38.2, 41.6, 44.6, 51.6, 54.0, 115.9, 119.9, 124.8, 128.7, 130.3, 142.6, 144.6, 149.2, 152.3, 156.2, 172.1. MS: m/e = 417 (M^+, 44%); *Analysis Calcd* for $C_{25}H_{27}N_3OS$: C, 71.91; H, 6.52; N, 10.06; S, 7.68%. Found: C, 72.27; H, 6.79; N, 9.84; S, 7.92%.

Compound **12b**: HPLC purity = 72% (C-18 NovaPak column; MeOH:H_2O/87:13), t_r = 21 min; yellow crystals from EtOAc:MeOH (73%), m.p. 189–192 °C; IR (KBr) cm^{-1}: 3551–3442, 3056, 2937, 1660, 1634, 1560; ^{1}H-NMR ($CDCl_3$): δ 0.80 (s, 3H), 1.33–1.60 (m, 5H), 1.66 (dt, J = 7.2, 2.7 Hz, 3H), 1.70–1.88 (m, 2H), 2.87–2.91 (m, 2H), 2.95–3.06 (m, 2H), 6.34 (d, 1H, J = 4.2 Hz), 6.62 (dd, 1H, J = 7.6 Hz and J = 4.2 Hz), 7.28–7.36 (m, 10H), 8.22 (s, 1H, D_2O exchangeable), 9.05 (s, 1H, D_2O exchangeable); ^{13}C-NMR ($CDCl_3$): δ 13.9, 19.5, 21.6, 22.9, 23.9, 25.8, 31.6, 38.2, 41.4, 44.6, 51.6, 54.3, 115.3, 120.3, 123.4, 123.9, 125.3, 128.4, 133.8, 134.7, 140.6, 142.8, 143.8, 149.0, 152.6, 156.5, 172.1. MS: m/e = 493 (M^+, 100%); *Analysis Calcd* for $C_{31}H_{31}N_3OS$: C, 75.42; H, 6.33; N, 8.51; S, 6.50%. Found: C, 75.63; H, 6.50; N, 8.31; S, 6.73%.

*2.1.6. (8R,9S,13S,14S)-16-bromo-3-hydroxy-13-methyl-7,8,9,11,12,13,15,16-octahydro-6H-cyclopenta[a]phenanthen-17(14H)-one (**13**)*

A solution of estrone (0.270 g, 1 mmol) in glacial acetic acid (40 mL) was heated to 60 °C then a solution of bromine (0.160 g, 1 mmol) in acetic acid (5 mL) was added drop wise with continuous stirring. The reaction mixture was stirred at 60 °C for an additional 1 h and the solid product, so formed, upon pouring onto ice/water was collected by filtration.

Compound **13**: HPLC purity = 77% (C-18 NovaPak column; MeOH:H_2O/88:12), t_r = 19 min; yellow crystals from acetone (89%), m.p. 189–192 °C; IR (KBr) cm^{-1}: 3542–3385, 3056, 1720, 2920, 1631, 1538; ^{1}H-NMR ($CDCl_3$): δ 0.89 (s, 3H), 1.30–1.60 (m, 5H), 1.68 (dt, J = 7.0, 2.7 Hz, 3H), 1.71–1.86 (m, 2H), 2.90–2.96 (m, 2H), 2.98–3.10 (m, 2H), 5.04 (s, 1H), 6.34 (d, 1H, J = 2.8 Hz), 6.60 (dd, 1H, J – 8.4 Hz and J = 2.8 Hz), 9.05 (s, 1H, D_2O exchangeable); ^{13}C-NMR ($CDCl_3$): δ 13.9, 23.9, 25.5, 26.6, 29.8, 31.6, 38.2, 43.2, 44.6, 46.2, 47.2, 48.6, 122.3, 128.4, 134.7, 154.8, 156.5, 180.6. MS: m/e = 349 (M^+, 28%); *Analysis Calcd* for $C_{18}H_{21}BrO_2$: C, 61.90; H, 6.06; Br, 22.88%. Found: C, 62.11; H, 6.29; Br, 23.17%.

*2.1.7. (6bS,8aS,12aS,12bR)-10-amino-8a-methyl-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[1,2-d]thiazol-4-ol (**14**)*

To a solution compound **13** (0.349 g, 1 mmol) in absolute ethanol (40 mL), thiourea (0.76 g, 1 mmol) was added. The reaction mixture was heated under reflux for 3 h then left to cool. The formed solid product was collected by filtration dried and monitored through TLC for the purity.

Compound **14**: HPLC purity = 81% (C-18 NovaPak column; MeOH:H_2O/90:10), t_r = 20 min; yellow crystals from acetone (83%), m.p. 220–223 °C; IR (KBr) cm^{-1}: 3560–3325, 3053, 2923, 1633, 1532; ^{1}H-NMR (DMSO): δ 0.84 (s, 3H), 1.33–1.56 (m, 5H), 1.64 (dt, J = 7.1, 2.9 Hz, 3H), 1.71–1.88 (m, 2H), 2.87–2.93 (m, 2H), 2.96–3.04 (m, 2H), 4.28 (s, 2H, D_2O exchangeable), 6.35 (d, 1H, J = 2.8 Hz), 6.63 (dd, 1H, J = 7.8 Hz and J = 2.8 Hz), 9.03 (s, 1H, D_2O exchangeable); ^{13}C-NMR (DMSO): δ 14.2, 24.3, 25.8, 26.6, 29.8, 31.4, 38.4, 43.6, 44.8, 48.8, 120.3, 125.8, 128.6, 130.5, 134.7, 140.3, 154.7, 156.6, 160.4. MS: m/e = 326 (M^+, 55%); *Analysis Calcd* for $C_{19}H_{22}N_2OS$: C, 69.90; H, 6.79; N, 8.58; S, 9.82%. Found: C, 69.84; H, 6.82; N, 8.47; S, 10.03%.

*2.1.8. 2-((6bS,8aS,12aS,12bR)-4-Hydroxy-8a-methyl-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[1,2-d]thiazol-10-yl) acetonitrile (**16**)*

To a solution of compound **6a** (0.416 g, 1 mmol) in ethanol (40 mL), cyanothioacetamide (0.10 g, 1 mmol) was added. The reaction mixture was heated under reflux for 4 h then evaporated under vacuum. The remaining product was triturated with diethyl ether and the formed solid product was collected by filtration.

Compound **16**: HPLC purity = 85% (C-18 NovaPak column; MeOH:H_2O/82:18), t_r = 22 min; pale yellow crystals from EtOAc:hexane (88%), m.p. 288–290 °C; IR (KBr) cm^{-1}: 3558–3422, 3053, 2932, 2220, 1642, 1560; ^{1}H-NMR ($CDCl_3$): δ 0.82 (s, 3H), 1.33–1.60 (m, 5H), 1.63 (dt, J = 7.3, 3.3 Hz, 3H), 1.70–1.88 (m, 2H), 2.87–2.95 (m, 2H), 2.91–2.99 (m, 2H), 4.99 (s, 2H), 6.43 (d, 1H, J = 3.4 Hz), 6.60 (dd, 1H, J = 8.5 Hz and J = 3.4 Hz), 9.02 (s, 1H, D_2O exchangeable); ^{13}C-NMR ($CDCl_3$): δ 14.1, 19.9, 21.9, 22.8, 23.5, 27.9, 30.8, 31.6, 38.8, 40.9, 44.3, 50.5, 54.2, 116.8, 124.2, 126.8, 129.9, 142.3, 143.0, 154.6, 163.0. MS: m/e = 350 (M^+, 100%); *Analysis Calcd* for $C_{21}H_{22}N_2OS$: C, 71.97; H, 6.33; N, 7.99; S, 9.15%. Found: C, 72.29; H, 6.58; N, 8.21; S, 9.28%.

*2.1.9. (6bS,8aS,12aS,12bR,Z)-4-hydroxy-8a-methyl-N′-phenyl-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[1,2-d]triazole-10-carbohydrazonoyl cyanide(**18a**), (6bS,8aS,12aS,12bR,Z)-4-hydroxy-8a-methyl-N′-(p-tolyl)-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho [2′,1′:4,5] indeno[1,2-d]triazole-10-carbohydrazonoyl cyanide (**18b**), (6bS,8aS,12aS,12bR,Z)-4-hydroxy-8a-methyl-N′-(4-chlorophenyl)-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho [2′,1′:4,5] indeno[1,2-d]triazole-10-carbohydrazonoyl cyanide (**18c**) and (6bS,8aS,12aS,12bR,Z)-4-hydroxy-8a-methyl-N′-(4-methoxyphenyl)-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho [2′,1′:4,5] indeno[1,2-d]triazole-10-carbohydrazonoyl cyanide (**18d**)*

General procedure: To a solution of compound **16** (0.350 g, 1 mmol) in ethanol (30 mL) containing sodium acetate (2.5 g), either of benzenediazonium chloride (0.01 mol), 4-methylbenzenediazonium chloride (1 mmol), 4-chlorobenzenediazonium chloride (1 mmol) or 4-methoxybenzenediazonium chloride [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 N) 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.

Compound **18a**: HPLC purity = 88% (C-18 NovaPak column; MeOH:H_2O/85:15), t_r = 20 min; orange crystals from 1,4-dioxan (78%), m.p. 172–174 °C; IR (KBr) cm^{-1}: 3548–3430, 3056, 2928, 2223, 1633, 1562; ^{1}H-NMR (DMSO): δ 0.83 (s, 3H), 1.31–1.61 (m, 5H), 1.62 (dt, J = 7.1, 3.1 Hz, 3H), 1.71–1.89 (m, 2H), 2.84–2.95 (m, 2H), 2.97–3.02 (m, 2H), 6.42 (d, 1H, J = 2.9 Hz), 6.62 (dd, 1H, J = 6.5 Hz and J = 2.9 Hz), 7.03–7.38 (m, 5H), 8.22 (s, 1H, D_2O exchangeable), 9.05 (s, 1H, D_2O exchangeable); ^{13}C-NMR (DMSO): δ 14.0, 19.7, 21.7, 22.9, 27.6, 31.6, 38.8, 40.9, 44.3, 50.5, 116.6, 120.3, 122.6, 124.8, 126.4, 130.6, 135.2, 138.3, 140.8, 142.3,

143.0, 149.4, 152.6, 163.0, 165.8. MS: *m*/*e* = 454 (M^+, 44%); *Analysis Calcd* for $C_{27}H_{26}N_4OS$: C, 71.34; H, 5.76; N, 12.32; S, 7.05%. Found: C, 71.49; H, 5.92; N, 12.19; S, 6.88%.

Compound **18b**: HPLC purity = 88% (C-18 NovaPak column; MeOH:H_2O/85:15), t_r = 20 min; orange crystals from 1,4-dioxan (78%), m.p. 172–174 °C; IR (KBr) cm^{-1}: 3548–3430, 3056, 2928, 2223, 1633, 1562; ^{1}H-NMR (DMSO): δ 0.83 (s, 3H), 1.31–2.83 (m, 5H), 2.82–2.97 (m, 4H), 3.11 (s, 3H), 3.33 (s, 1H), 6.42 (d, 1H, *J* = 2.9 Hz), 6.61 (dd, 1H, *J* = 7.5 Hz and *J* = 2.9 Hz), 7.03–7.38 (m, 7H), 8.24 (s, 1H, D_2O exchangeable), 9.06 (s, 1H, D_2O exchangeable); ^{13}C-NMR (DMSO): δ 13.9, 19.9, 21.5, 22.8, 23.1, 27.8, 31.8, 38.6, 40.9, 44.6, 50.8, 116.8, 120.3, 122.6, 124.8, 126.6, 130.9, 135.2, 137.4, 140.8, 142.3, 143.3, 148.8, 152.8, 162.8, 166.0. MS: *m*/*e* = 468 (M^+, 28%); *Analysis Calcd* for $C_{28}H_{28}N_4OS$: C, 71.76; H, 6.02; N, 11.96; S, 6.84%. Found: C, 71.63; H, 5.84; N, 12.24; S, 6.77%.

Compound **18c**: HPLC purity = 74% (C-18 NovaPak column; MeOH:H_2O/90:10), t_r = 19 min; orange crystals from ethanol (80%), m.p. 145–148 °C; IR (KBr) cm^{-1}: 3555–3426, 3054, 2931, 2221, 1635, 1560; ^{1}H-NMR (DMSO): δ 0.84 (s, 3H), 1.30–1.61 (m, 5H), 1.60 (dt, *J* = 7.2, 3.1 Hz, 3H), 1.73–1.88 (m, 2H), 2.44–2.68 (m, 2H), 2.90–2.95 (m, 2H), 6.43 (d, 1H, *J* = 3.4 Hz), 6.59 (dd, 1H, *J* = 7.4 Hz and *J* = 3.4 Hz), 7.28–7.39 (m, 4H), 8.24 (s, 1H, D_2O exchangeable), 9.03 (s, 1H, D_2O exchangeable); ^{13}C-NMR (DMSO): δ 13.5, 19.9, 21.7, 23.5, 27.9, 31.8, 38.6, 41.6, 44.6, 50.6, 116.9, 120.6, 122.8, 124.3, 128.8, 130.8, 132.6, 134.8, 138.8, 140.6, 145.6, 148.8, 153.5, 162.4, 166.2. MS: *m*/*e* = 489 (M^+, 31%); *Analysis Calcd* for $C_{27}H_{25}ClN_4OS$: C, 66.31; H, 5.15; Cl, 7.25; N, 11.46; S, 6.56%. Found: C, 66.28; H, 5.30; Cl, 7.40; N, 11.59; S, 6.70%.

Compound **18d**: HPLC purity = 79% (C-18 NovaPak column; MeOH:H_2O/90:10), t_r = 23 min; orange crystals from ethanol (83%), m.p. 250–253 °C; IR (KBr) cm^{-1}: 3512–3427, 3053, 2929, 2223, 1635, 1562; ^{1}H-NMR (DMSO): δ 0.84 (s, 3H), 1.30–1.60 (m, 5H), 1.62 (dt, *J* = 7.3, 3.2 Hz, 3H), 1.73–1.89 (m, 2H), 2.84–2.89 (m, 2H), 2.92–2.99 (m, 2H), 3.01 (s, 3H), 6.40 (d, 1H, *J* = 3.2 Hz), 6.63 (dd, 1H, *J* = 7.9 Hz and *J* = 3.2 Hz), 7.28–7.42 (m, 4H), 8.24 (s, 1H, D_2O exchangeable), 9.04 (s, 1H); ^{13}C-NMR (DMSO): δ 14.1, 19.6, 21.7, 23.3, 27.4, 31.9, 38.6, 41.2, 44.6, 51.4, 54.8, 117.3, 120.6, 123.1, 124.6, 125.4, 129.4, 135.2, 140.8, 142.6, 143.3, 149.5, 153.5, 154.9, 163.6, 166.0. MS: *m*/*e* = 484 (M^+, 35%); *Analysis Calcd* for $C_{28}H_{28}N_4O_2S$: C, 69.40; H, 5.82; N, 11.56; S, 6.62%. Found: C, 69.53; H, 5.65; N, 11.71; S, 6.87%.

*2.1.10. 6-((6bS,8aS,12aS,12bR)-4-hydroxy-8a-methyl-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[1,2-d]thiazol-10-yl)-5-imino-2,4-diphenyl-4,5-dihydro-1,2,4-triazine-3(2H)-thione (**19a**), 6-((6bS,8aS,12aS,12bR)-4-hydroxy-8a-methyl-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho [2′,1′:4,5] indeno[1,2-d]thiazol-10-yl)-5-imino-4-phenyl-2-(p-tolyl)-4,5-dihydro-1,2,4-triazine-3(2H)-thione (**19b**), 6-((6bS,8aS,12aS,12bR)-4-hydroxy-8a-methyl-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho [2′,1′:4,5] indeno[1,2-d]thiazol-10-yl)-5-imino-4-phenyl-2-(p-chlorophenyl)-4,5-dihydro-1,2,4-triazine-3(2H)-thione (**19c**), 6-((6bS,8aS,12aS,12bR)-4-hydroxy-8a-methyl-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho [2′,1′:4,5] indeno[1,2-d] thiazol-10-yl)-5-imino-4-phenyl-2-(p-methoxyphenyl)-4,5-dihydro-1,2,4-triazine-3(2H)-thione (**19d**)*

General procedure: To a solution of either compound **18a** (0.454 g, 1 mmol), **18b** (0.468 g, 1 mmol), **18c** (0.489 g, 1 mmol) or **18d** (0.484 g, 1 mmol) in 1,4-dioxan (30 mL) containing triethylamine (0.250 mL) phenylisothiocyanate (0.130 g, 1 mmol) was added. The whole reaction mixture, in each case was heated under reflux for 4 h then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

Compound **19a**: HPLC purity = 90% (C-18 NovaPak column; MeOH:H_2O/90:10), t_r = 21 min; orange crystals from ethanol (72%), m.p. 210–212 °C; IR (KBr) cm^{-1}: 3531–3424, 3054, 2925, 1670, 1632, 1560; ^{1}H-NMR (DMSO): δ 0.82 (s, 3H), 1. .30–1.58 (m, 5H), 1.61 (dt, *J* = 7.0, 3.1 Hz, 3H), 1.72–1.89 (m, 2H), 2.82–2.88 (m, 2H), 2.92–2.95 (m, 2H), 6.40 (d, 1H, *J* = 3.3 Hz), 6.62 (dd, 1H, *J* = 8.2 Hz and *J* = 3.3 Hz), 7.26–7.44 (m, 10H), 8.22, (s, 1H, D_2O exchangeable), 9.03 (s, 1H, D_2O exchangeable); ^{13}C-NMR (DMSO): δ 14.2, 19.5, 21.9, 22.5, 23.7, 27.3, 30.4, 31.7, 38.3, 41.1, 44.3, 50.5, 119.3, 121.4, 122.6, 124.3, 125.7, 126.4, 128.0, 129,8, 130.8, 133.2, 135.0, 140.8, 142.6, 143.4, 149.2, 152.8, 163.3, 166.0, 168.3, 179.3 MS: *m*/*e* = 589 (M^+, 28%); *Analysis Cald* for $C_{34}H_{31}N_5OS_2$: C, 69.24; H, 5.30; N, 11.87; S, 10.87%. Found: C, 69.44; H, 5.52; N, 11.64; S, 10.73%.

Compound **19b**: HPLC purity = 87% (C-18 NovaPak column; MeOH:H_2O/90:10), t_r = 19 min; red crystals from ethanol (80%), m.p. 189–191 °C; IR (KBr) cm^{-1}: 3545–3427, 3053, 2932, 1677, 1631, 1560; ^{1}H-NMR (DMSO): δ 0.83 (s, 3H), 1.30–1.55 (m, 5H), 1.60 (dt, *J* = 7.2, 2.6 Hz, 3H), 1.72–1.90 (m, 2H), 2.82–2.89 (m, 2H), 2.91–2.96 (m, 2H), 3.09 (s, 3H), 6.45 (d, 1H, *J* = 3.2 Hz), (dd, 1H, *J* = 7.6 Hz and *J* = 3.2 Hz), 7.28–7.43 (m, 9H), 8.24 (s, 1H, D_2O exchangeable), 9.04 (s, 1H, D_2O exchangeable); ^{13}C-NMR (DMSO): δ 14.1, 19.5, 21.6, 22.9, 23.4, 23.7, 24.6, 28.4, 30.9, 31.7, 38.5, 40.9, 44.8, 50.6, 120.4, 123.9, 124.4, 125.3, 126.6, 128.3, 130.6, 133.6, 135.0, 138.5, 140.6, 142.2, 143.6, 148.8, 152.7, 154.6, 163.0, 166.3, 172.5, 180.3. MS: *m*/*e* = 603 (M^+, 28%); *Analysis Calcd* for $C_{35}H_{33}N_5OS_2$: C, 69.62; H, 5.51; N, 11.60; S, 10.62%. Found: C, 69.82; H, 5.73; N, 11.53; S, 10.85%.

Compound **19c**: HPLC purity = 89% (C-18 NovaPak column; MeOH:H_2O/90:10), t_r = 22 min; yellow crystals from ethanol (83%), m.p. 122–124 °C; IR (KBr) cm^{-1}: 3555–3441, 3056, 2940, 1666, 1635, 1563; ^{1}H-NMR (DMSO): δ 0.82 (s, 3H), 1.31–1.59 (m, 5H), 1.63 (dt, *J* = 7.2, 3.0 Hz, 3H), 1.72–1.89 (m, 2H), 2.81–2.88 (m, 2H), 2.92–2.97 (m, 2H), 6.46 (d, 1H, *J* = 3.2 Hz), 6.62 (dd, 1H, *J* = 8..1 Hz and *J* = 3.2 Hz), 7.31–7.44 (m, 9H), 8.26 (s, 1H, D_2O exchangeable), 9.04 (s, 1H, D_2O exchangeable); ^{13}C-NMR (DMSO): δ 13.8, 19.5, 21.7, 22.8, 23.7, 28.5, 30.5, 38.8, 41.6, 44.6, 50.6, 119.4, 120.6, 122.8, 123.7, 124.3, 125.6, 127.4, 128.9, 133.9, 134.2, 138.4, 142.3, 145.8, 148.8, 153.2, 155.1, 162.3, 166.4, 172.4, 180.4. MS: *m*/*e* = 624 (M^+, 28%); *Analysis Calcd* for $C_{34}H_{30}ClN_5OS_2$: C, 65.42; H, 4.84; Cl, 5.68; N, 11.22; S, 10.27%. Found: C, 64.53; H, 5.08; Cl, 5.82; N, 11.36; S, 10.36%.

Compound **19d**: HPLC purity = 89% (C-18 NovaPak column; MeOH:H_2O/85:15), t_r = 19 min; pale yellow crystals from ethanol (73%), m.p. > 300 °C; IR (KBr) cm^{-1}: 3532–3451, 3056, 2925, 1635, 1558; ^{1}H-NMR (DMSO): δ 0.82 (s, 3H), 1.28–2.82 (m, 5H), 1.60 (dt, *J* = 6.8, 3.3 Hz, 3H), 1.702–1.89 (m, 2H), 2.81–2.87 (m, 2H), 2.90–2.93 (m, 2H), 3.26 (s, 3H), 6.37 (d, 1H, *J* = 3.1 Hz), 6.63 (dd, 1H, *J* = 6.3 Hz and *J* = 3.1 Hz), 7.31–7.46 (m, 9H), 8.23 (s, 1H, D_2O exchangeable), 9.02 (s, 1H, D_2O exchangeable); ^{13}C-NMR (DMSO): δ 14.0, 19.7, 21.9, 22.3, 23.6, 27.6, 31.1, 38.6, 41.2, 44.8, 51.4, 54.3, 119.8, 120.3, 122.7, 123.7, 127.2, 129.2, 135.0, 138.7, 141.5, 142.8, 143.1, 149.7, 153.6, 154.9, 163.4, 166.4, 172.0, 180.2. MS: *m*/*e* = 619 (M^+, 26%); *Analysis Calcd* for $C_{35}H_{33}N_5O_2S_2$: C, 67.82; H, 5.37; N, 11.30; S, 10.35%. Found: C, 67.68; H, 5.49; N, 11.59; S, 10.42%.

3. Results and discussion

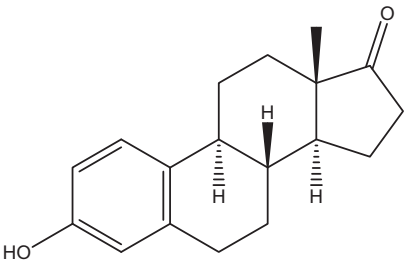
3.1. Chemistry

Although much attention has been directed to study the uses of estrone in heterocyclic synthesis [22–25], 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 estrone 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 [26,27]. In the present work, we studied the uses of estrone in heterocyclic chemistry through the one pot reaction to form novel E-heterocyclic rings of estrone. The obtained products were good candidates, in a different strategy, were used to obtain new heterocyclic derivatives of estrone for comparison with their cytotoxic activities towards human cancer and normal cell lines. Thus, the one pot reaction of estrone (**1**) with either of benzaldehyde (**2a**), 4-chlorobenzaldehyde (**2b**) or 4-methoxybenzaldehyde (**2c**) and either malononitrile (**3a**) or ethyl cyanoacetate (**3b**) in ethanol containing triethylamine gave the pyran derivatives **4a–f**, respectively (Scheme 1). The analytical and spectral data of the synthesized products were based on their analytical and spectral data. Thus, the ^{1}H NMR spectrum of **4a** (as an example) showed beside the expected signals for estrone, a singlet at δ 4.44 ppm (D_2O exchangeable) corresponding to the NH_2 group, a singlet at δ 5.77 ppm indicating the pyran H-4, a multiplet at δ 6.88–7.36 ppm indicating the phenyl protons. Moreover, the ^{13}C NMR spectrum showed δ 117.8 corresponding to the CN group and signals at δ 120.3, 124.2, 126.3, 128.6, 129.0, 135.4, 136.9, 140.3, 142.6, 146.8, 148.5, 149.5, 154.3, 155.9, 160.3 and 196.6 for the pyran and phenyl C. Formation of the pyran derivatives using one pot reaction of cycloketone and cyanomethylene reagents were reported in literature [28,29].

Next, our program moved towards studying the reaction of estrone with the aromatic aldehydes **2a–c** and thiourea in ethanol containing triethyl amine gave the pyrimidine derivatives **6a–c**, respectively. The ^{1}H-NMR and ^{13}C-NMR data of **6a–c** were the basis of their structure elucidation. Thus, the ^{1}H NMR spectrum of compound **6a** (as an example) showed a singlet at δ 1.03 ppm indicating the SH group, a singlet at δ 5.73 for the pyrimidine H-4, a multiplet at δ 6.93–7.42 ppm for the phenyl protons a singlet at δ 8.22 (D_2O exchangeable) corresponding to the presence of the NH group. Moreover, the ^{13}C NMR spectrum showed δ 112.6, 113.8, 121.9, 124.8, 126.4, 127.2, 129.5, 135.4, 146.8, 153.0 154.8, 162.9 for the pyrimidine C-4 and aromatic carbons.

Compounds **6a–c** with their high yields encouraged us to use them as good candidates for fused thiazolopyrimidine formation. Thus, the reaction of either of compounds **6a**, **6b** or **6c** with any of the α-haloketones namely 2-bromo-1-phenylethanone (**7a**), 2-bromo-1-(4-chlorophenyl)ethanone (**7b**), 2-bromo-1-p-tolylethanone (**7c**) and 2-bromo-1-(4-methoxy)ethanone (**7d**) in ethanol solution under reflux gave the 3,7-diphenyl-7H-thiazolo[3,2-*a*]



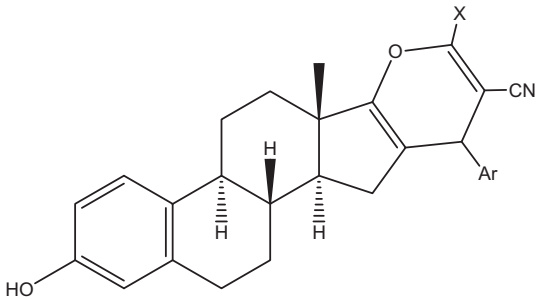
1

+ Ar-CHO + H_2C—CN
|
X

2a, Ar = C_6H_5
b, Ar = 4-Cl-C_6H_4
c, Ar = 4-CH_3O-C_6H_4

3a, X = CN
b, X = $COOC_2H_5$

EtOH/Et_3N



4	Ar	X
a	C_6H_5	NH_2
b	C_6H_5	OH
c	4-Cl-C_6H_4	NH_2
d	4-Cl-C_6H_4	OH
e	4-CH_3O-C_6H_4	NH_2
f	4-CH_3O-C_6H_4	OH

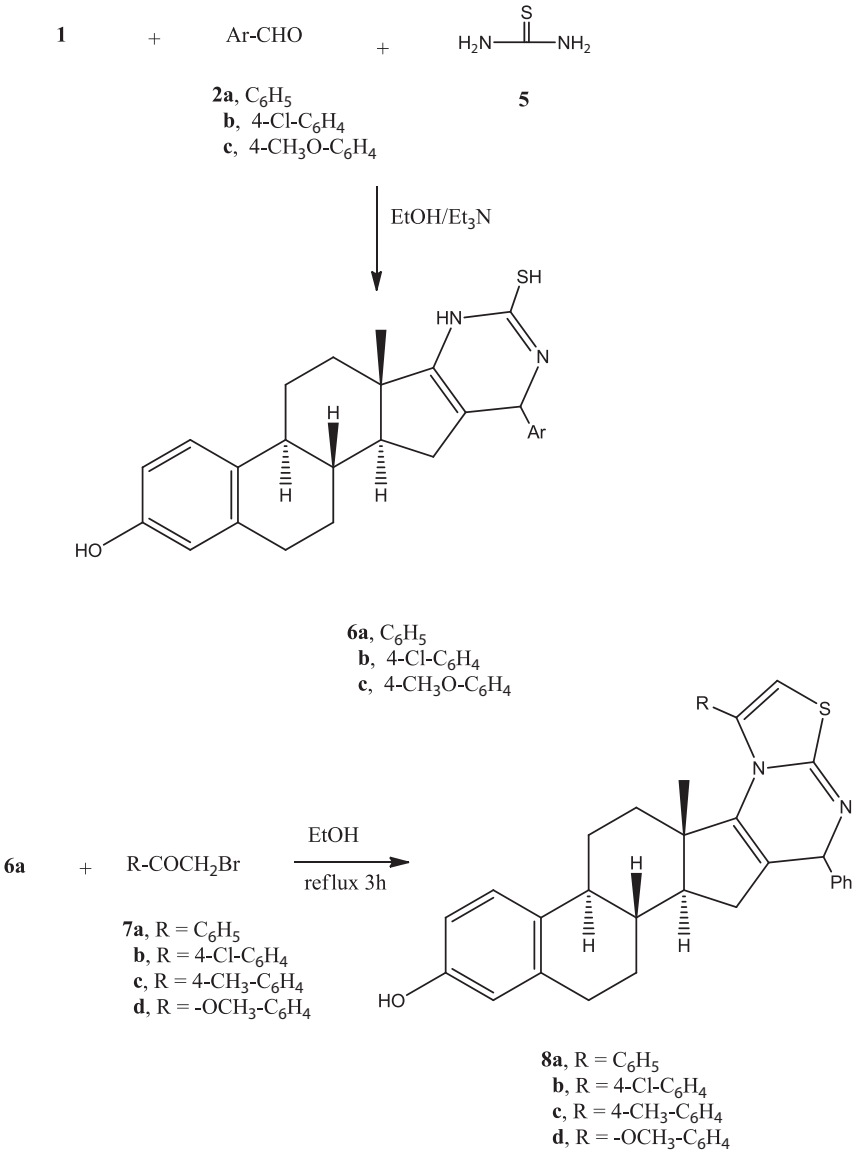
Scheme 1. Synthesis of compounds **4a-f**.

pyrimidine derivatives **8a–d**, respectively (Scheme 2). Formation of these thiazole derivatives took place in analogy to the well known the Hantzsch thiazole synthesis which involves the condensation of α-haloketones with thioureas [30–33]. The obtained analytical and spectral data of compounds **8a–d** were in agreement with their respective structures (see Section 2).

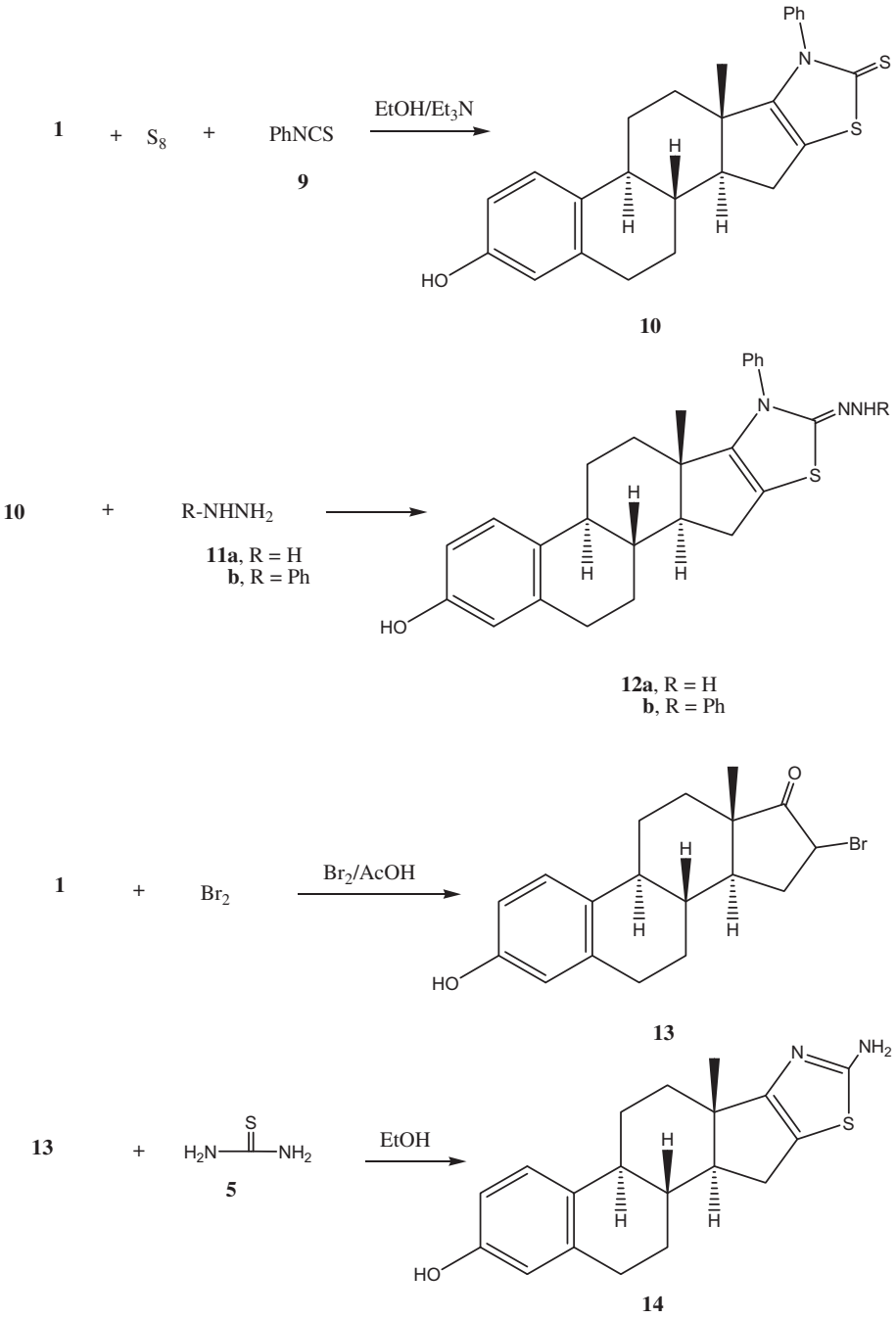
The reaction of estrone with phenylisothiocyanate and elemental sulphur in ethanol and triethyl amine gave the thiazole derivative **10**. The reaction of compound **10** with either hydrazine hydrate or phenylhydrazine gave the hydrazone derivatives **12a** and **12b**, respectively.

The reaction of estrone with bromine has been reported before under different reaction conditions. Thus, its reaction with N-bromosuccinimide and refluxing chloroform [34,35] the product was the 2,4-dibromo derivative which means that the reaction occurred at phenolic ring A of estrone. On the other hand, in other reports carrying the reaction of estrone acetate with bromine in chloroform solution gave the 16-bromo-estrone acetate [36]. In the present work direct bromination of estrone was carried out to give the corresponding 16-bromo derivative by the shortest route. Thus, estrone reacted with bromine in acetic acid at 60 °C to give the α-halocarbonyl compound **13** together with other unidentified products. The pure 16-bromoestrone derivative was separated via column chromatography and identified via the analytical and spectral data. Compound **13** reacted with thiourea in ethanol gave the 2-aminothiazole derivative **14** (Scheme 3). The structure of compound **14** was established on the basis of analytical and spectral data. Thus, the ^{1}H NMR spectrum showed a singlet at δ 4.28 (D_2O exchangeable) indicating the NH_2 group, and the ^{13}C NMR spectrum showed δ at 120.3, 125.8, 128.6, 130.5, 134.7, 140.3, 154.7, 156.6 and 160.4 corresponding to the thiazole and phenyl carbons.

Similarly, the reaction of compound **13** with 2-cyanoethanethioamide (**15**) in ethanol solution gave the thiazole derivative **15**. The cyanomethylene moiety present in compound **15** showed interesting reactivity. Thus, compound **15** reacted with the aryldiazonium salts **17a–d** in ethanol containing sodium acetate to give the arylhydrazone derivatives **18a–d**, respectively. The reaction of the latter compounds with phenylisothiocyanate (**9**) in ethanol containing triethylamine gave the 1,2,4-triazin-6-thiazolyl derivatives **19a–d**, respectively (Scheme 4). The analytical and spectral data of the latter products are consistent with their respective



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



Scheme 3. Synthesis of **10**, **12a,b**; **13** and **14**.

structures. Thus, the ^{1}H NMR spectrum of **19a** showed the presence of a multiplet at δ 7.26–7.44 ppm for the phenyl protons and a singlet at δ 8.22 (D_2O exchangeable) for the NH group. Moreover, the ^{13}C NMR spectrum showed δ 119.3, 121.4, 122.6, 124.3, 125.7, 126.4, 128.0, 129.8, 130.8, 133.2, 135.0, 140.8, 142.6, 143.4, 149.2, 152.8, 163.3, 166.0 corresponding to phenyl carbons, δ 168.3 corresponding to the exocyclic C=N group and δ 179.3 indicating the C=S group. It is clear from the demonstrated reactions especially formation of **4a–f**, **6a–c**, **10** and **13** occurred at the ring D of estrone and this is explained in terms of the reactivity of the cyclopentanone moiety towards the respective reagents used for each reaction. More support for such findings were demonstrated through the previous reported work [10,37–39].

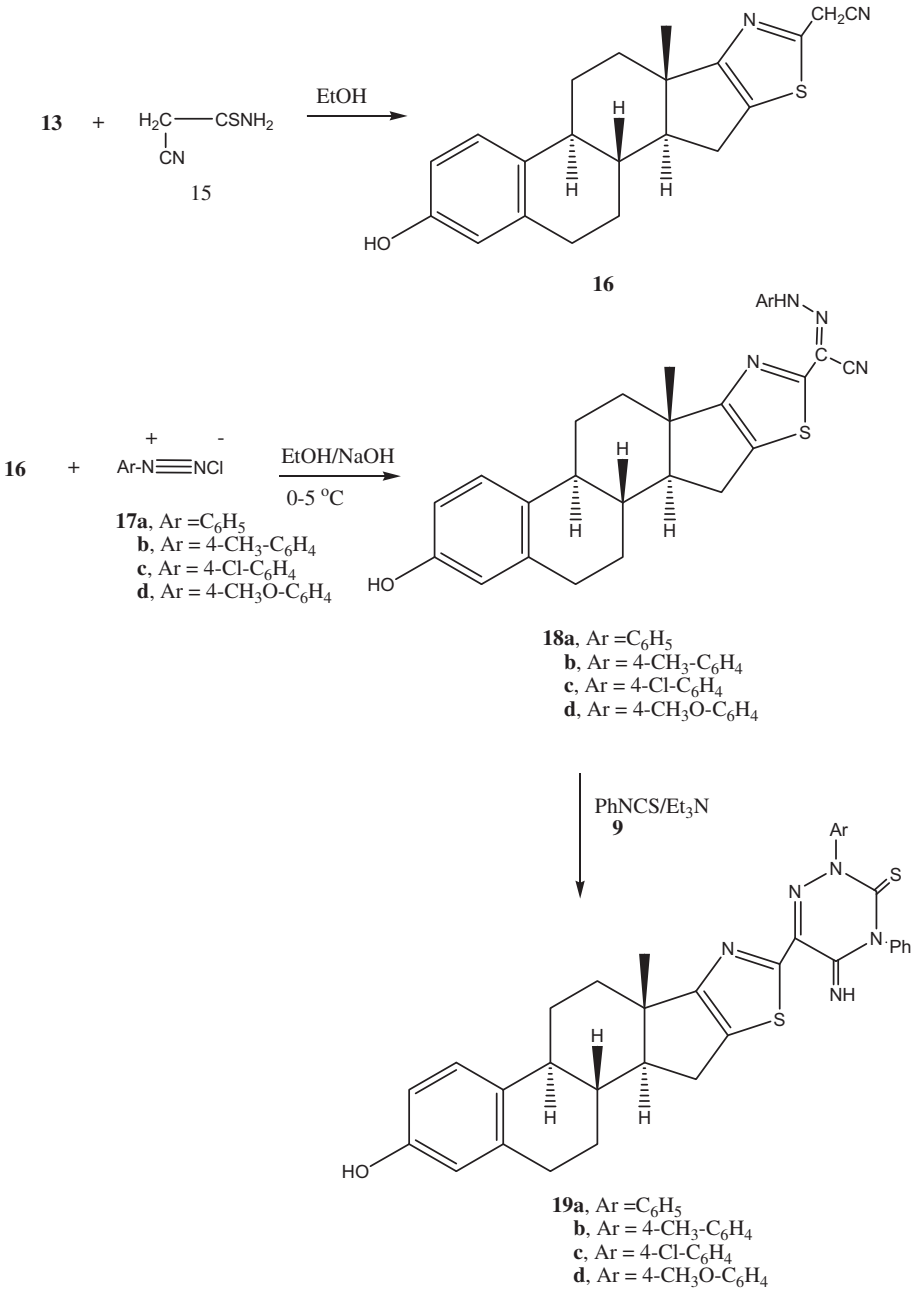
3.2. In vitro cytotoxic assay

3.2.1. 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).

3.2.2. 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



Scheme 4. Synthesis of compounds **16**; **18a-d** and **19a-d**.

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 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 estrone 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). In this study the optimal cytotoxicity effects of the newly synthesized products were obtained using such receptors. Moreover, in this study the receptor content being of the same content among all cell lines. All of IC_{50} values were listed in Table 1. Some heterocyclic compounds were 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 much lesser extent (IC_{50} > 10,000 nM). The reference compound used is the CHS-828 which is a pyridyl cyanoguanidine anti-tumor agent.

3.2.3. Structure activity relationship

From Table 1 it is clear that the estrone moiety was found to be crucial for the cytotoxic effect of cyclic compounds **4a–f–19a–d**. Compounds **4c**, **4f**, **6b**, **8b**, **8c**, **10**, **13**, **16**, **18c** and **19c** exhibited

Table 1
Cytotoxicity of novel estrone derivatives against a variety of cancer cell lines[a] [IC_{50}[b] (nM)].

Compd	Cytotoxicity (IC_{50} in nM)						
	NUGC	DLDI	HA22T	HEPG2	HONE1	MCF	WI38
4a	822	2355	2210	2655	1642	1880	na
4b	455	378	1920	288	180	2885	na
4c	26	53	58	22	380	na	na
4d	214	120	1273	309	1266	1655	na
4e	2253	2165	1988	1438	1340	1088	na
4f	120	60	85	1233	1319	38	na
6a	3472	2549	3477	2193	2880	1320	na
6b	160	42	32	304	1246	180	na
6c	227	1284	1577	287	1880	1422	na
8a	2130	1620	1286	1896	386	827	na
8b	138	46	328	30	48	1288	na
8c	488	129	884	485	2865	633	na
8d	480	255	1820	1276	1286	664	na
10	2810	1266	3228	2275	4822	1117	na
12a	3277	1283	1089	774	450	2866	na
12b	1154	960	46	29	166	3277	na
13	38	38	684	1260	2416	na	na
14	1680	2055	468	138	188	1640	na
16	140	150	3163	2788	2188	39	na
18a	2279	2560	780	120	2788	2876	na
18b	660	55	166	255	70	264	na
18c	122	44	548	894	1722	28	na
18d	489	664	3220	3866	2877	1189	na
19a	3344	2363	2298	2784	1922	2297	na
19b	1666	3280	42	1829	320	3283	na
19c	130	180	829	2729	350	4920	na
19d	2781	620	1466	3276	1886	695	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.
CHS-828 is a pyridyl cyanoguanidine anti-tumor agent.
[b] The sample concentration produces a 50% reduction in cell growth.

optimal cytotoxic effect against cancer cell lines, with IC_{50}'s in the nM range. Comparing the cytotoxicity of the decahydronaphtho [2′,1′:4,5] indeno[1,2-*b*]pyran derivatives **4a–f**, it is obvious that the cytotoxicity of **4c**, **4d** and **4f** are higher than those of **4a**, **4b** and **4e**. The presence of the chloro group and either the NH_2 or the OH groups are responsible for the high potency of **4c** and **4d**. The presence of the CH_3O and the OH groups are responsible for the reactivity of **4f**, such high cytotoxicity is remarkable against MCF cell line. On the other hand, considering the decahydro-naphtho [2′,1′:4,5] indeno[1,2-*d*]pyrimidine derivatives **6a–c**, it is clear that the un-substituted 4-phenyl group in **6b** showed the less potency. Compounds **6b** with the 4-chlorophenyl moiety showed more cytotoxicity than compound **6c** with the 4-CH_3O-phenyl moiety. The decahydronaphtho [2′,1′:4,5] indeno[2,1-*e*]thiazolo[3,2-*a*]pyrimidine derivatives **8a–d** showed higher cytotoxicity than the octahydro-1H-naphtho [2′,1′:4,5] indeno[1,2-*d*]thiazole derivative **10**. Such high cytotoxicity of **8a-d** is attributed to the thiazolo[3,2-*a*]pyrimidine moiety. For the 2-hydrazonothiazole derivatives **12a** and **12b**, it is clear that the phenylhydrazono derivative **12b** is more cytotoxic than the hydrazone derivative **12a** especially against the four cancer cell lines DLDI, HA22T, HEPG2 with IC_{50}'s values 960, 46, 29 and 166, respectively. The bromo derivative **13** showed high cytotoxicity against the cancer cell lines NUGC and DLDI and such cytotoxicity effects are the highest among the tested compounds. It is obvious that compounds **4c**, **4f**, **6b** and **12b** showed high cytotoxicity against the cancer cell lines DLDI, HA22T and HEPG2 and that they are more potent than the reference CHS 828. The 2-aminothiazole derivative **14** showed low cytotoxicity against NUGC,DLDI cell lines and high cytotoxicity against HA22T, HEPG2 and HONE1. On the other hand, the octahydro-1H-naphtho [2′,1′:4,5] indeno[1,2-*d*]thiazol-10-yl)acetonitrile **16** has higher cytotoxicity against the cell line DLDI than CHS 828. Next, for the arylhydrazo derivatives **18a–d**, it is clear that the substituted aryl derivatives **18b**, **18c** and **18d** are more potent than the unsubstituted aryl derivative **18a**. Moreover, compound **18c** showed the highest cytotoxicity against NUGC, DLDI and MCF cell lines. The 1,2,4-triazine derivative **19c** showed the highest cytotoxicity among **19a–d**, such high cytotoxicity is attributed to the presence the 4-Cl-phenyl group. Our results showed that the electronegative CN and Cl hydrophobic groups in the thiophene derivative might play a very important role in enhancing the cytotoxic effect. The decahydronaphtho [2′,1′:4,5] indeno[1,2-*b*]pyran derivative **4c** selectively exhibited the maximum potency cytotoxic activity against human gastric cancer NUGC, human colon cancer cell lines (DLD1), and the human liver cancer HEPG2 with IC_{50}'s of 26, 53, 58 nM and 22 nM, respectively.

4. Conclusions

In summary, we have shown herein that our strategy is compatible with the synthesis of a wide range of steroids and particularly steroids possessing a puran, thiazole, 1,2,4-triazine and some of their derivatives as in a biologically important position C-17 of estrone skeleton. 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 **4c**, **4f**, **6b**, **8b**, **8c**, **10**, **13**, **16**, **18c** and **19c** exhibited optimal cytotoxic effect against cancer cell lines, with IC_{50}'s in the nM range.

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