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Synthesis and cytotoxic evaluation of novel hybrid estrane heterocycles as chemotherapeutic anti-cancer agents

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ABSTRACT

New synthesized hybrid steroidal heterocyclic compounds have received a lot of attention in view of their biological activities as anticancer agents. In this study, a novel class of hybrid estrane heterocyclic compounds were synthesized and evaluated by analytical and spectral data which proved the validity of these derivatives. The cytotoxicity of synthesized compounds **2a, 2b, 2c, 3b, 8, 10a, 10b, 13, 14, 16a** and **19** against three human cell lines: breast cancer cells (MCF-7), prostate cancer cells (PC3), and liver cancer cells (HepG2) has been tested using MTT assay. Compounds **10a**, **10b**, **2c**, and **14** revealed more inhibitory influence on MCF7, PC3 and HepG2 growth than the reference drug doxorubicin (Dox) after 24 h incubation. Noteworthy, the tested compounds **10a**, **10b**, **2c**, and **14** exhibited the most pronounced effect in this respect. The results were confirmed by morphology study.

1. Introduction

Cancer is a multicellular disease in which abnormal cells divide without control and can invade nearby tissues [1]. It is one of the leading causes of death worldwide according to World health organization (WHO) [2]. Although, chemotherapy has confirmed a new period of molecularly targeted therapeutics but the effectiveness of existing drugs for treating cancer is still limited [3]. Therefore, there is an urgent need to synthesize new, efficient and less toxic anticancer agents to overcome the current therapy inclusive the development of drug resistance [4,5]. steroids have always attracted great attention because of their remarkable structural design and their impressive variety of biological activities. Accurate variation in the steroid molecule can result significant therapeutic action [6]. The heterocyclic rings play the most important role in the therapeutic function of compounds due to their potent receptor binding properties [7]. The proportional ease of heterocyclic rings that can be modulated with additional substituent let them to cover a broad area of chemical space, more over considering as excellent starting point for anticancer drug development [8]. Combination of steroids with heterocyclic rings enhanced a change of their physiological and various biological activities [9]. These heterocyclic steroid derivatives appear to be promising in the treatment of various types of cancer including liver cancer, breast cancer, prostate cancer, etc [10–14]. The present investigation aimed to test the effect of newly synthesized heterocyclic estrane derivatives on breast (MCF7), prostate (PC3) and liver (HepG2) human cancer cell lines and the results were confirmed by morphology study.

2. Materials and methods

2.1. Synthetic methods, analytical and spectral data

The hormone used as starting material (estrone) was purchased from sigma company (Tables 1 and 2), St. Louis, MO, USA. The used solvents were an hydrated by distillation prior. All the melting points of products were identified by using electro thermal apparatus and are uncorrected. The IR spectra were identified by (KBr discs) on a Shimadzu FT-IR 8201 PC spectrometer and measured by cm^{-1} unit. The ^{1}H NMR was

Abbreviations: Dox, Doxorubicin; WHO, World health organization; TLC, Thin layer chromatography; VACSERA, The holding company for biological products & vaccines; RPMI, Roswell Park Memorial Institute; DPBS, Dulbecco's phosphate buffered saline; MTT, (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; DMSO, dimethyl sulfoxide; DMF-DMA, dimethyl formamide dimethyl acetal.

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Table 1
The in vitro cytotoxic activity of synthesized compounds on MCF-7 cancer cell line.

Compd.No	IC_{50} (μg/ml)	Compd.No	IC_{50} (μg/ml)
2a	616.69	**10b**	18.27
2b	141.65	**13**	172.07
2c	54.21	**14**	24.15
3b	156.5	**16a**	92.53
8	77.12	**19**	26.61
10a	18.26		
Doxorubicin	93.08	**Doxorubicin**	93.08

IC_{50}: Concentration required to inhibit cell viability by 50%.

Table 2
The in vitro cytotoxic activity of synthesized compounds on PC3 cancer cell line.

Compd.No	IC_{50} (μg/ml)	Compd.No	IC_{50} (μg/ml)
2a	690.41	**10b**	38.4
2b	202.5	**13**	164.61
2c	22.89	**14**	48.11
3b	331.3	**16a**	164.86
8	92.77	**19**	111.17
10a	31.75		
Doxorubicin	72.39	**Doxorubicin**	72.39

IC_{50}: Concentration required to inhibit cell viability by 50%

identified by Joel instrument (Japan). At 300 MHz, in DMSO-d_6 as solvent and chemical shifts were measured by ppm relative to TMS. The spin multiplicities were abbreviated by the letters: s-singlet, d-doublet, *t*-triplet, q-quartet and m (multiplet, more than quartet). Mass spectra were recorded on GCMS-QP 1000ex spectra mass spectrometer working at 70 EV and VPLCMs / MS "water" 3100 "USA" spectra mass spectrometer working at 30 EV. Elemental analysis was carried by the Micro analytical data unit at Cairo University, Giza, Egypt and the Micro-analytical data unit at the National Research Center, Giza, Egypt. All reactions were observed by using thin layer chromatography (TLC) which was executed using Merk 60 F254 aluminum sheets and visualized by UV light (254 nm). The mixtures were separated by preparative TLC and gravity chromatography.

2.1.1. General procedure for synthesis of compounds 2a, 2b and 2c

A mixture of estrone **(1)** (0.27 g, 1 mmol) with benzaldehyde (0.106 g, 1 mmol), 4-fluorobenzaldehyde (0.124 g, 1 mmol) or 4-methoxybenzaldehyde (0.136 g, 1 mmol) and thiourea (0.078 g, 1 mmol) in absolute ethanol was heated under reflux for 3–6 hrs until all the starting materials were disappeared as indicated by TLC. The reaction mixture was treated with ice/water. The formed solid of each product was collected by filtration and crystallized from absolute ethanol.

10-mercapto-8a-methyl-12-phenyl-2,6b,7,8,8b,9,12,12a,13,13a,13b-dodecahydro-1H-naphtho[2′,1′:4,5]indeno[1,2-d]pyrimidin-4-ol (2a)

White powder from absolute ethanol, yield 0.24 g (58.5%); mp 75–77 ^{0}C; IR (KBr, cm^{-1}): ν 3855–3751 (SH, NH), 2930 (CH_3), 2869 (CH_2), 1718 (C═N), 1618 (C═C).^{1}H NMR (DMSO – d_6, ppm): $\delta = 0.809$ (s, 3H, 18- CH_3), 0.809–2.739 (m, estrane moiety), 1.34 (m, 1H, 14- CH), 1.46 (S, 1H, SH), 1.72 (m, 1H, 16- CH), 2.065 (m, 2H, 15- CH_2), 2.49 (d, 1H, 17- CH), 2.74 (d, 1H, 22– CH), 6.44 (s, 1H, NH, D_2O exchangeable), 7.028 (d, 2H, phenyl group), 7.044 (m, 3H, phenyl group), 9.02 (s, 1H, OH, D_2O exchangeable) MS (EI) *m/z* (%): 418 [M^+, 20%], 366 (15), 341 (30), 338 (17). Calc for $C_{26}H_{30}N_2O_5$ (418.2): C, 74.60; H, 7.22; N, 6.69; S, 7.62%, found: C, 74.55; H, 7.25; N, 6.54; S, 7.65%

12-(4-fluorophenyl)-10-mercapto-8a-methyl-2,6b,7,8,8a,8b,9,12,12a,13,13a,13b-dodecahydro-1H-naphtho[2′,1′,4,5]indeno[1,2-d]pyrimidin-4-ol (2b)

Pale yellow crystals from absolute ethanol, yield 0.41 g (95.3%);mp 62–64 °C; IR (KBr, cm^{-1}):ν 3855–3343 (SH, NH), 3213 (OH), 2937 (CH_3), 2864 (CH_2), 1718 (C═N), 1595 (C═C).^{1}H NMR (DMSO – d_6, ppm)$\delta = 0.811$ (s, 3H, 18- CH_3), 0.811–2.490 (m, estrane moiety), 1.292 (m,1H, 14-CH), 1.518 (s, 1H, SH), 1.719 (m, 1H, 16-CH), 2.483 (m, 2H, 15-CH_2), 2.490 (d, 1H, 17-CH), 2.728 (d, 1H, 22-CH), 6.437 (S, 1H, NH, D_2O exchangeable), 7.029 (d, 2H, phenyl group), 7.326 (d, 2H, phenyl group), 9.02 (s, 1H, OH, D_2O exchangeable), Calc for $C_{26}H_{29}FN_2OS$ (436.20): C, 71.5; H, 6.70; F, 4.35; N, 6.42; S, 7.34% found: C, 71.46; H, 6.63; F, 4.38; N, 6.37; S, 7.28%

10-mercapto-12-(4-methoxyphenyl)-8a-methyl-2,6b,7,8,8a,8b,9,12,12a,13,13a,13b-dodecahydro-1H-naphtho[2′,1′:4,5]indeno[1,2-d]pyrimidin-4-ol (2c)

White powder from absolute ethanol, yield 0.43 g (95.5%); mp 70–72 ^{0}C; IR (KBr, cm^{-1}): ν 3858 (SH), 3343 (NH), 3216 (OH), 2934 (CH_3), 2862 (CH_2), 1718 (C═N), 1593 (C═C). ^{1}H NMR (DMSO – d_6, ppm)$\delta = 0.813$ (s, 3H, 18- CH_3), 0.813–2.561 (m, estrane moiety), 1.310 (m, 1H, 14- CH), 1.481 (S,1H, SH), 1.75 (m, 1H, 16-CH), 2.231 (m, 2H, 15- CH_2), 2.480 (d, 1H, 17-CH), 2.731 (d, 1H, 22-CH), 3.231(s, 3H, CH_3OH), 6.531 (s, 1H, NH, D_2O exchangeable), 7.121 (d, 2H, phenyl group), 7.282 (d, 2H, phenyl group)9.02 (s, 1H, OH, D_2O exchangeable), MS (EI) *m/z* (%): 448.63 [M^{+1}, 16.3%], 348 (25), 341 (32), 339 (95), 322 (90); Calc for $C_{27}H_{32}N_2O_2S$ (448.22): C, 72.29; H, 7.19; N, 6.24; S, 7.15%; found: C, 72.13; H, 7.15; N, 6.29; S, 7.

2.1.2. General procedure for synthesis of compounds3a, 3b and 3c

To a solution of a product **2a, 2b or 2c** (0.104, 0.109 or 0.11 g, 0.4 mmol) in absolute ethanol (25–30 mL), phenacylbromide (0.049 g, 4 mmol) was added. The reaction mixture, in each case was heated under reflux for 4 hrs until the starting material was disappeared as indicated by TLC. The reaction mixture was treated with ice/water mixture. The formed solid product was collected by filtration and crystallized from absolute ethanol.

8a-methyl-10,14-diphenyl-1,2,6b,7,8,8a,8b,14,14a,15,15a,15b-dodecahydronaphtho[2′,1′:4,5]indeno[2,1-e]thiazolo[3,2-a]pyrimidin-4-ol (3a)

White powder from absolute ethanol, yield 0.07 g (54.26%); mp 256–258 °C; IR (KBr, cm^{-1}): ν 3418 (OH), 2928 (CH_3), 2867 (CH_2), 1718 (C═N), 1621 (C═C).^{1}H NMR (DMSO – d_6, ppm): $\delta = 0.811$(s, 3H, 18-CH_3), 0.811–2.740 (m, estrane moiety), 1.341 (m, 1H, 14-CH), 1.46 (m, 1H, 16-CH), 2.303 (m, 2H, 15-CH_2), 2.490 (d, 1H, 17-CH), 2.728 (d, 1H, 25-CH), 7.029 (d, 2H, phenyl ring), 7.046 (m, 3H, phenyl ring), 7320 (d, 2H, phenyl ring), 7.650 (m, 3H, phenyl ring), 9.015 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 518 [M^+, 5%], 466 (10), 414 (20), 307 (12), 254 (39); Calc for $C_{34}H_{34}N_2OS$ (518.72): C, 78.73; H, 6.61; N, 5.40; S, 6.18%; found: C, 78.65; H, 6.52 N, 5.31; S, 6.11%

14-(4-fluorophenyl)-8-methyl-10-phenyl-1,2,6b,7,8,8a,8b,14,14a,15,15a,15b-bodecahydonphtho[2′,1′:4,5]ineno[2,1-e]thizolo[3,2-a]pyrimidin-4-ol (3b)

Colorless crystals from absolute ethanol, yield 0.07 g (52%); mp 248–250 ^{0}C; IR (KBr, cm^{-1}): ν 3343 (OH), 2938 (CH_3), 2864 (CH_2), 1718 (C═N), 1619 (C═C).^{1}H NMR (DMSO – d_6 ppm): $\delta = 0.807$ (s, 3H, 18-CH_3), 0.807–2.831 (m, estrane moiety), 1.312 (m, 1H, 14-CH), 1.441 (m, 1H,16-CH), 2.185 (m, 2H, 15-CH_2), 2.320 (d, 1H, 17-CH), 2.683 (d, 1H, 25-CH), 7.013 (d, 2H, phenyl ring), 7.026 (d, 2H, phenyl ring), 7.208 (d, 2H, phenyl ring), 7.613 (m, 3H, phenyl ring), 9.015 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 440 [M^+, 8%], 449 (10), 414 (8), 214 (30); Calc for $C_{34}H_{33}FN_2OS$ (536.23)C, 76.09; H, 6.20; F, 3.54;N, 5.22; S, 5.97%, found: C,76.13; H, 6.25;F, 3.51; N, 5.17; S, 5.84%

14(4-methoxyphenyl)-8a-methyl-10-phenyl-1,2,6b,7,8,8a,8b,14,14a,15,15a,15b,dodecahydronaphtho[2′,1′:4,5]indeno[2,1-e]thiazolo[3,2-a]pyrimidin-4-ol (3c)

Pale yellow crystals from absolute ethanol, yield 0.05 g (36.5%); mp 204–206 ^{0}C; IR (KBr, cm^{-1}): ν 3302 (OH), 2928 (CH_3), 2868 (CH_2), 1717 (C═N), 1618 (C═C); ^{1}H NMR (DMSO – d_6, ppm): $\delta = 0.812$ (s, 3H, 18-CH_3), 0.812–2.740 (m, estrane moiety), 1.341 (m, 1H, 14-CH), 1.468 (m, 1H, 16-CH), 2.359 (m, 2H, 15-CH_2), 2.90 (d, 1H,17-CH), 2.740 (d, 2H, 25-CH), 3.327 (s, 3H, CH_3CO), 7.029 (d, 2H, phenyl ring), 7.046 (d,

2H, phenyl ring), 7.320 (d, 2H, phenyl ring), 7.650 (m, 3H, phenyl ring), 9.013 (s, 1H, OH, D_2O exchangeable). Calc for $C_{35}H_{36}N_2O_2S$ (548.25): C, 76.61; H, 6.61; N, 5.11; S, 5.4%; found: C, 76.58; H, 6.54; N, 503; S, 5.78%

2.1.3. General procedure for synthesis of compound 4a, 4b and 4d

A mixture of estrone **1** (0.279 g, 1 mmol), benzaldehyde (0.106 g, 1 mmol), 4-flourobenzaldehyde (0.124 g, 1 mmol), 4-methoxybenzaldehyde (0.13 g, 1 mmol) and guanidine (0.136 g, 1 mmol) in absolute ethanol was heated under reflux for 4–6 hrs until all the starting materials were disappeared as indicated by TLC. The reaction mixture was treated with ice/water, the formed solid of each product was collected by filtration and crystallized from absolute ethanol.

10-amino-8a-methyl12-phenyl-2,6b,7,8,8a,8b,9,12,12a,13,13a,13b-dodecahydro-1H-naphtho[2′,1′:4,5]indeno[1,2-d]pyrimidin-4-ol (4a)

Pale yellow crystals from absolute ethanol, yield 0.37 g (92.2%); mp 64–66 °C; IR (KBr, cm^{-1}): ν 3214 (NH_2), 3117 (NH), 3043 (OH), 2978 (CH_3), 2867 (CH_2), 1716 (C═N), 1596 (C═C); ^{1}H NMR (DMSO – d_6, ppm): δ = 0.809 (s, 3H, 18-CH_3), 0.809–2.728 (m, estrane moiety), 1.292 (m, 1H, 14-CH), 1.321 (m, 1H, 16-CH), 2.490 (m, 2H, 15-CH_2), 2.728 (d, 1H, 17-CH), 2.813 (d, 1H, 22-CH), 4.507 (s, 1H, NH, D_2O exchangeable), 6.511 (s, 2H, NH_2, D_2O exchangeable), 7.127 (d, 2H, phenyl ring), 7.333 (m, 3H, phenyl ring), 9.008 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 373[M, 0.5%], 348 (0.7), 299 (0.3), 269 (7), 93 (100); Calc for $C_{26}H_{31}N_3O$ (401.25): C, 77.77; H, 7.78; N, 10.46%; found: C, 77.68; H, 7.5; N, 10.49%

10-amino-12(4-flourophenyl)-8a-methyl-2,6b,7,8,8a,8b,9,12,12a,13,13a,13b-dodecahydro-1 h-naphtho [2′,1′:4,5]indeno[1,2-d]pyrimidin-4-ol (4b)

Pale yellow crystals from absolute ethanol, yield 0.39 g (93%); mp 78–80 °C; IR (KBr, cm^{-1}): ν 3751 (NH_2), 3678 (NH), 3290 (OH), 2929 (CH_3), 2868 (CH_2), 1717 (C═N), 1618 (C═C); ^{1}H NMR (DMSO – d_6, ppm): δ = 0.808 (s, 3H, 18-CH_3), 0.808–2.740 (m, estrane moiety), 1.292 (m, 1H, 14-CH), 1.340 (m, 1H, 16-CH), 2.490 (m, 2H, 15-CH_2), 2.740 (d, 1H, 17-CH), 2.820 (d, 1H, 22-CH), 4.509 (s, 1H, NH, D_2O exchangeable), 6.511 (s, 2H, NH_2, D_2O exchangeable), 7.028 (d, 2H, phenyl group), 7.631 (d, 2H, phenyl group), 9.012 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 419 [M^{+2}, 0.7%], 299 (12), 269 (7), 228 (10); Calc. for $C_{26}H_{30}FN_3O$ (419.24): C, 74.43; H, 7.21; F, 4.53; N, 10.02%; found: C, 74.36; H, 7.25; F, 4.57; N, 9.98%

10-amino-12-(4-methoxyphenyl)-8a-methyl-2,6b,7,8,8a,8b,9,12,12a,13,13a,13b-dodecahydro-H-naphtho[2′,1′:4,5] indeno[1,2-d]pyrimidin-4-ol (4c)

White crystals from absolute ethanol, yield 0.40 g (92.8%); mp 60–62 °C; IR (KBr, cm^{-1}): ν 3751 (NH_2), 3345 (NH), 3215 (OH), 2937 (CH_3), 2863 (CH_2), 1718 (C═N), 1595 (C═C); ^{1}H NMR (DMSO – d_6, ppm): δ = 0.811 (s, 3H, 18-CH_3), 0.811–2.720 (m, estrane moiety), 1.252 (m, 1H, 14-CH), 1.320 (m, 1H, 16-CH), 2.421 (m, 2H,15-CH_2), 2.738 (d, 1H, 17-CH), 2.791 (d,1H, 22-CH), 3.401 (s, 3H, CH_3OH), 4.482 (s, 1H, NH, D_2O exchangeable), 6.450 (s, 2H, NH_2), 7.028 (d, 2H, phenyl group), 7.620 (d, 2H, phenyl group), 9.012 (s, 1H, OH); MS (EI) *m/z* (%): 431 [M^+, 5%], 324 (35), 307 (43), 299 (7), 269 (40); Calc. for $C_{27}H_{33}N_3O_2$ (431.26): C, 75.14; H, 7.71; N, 9.74%; found: C, 75.19; H, 7.78; N, 9.78%

2.1.4. General procedure for synthesis of compounds 5a, 5b and 5c

To a solution of a product **4a, 4b or 4c** (0.200, 0.209 or 0.215 g, 2 mmol) in absolute ethanol (25–30 mL), phenacylbromide (0.99 g, 2 mmol) was added. The reaction mixture, in each case was heated under reflux for 3 hrs until the starting materials were disappeared as indicated by TLC. The reaction mixture was treated with ice/water mixture. The formed solid product was collected by filtration and crystallized from absolute ethanol.

8a-methyl-10,14-diphenyl-2,6b,7,8,8a,8b,12,14,14a,15,15a,15b-dodechyro-1H-imidazo[1,2-*a*]naphtha[2′,1′:4,5]indeno[2,1-e]pyrimidin-4-ol (5a)

Pale yellow crystals from absolute ethanol, yield 0.05 g (20%); mp 218–220 °C; IR (KBr, cm^{-1}): ν 3752 (NH), 3343 (OH), 2936 (CH_3), 2864 (CH_2), 1718 (C═N), 1619 (C═C); ^{1}H NMR (DMSO – d_6, ppm): δ = 0.812 (s, 3H, 18-CH_3), 0.812–2741 (m, estrane moiety), 1.343 (m, 1H, 14-CH), 1.360 (m, 1H, 16-CH), 2.483 (s, 1H, NH, D_2O exchangeable), 2.487 (m, 2H, 15-CH_2), 2.490 (d, 1H, 17-CH), 2.494 (d, 1H, 25-CH), 7.029 (m, 3H, phenyl group),7.046 (d, 2H, phenyl group), 7.230 (m, 3H, phenyl group), 7.242 (d, 2H, phenyl group), 7.359 (s, 1H, 21-CH), 9.291 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 501 [M^{+4}, 7], 399 (14), 353 (17), 324 (100);Calc. for $C_{34}H_{35}N_3O$ (501.28):C, 81.40; H, 7.03; N, 8.38%; found: C, 81.32; H, 7.03; N, 8.38%

14-(4-fourophenyl)-8a-methyl-10-phenyl-2,6b,7,8,8a,8b,12,14,14a,15,15a,15b-dodecahydro-1H-imidazo[1.2-a] naphtha[2′,1′:4,5]indeno[2,1-e]pyrimidin-4-ol (5b)

Yellow crystals from absolute ethanol, yield 0.05 g (19%); mp 194–196 °C; IR (KBr, cm^{-1}): ν 3751 (NH), 3344 (OH), 2936 (CH_3), 2863 (CH_2), 1718 (C═N), 1619 (C═C); ^{1}H NMR (DMSO – d_6 ppm): δ = 0.811 (s, 3H, 18-CH_3), 0.811–2.740 (m, estrane moiety), 1.343 (m, 1H, 14-CH), 1.720 (m, 1H, 16-CH), 2.067 (s, 1H, NH, D_2O exchangeable), 2.490 (m, 2H, 15-CH_2), 2.740 (d, 1H, 17-CH), 2.764 (d, 1H, 25-CH), 7.029 (d, 2H, phenyl ring), 7.046 (d, 2H, phenyl ring), 7.242 (m, 3H, phenyl ring), 7.342 (d, 2H, phenyl ring), 7.359 (s, 1H, 21-CH), 9.015 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 512 [M^+, 25%], 399 (15), 387 (23), 347 (13); Calc. for $C_{34}H_{34}FN_3O$ (519.27): C, 78.58; H, 6.60; F, 3.66; N, 8.09%; found: C, 78.51; H, 6.53; F, 3.51; N, 8.01%

14-(4-methoxypheyl)-8a-methyl-10-phenyl-2,6a,7,8,8a,8b,12,14,14a,15,15a15b-dodecahydro-1H-imidazol[1,2-a] naphtha[2′,1′:4,5]indeno[2,1-e]pyrimidin-4-ol (5c)

Yellow crystals from absolute ethanol, yield 0.05 g (19%); mp 194–196 ^{0}C; IR (KBr,cm^{-1}): ν 3751 (NH), 3344 (OH), 2936 (CH_3), 2863 (CH_2), 1718 (C═N), 1619 (C═C); ^{1}H NMR (DMSO – d_6, ppm): δ = 0.811 (s, 3H, 18-CH_3), 0.811–2.740 (m, estrane moiety, 1.341 (m, 1H, 14-CH), 1.359 (m, 1H, 16-CH), 2.067 (s, 1H, NH, D_2O exchangeable), 2.490 (m, 2H, 15-CH_2), 2.740 (d, 1H, 17-CH), 2.763 (d, 1H, 16-CH), 2.764 (d, 1H, 25-CH), 3.213 (s, 3H,CH_3OH), 7.029 (d, 2H, phenyl group), 7.046 (d, 2H, phenyl group), 7.180 (m, 3H, phenyl group), 7.242 (d, 2H, phenyl group), 7.359 (s, 1H, 21-CH), 9.017 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 531 [M^{+4}, 30%], 403 (25), 347 (37), 29 (13); Calc for $C_{35}H_{37}N_3O_2$ (531.29): C, 79.06; H, 7.01; N, 7.90%; found: C, 79.01; H, 6.96; N, 7.90%

2.1.5. General procedure for synthesis of compounds 6a, 6b and 6c

A mixture of estrone**1** (0.27 g, 1 mmol) benzaldehyde (0.106 g, 1 mmol), 4-flourobenzaldehyde (0.124 g, 1 mmol) or 4-methoxybenzaldehyde (0.136 g, 1 mmol) ad urea (0.06 g, 1 mmol) in absolute ethanol. The reaction mixture was heated under reflux for 4–6 hrs until all the starting materials were disappeared as indicated by TLC. The reaction mixture was treated with ice/water. The formed solid of each product as collected by filtration and crystallized from absolute ethanol.

8a-methyl-12-phenyl-2,6b,7,8,8a,8b,9,12,12a,13,13a,13b-dodecahydro-1H-naphtho[2′,1′:4,5]indeno[1,2-dpyrimidine-4,10-diol (6a)

White powder from absolute ethanol, yield 0.38 g (94.5%); mp 150–152 °C; IR (KBr, cm^{-1}): ν 3751 (NH), 3051 (2OH), 2930 (CH_3), 2868 (CH_2), 1717 (C═N), , 1618 (C═C); ^{1}H NMR (DMSO – d_6 ppm): δ = 0.809 (s, 3H, 18-CH_3), 0.809–2.740 (m, estrane moiety), 1.292 (m, 1H, 14-CH), 1.465 (m, 1H, 16-CH), 2.487 (m, 2H, 15-CH_2), 2.490 (d, 1H, 17-CH), 2.494 (d, 1H, 22-CH), 4.507 (s, 1H, NH, D_2O exchangeable), 7.028 (d, 2H, phenyl group), 7.325 (m, 3H, phenyl group), 9.011 (s, 1H, OH, D_2O exchangeable), 11.051 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 402 [M^{+4}, 5], 325 (25), 269 (100), 228 (54); Calc. for $C_{26}H_{30}N_2O_2$ (402.54); c, 77.58; H, 7.51; N, 9.96%; found: C, 77.61; H, 7.54; N, 9.99%

12-(4-fluorophenyl)-8a-methyl-2,6b,7,8,8a,8b,9,12,12a,13,13a,13b,dodecahydro-1H-naphtho [2′,1′:4,5]indeno[1,2-d]pyrimidine-4,10-diol (6b)

White powder from absolute ethanol, yield 0.25 g (59.5%); mp 210–212 °C; IR (KBr, cm^{-1}):ν 3751 (NH), 3302 (2OH), 2929 (CH_3), 2868

(CH_2), 1717 (C═N), 1620 (C═C); ^{1}H NMR (DMSO – d_6, ppm): $\delta = 0.801$ (s, 3H, 18-CH_3), 0.801–2.732 (m, estrane moiety), 1.286 (m, 1H, 14-CH), 1.461 (m, 1H, 16-CH), 2.482 (m, 2H, 15-CH_2), 2.485 (d, 1H, 17-CH), 2.46 (d, 1H, 22-CH), 4.503 (s, 1H, NH, D_2O exchangeable), 7.025 (d, 2H, phenyl group), 7.313 (d, 2H, phenyl group), 9.003 (s, 1H, OH, D_2O exchangeable), 11.031 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 420[M^{+3}, 34%], 346 (15), 313 (5); Calc for $C_{26}H_{29}FN_2O_2$ (420.22); c, 74.26; H, 6.26; F, 4.52; N, 6.66%; found: C, 74.21; H, 6.92; F,4.47; N, 6.62%

12-(4-methoxyphenyl)-8a-methyl-2,6b,7,8,8a,8b,9,12,12a,13,13a,13b,dodecahydro-1H-naphtho[2′,1′:4,5]indeno[1,2-d]pyrimidine-4,10-diol (6c)

Pale brown powder from absolute ethanol, yield 0.29 g (67.13%); mp 233–235 °C; IR (KBr, cm^{-1}):ν 3752 (NH), 3316 (2OH), 2928 (CH_3), 2863 (CH_2), 1718 (C═N), 1621 (C═C); MS (EI) *m/z* (%): 432[M^{+1}, 14%], 350 (25), 348 (3); ^{1}H NMR (DMSO – d_6, ppm): $\delta = 0.813$ (s, 3H, 18-CH_3), 0.813–2.754 (m, estrane moiety), 1.291 (m, 1H, 14-CH), 1.464 (m, 1H, 16-CH), 2.493 (m, 2H, 15-CH_2), 2.491(d, 1H, 17-CH), 2.49 (d, 1H, 22-CH), 4.598 (s, 1H, NH, D_2O exchangeable), 3.124 (s, 3H, CH_3OH), 7.021(d, 2H, phenyl group), 7.318 (d, 2H, phenyl group), 9.003 (s, 1H, OH, D_2O exchangeable), 11.112 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 432[M^{+1}, 14%], 350 (25), 348 (3); Calc. for $C_{27}H_{32}N_2O_3$ (432.24); c, 74.97; H, 7.46; N, 6.48%; found: C, 74.92; H, 7.41; N, 6.43%

2.1.6. General procedure for synthesis of compounds 7a, 7b and 7c

To a solution of **6a, 6b or 6c** (0.1005, 0.105 or 0.108 g, 4 mmol) in absolute ethanol (25–30 mL), phenacylbromide (0.049 g, 4 mmol) was added. The reaction mixture, in each case, was heated under reflux for 4 hrs until the starting materials were disappeared as indicated by TlC. The reaction mixture was treated with ice/water mixture. The formed solid product was collected by filtration and crystallized from absolute ethanol

8a-methyl-10,14-diphenyl-1,2,6b,7,8,8a,8b,14,14a,15,15a,15b-dodecahydronaphtho[2′,1′:4,5]indeno[2,1-e]oxazolo[3,2-a]pyrimidin-4-ol (7a)

White powder from absolute ethanol, yield 0.05 g (39.8%); mp210-212 °C; IR (KBr, cm^{-1}): ν 3409 (OH), 2929 (CH_3), 2868 (CH_2), 1718 (C═N), 1595 (C═C), 1199 (C—O); ^{1}H NMR (DMSO – d_6, ppm): $\delta = 0.811$ (s, 3H, 18-CH_3), 0.811–2.742 (m, estrane moiety), 1.340 (m, 1H, 14-CH), 1.361 (m, 1H, 16-CH), 2.487 (m, 2H, 15-CH_2), 2.485 (d, 1H, 17-CH), 2.493 (d, 1H, 25-CH), 7.029 (s, 1H, 21-CH), 7.038 (m, 3H, phenyl group), 7.029 (s, 1H, 21-CH), 7.038 (m, 3H, phenyl group), 7.046 (d, 2H, phenyl group), 7.220 (m, 3H, phenyl group), 7.224 (d, 2H, phenyl group), 9.011 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 502 [M^{+2}, 12], 425 (0.5), 370 (0.7), 357 (15), 254 (3); Calc. for $C_{34}H_{34}N_2O_2$ (502.26): C, 81.24; H, 6.28; N, 5.57; %; found: C, 81.18; H, 6.23; N, 5.52; %

14-(4-fluorophenyl)-8a-methyl-10-phenyl-1,2,6b,7,8,8a,8b,14,14a,15,15a,15b-dodecahydronaphtho[2′,1′:4,5]indeno[2,1-e]oxazolo[3,2-a]pyrimidin-4-ol (7b)

White powder from absolute ethanol, yield 0.178 g (57.4%); mp 232–234 °C; IR (KBr, cm^{-1}):ν 3396 (OH), 2928 (CH_3), 2867 (CH_2), 1718 (C═N), 1622 (C═C), 1155 (C—O); ^{1}H NMR (DMSO – d_6 ppm): $\delta = 0.812$ (s, 3H, 18-CH_3), 0.811–2.740 (m, estrane moiety), 1.341 (m, 1H, 14-CH), 1.359 (m, 1H, 16-CH), 2.068 (m, 2H, 15-CH_2), 2.490 (d, 1H, 17-CH), 2.740 (d, 1H, 25-CH), 7.029 (s, 1H, 21-CH), 7.037 (d, 2H, phenyl group), 7.046 (d, 2H, phenyl group), 7.210 (m, , 3H, phenyl group), 7.223 (d, 2H, phenyl group), 9.012 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 520 [M^+, 10], 425 (12), 400 (15), 357 (5); Calc. for $C_{34}H_{33}FN_2O_2$ (520.25): C, 78.44; H, 6.39; F, 3.65; N, 5.38%; found: C, 78.48; H, 6.35; F, 3.69; N, 5.32%

14-(4-methoxyphenyl)-8a-methyl-10-phenyl-1,2,6b,7,8,8a,8b,14,14a,15,15a,15b-dodecahydronaphtho[2′,1′:4,5]indeno[2,1-e]oxazolo[3,2-a]pyrimidin-4-ol (7C)

White powder from absolute ethanol, yield 0.06 g (45.11%); mp 244–246 °C; IR (KBr, cm^{-1}):ν 3307 (OH), 2928 (CH_3), 2868 (CH_2), 1717 (C═N), 1619 (C═C), 1155 (C—O); ^{1}H NMR (DMSO – d_6, ppm): $\delta = 0.812$ (s, 3H, 18-CH_3), 0.812–2.740 (m, estrane moiety), 1.326 (m, 1H, 14-CH), 1.341 (m, 1H, 16-CH), 3.324 (s, 3H, 29- CH_3OH), 7.029 (s, 1H, 21-CH), 7.037 (d, 2H, phenyl group), 7.046 (d, 2H, phenyl group), 7.210 (m, 3H, phenyl group), 7.221 (d, 2H, phenyl group), 9.009 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 532[M^{+3}, 15], 400 (5), 357 (3), 293 (100), 254 (4); calc. for $C_{35}H_{36}N_2O_3$ (534.28): C, 78.92; H, 6.81; N, 5.26%; found: C, 78.97; H, 6.77; N, 5.25%

2.1.7. Synthesis of 4-hydroxy-8a-methyl-9-phenyl-1,2,6b,7,8,8a,9,12,12a,12b-decahydro-10H-naphtho[2′,1′:4,5]indeno[1,2-d]thiazole-10-thione (8)

To a solution of estrone**(1)** (0.135 g, 2 mmol) in absolute ethanol, Sulphur (0.016 g, 2 mmol), phenylisothiocyanate (0.0675 g, 2 mmol) and drop wise of triethylamine were added, the reaction mixture was heated under reflux for 12 hrs until all the starting materials were disappeared as indicated by TLC. The reaction mixture was treated with ice/water mixture. The formed solid product was collected by filtration and crystallized from absolute ethanol to form pale brown powder. yield 0.201 g (96.17%); mp 130–132 °C; IR (KBr, cm^{-1}): ν 3295 (OH), 2931 (CH_3), 2869 (CH_2), 1717 (C═S), 1594 (C═C); ^{1}H NMR (DMSO – d_6, ppm): $\delta = 0.811$ (s, 3H, 18-CH_3), 0.811–2.741 (m, estrane moiety), 1.292 (m, 1H, 14-CH), 2.491 (d, 2H, 15-CH_2), 7.029 (d, 2H, phenyl group), 7.325 (m, 2H, phenyl group), 9.011 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 419 [M^{+1}, 10], 403 (2), 368 (5), 284 (96), 226 (8), 213 (15); Calc for $C_{25}H_{25}NOS_2$ (419.14): C,17.56; H, 6.01; N, 3.34; S, 15.28%; found: C, 17.66; H, 6.11; N, 3.44; 15.32%

2.1.8. Synthesis of 16-bromo-3-hydroxy-13-methyl-6,7,8,9,11,12,13,14,15,16-decahydro-17H-cyclopenta[a]phenanthren-17-one (9)

To a solution of estrone **(1)** (0.135 g, 2 mmol) in absolute ethanol, cupper bromide (0.112 g, 2 mmol) was added, the reaction mixture was heated under reflux for 2 hrs until all the starting materials were disappeared as indicated by TLC. The reaction mixture was treated with ice/water mixture. The precipitate was filtered off and crystalized from absolute ethanol as pale green powder, yield 0.171 g(98.27%); mp 176–178 °C; IR (KBr, cm^{-1}): ν 3403 (OH), 2928 (CH_3), 2866 (CH_2), 1722 (C═O), 1612 (C═C); ^{1}H NMR (DMSO – d_6, ppm): $\delta = 0.802$ (s, 3H, 18-CH_3), 0.802–2.490 (m, estrane moiety), 1.340 (m, 1H, 14-CH), 2.490 (m, 2H, 15-CH_2), 5.1 (t, 1H, 16-CH), 9.852 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 349 [M^{+1}, 29%), 334 (5), 290 (8), 269 (10); Calc. for $C_{18}H_{21}BrO_2$ (349.27): C, 61.90; H, 6.06; Br, 22.88%; found: C, 61.86; H, 6.02; Br, 22.83%

2.1.9. General procedure for synthesis of compounds 10a, 10band 10c

A mixture of **(9)** (0.116 g, 3 mmol), thiourea (0.025 g, 3 mmol), guanidine (0.019 g, 3 mmol) or Urea (0.02 g, 3 mmol) and triethylamine (2–3 drops) in absolute ethanol (20–30 mL). The reaction mixture was heated and refluxed for 3–5 hrs until all the starting materials were disappeared as indicated by TLC. The reaction mixture was treated with ice/water mixture. The precipitate was filtered off and crystallized from absolute ethanol.

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 (10a)

Yellow crystals from absolute ethanol, yield 0.152 g (93.25%); mp 42–44 °C; IR (KBr, cm^{-1}): ν 3411 (NH_2), 3217 (OH), 2928 (CH_3), 2865 (CH_2), 1719 (C═N), 1596 (C═C); ^{1}H NMR (DMSO – d_6, ppm): $\delta = 0.804$ (s, 3H, 18-CH_3), 0.804–2.490 (m, estroid moiety), 1.457 (m, 1H, 14-CH), 2.490 (d, 2H, 15-CH_2), 4.510 (s, 2H, NH_2, D_2O exchangeable), 9.008 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 326 [M^+, 25%], 293 (5), 268 (7), 214 (10); Calc. for $C_{19}H_{22}N_2OS$ (326.15): C, 73.76; H, 7.49; N, 13.58%; found: C, 73.71; H, 7.43; N, 13.54%

10-amino-8a-methyl-1,2,6b,7,8,8a,11,12,12a,12b-decahydronaphtho[2′,1′:4,5]indeno[1,2-d]imidazole-4-ol (10b)

Yellow crystals from absolute ethanol, yield 0.09 g (87.37%); mp

44–46 °C; IR (KBr, cm^{-1}): ν 3752 (NH_2), 3217 (NH), 3046 (OH), 2926 (CH_3), 2862 (CH_2), 1721 (C═N), 1596 (C═C); ^{1}H NMR (DMSO – d_6, ppm): δ = 0.804 (s, 3H, 18-CH_3), 0.804–2.730 (m, estrane moiety), 2.490 (m, 1H, 14-CH), 2.730 (d, 2H, 15-CH), 7.631 (s, 2H, NH_2, D_2O exchangeable), 9.013 (s, 1H, OH, D_2O exchangeable), 11.058 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z* (%): 309 [M^+, 3], 294 (5), 266 (4); Calc. for $C_{19}H_{23}N_3O$ (309.41): C, 69.90; H, 6.79; N, 8.58; S, 9.82%; found: C, 69.94; H, 6.76; N, 8.53; S, 9.78%

10-amino-8a-methyl-1,2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[1,2-d]oxazol-4-ol (10c)

Yellow crystals from absolute ethanol, yield 0.097 g (94.17%); mp 46–48 °C; IR (KBr, cm^{-1}): ν 3418 (NH_2), 3052 (OH), 2926 (CH_3), 2861 (CH_2), 1722 (C═N), 1591 (C═C), 1292 (C—O); ^{1}H NMR (DMSO – d_6, ppm): δ = 0.801 (s, 3H, 18-CH_3), 0.801–2.725 (m, estrane moiety), 2.065 (m, 1H, 14-CH), 2.490 (d, 2H, 15-CH_2), 7.325 (s, 2H, NH_2, D_2O exchangeable), 9.850 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 293 [M^+, 5], 228 (7); Calc. for $C_{19}H_{22}N_2O_2$ (310.40): C, 73.52; H, 7.14; N, 9.03%; found: C, 73.55; H, 7.19; N, 8.07%

2.1.10. Synthesis of 2-(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 (11)

To a solution of **(9)** (0.1159 g, 3 mmol)in absolute ethanol, cyanothioacetamide (0.03 g, 3 mmol) was added, The reaction mixture was heated under reflux for 4–5 hr until all the starting materials were disappeared as indicated by TLC. The reaction mixture was treated with ice/water mixture. The formed solid product was collected by filtration and crystallized from ethanol to form dark brown powder,yield0.110 g (86.2%); mp over 300 °C; IR (KBr, cm^{-1}): ν 3416 (OH), 2926 (CH_3), 2862 (CH_2), 2203 (C≡N), 1720 (C═N), 1622 (C═C); ^{1}H NMR (DMSO – d_6, ppm): δ = 0.800 (s, 3H, 18- CH_3), 0.800–2.724 (m, estrane moiety), 2.299 (m, 1H, 14-CH), 2.724 (m, 2H, 15-CH), 3.335 (s, 2H, 22-CH_2, acetonitrile), 9.013 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 350[M^+,5], 335 (12); 294 (1.12), 292 (2.8); Calc. for $C_{21}H_{22}N_2OS$ (350.48): C, 71.97; H, 6.33; N, 7.99; S, 9.15%; found: C, 71.92; H, 6.30; N, 7.94; S, 8.98%

2.1.11. Synthesis of (E)-N-benzyl-4-hydroxy-8a-methyl-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[1,2-d] thiazole-10-carbohydrazonoyl cyanide (12)

By using 3 tubes (Azo dye test), in the first tube solid salt **11** (0.1167 g, 3 mmol) and sodium hydroxide solution 0.0133 g, 3 mmol) in water, in the second tube sodium nitrate (0.023 g, 3 mmol), in the third tube aniline hydrochloride (0.043 g, 3 mmol), The three tubes are dunked inice path for 10–15 min., the three tubes are poured in each other respectively, The mixture was treated with ice/water mixture. The formed product was extracted by chloroform (45 mL), sodium hydrogen carbonate was added and the mixture was stirred for 45 min and filtered. The chloroform layer was dried with anhydrous calcium chloride and evaporated till dryness as brown gum, yield 0145 g(92.94%); Oily product; IR (KBr, cm^{-1}): ν 3433 (NH), 3201 (OH), 2362 (C≡N), 1940 (C═S), 1726 (C═N), 1597 (C═C); ^{1}H NMR (DMSO – d_6, ppm): δ = 8.810 (s, 3H, 18-CH_3), 0.810–2.497 (m, estrane moiety), 2.013 (m, 1H, 14-CH), 2.234 (d, 2H, 15-CH_2), 3.319 (d, 2H, 25- CH_2), 7.020 (d, 2H, phenyl group), 7.358(m, 3H, phenyl group), 7.419 (d, 1H, NH, D_2O exchangeable), 9.003 (s, 1H, C-3, OH, D_2O exchangeable); MS (EI) *m/z* (%): 452[M^+, 20%], 377 (13), 321(7); Calc. for $C_{28}H_{28}N_4OS$ (468.62): C, 71.77; H, 6.02; N, 11.96; S, 6.84%; found: C, 71.75; H, 6.01; N,11.94; S, 6.81%

2.1.12. General procedure for synthesis of compounds 13, 14

A mixture of estrone**1** (0.270 g, 1 mmol), benzaldehyde (0.106 g, 1 mmol) and malononitrile (0.066 g, 1 mmol) in absolute ethanol (30 mL) in the presence of piperdine (0.085, 1 mmol) or 20% excess of ammonium acetate (0.0785 g, 1 mmol + 20%, 0.0157 g). The reaction mixture was heated under reflux for 4–5 hrs until all the starting materials were disappeared as indicated by TLC. The reaction mixture was treated with ice/water. The formed solid of each product was collected by filtration and crystallized from ethanol.

10-amino-4-hydroxy-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 (13)

Pale brown powder from absolute ethanol, yield 0.17 g (41%): mp 216–218 °C; IR (KBr, cm^{-1}): ν 3303 (NH_2), 3022 (OH), 2931 (CH_3), 2868 (CH_2), 2180 (C≡N), 1619 (C═C), 1288 (C—O); ^{1}H NMR (DMSO – d_6, ppm): δ = 0.826 (s, 3H, 18-CH_3), 0.826–2.752 (m, estrane moiety), 1.355 (m, 1H, 14-CH), 2.091 (d, 2H, 15-CH_2), 3.298 (s, 1H, 22-CH), 6.453 (s, 1H, NH_2, D_2O exchangeable), 7.032 (d, 2H, phenyl group), 7.060 (m, 3H, phenyl group), 8.977 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 395[M^+, 12%], 369 (5), 398 (15); Calc. for $C_{28}H_{28},N_2O_2$ (424.54): C, 79.22; H, 6.65; N, 6.60%; found: C, 79.18; H, 6.61; N, 6.56%

10-amino-4-hydroxy-8a-methyl-12-phenyl-2,6b,7,8,8a,9,10,11,12,13,13a,13b-dodecahydro-1H-naphtho[2′,1′:4,5] indeno[1,2-b]pyridine-11-carbonitrile (14)

Pale yellow powder from absolute ethanol, yield 0.423 g (94.5%): mp 194–196 °C; IR (KBr, cm^{-1}): ν 3450 (NH_2), 3316 (NH), 3027 (OH), 2930 (CH_3), 2868 (CH_2), 2221 (C≡N), 1619 (C═C); ^{1}H NMR (DMSO – d_6, ppm): δ = 0.822 (s, 3H, 18-CH_3), 0.822–2.760 (m, estrane moiety), 1.351 (m, 1H, 14-CH), 2.493 (s, 1H, NH, D_2O exchangeable), 2.498 (m, 2H, 15-CH_2), 2.503 (d, 1H, 21-CH), 2.760 (d, 1H, 22-CH), 3.295 (d, 1H, 20-CH), 7.623 (d, 2H, phenyl group), 7.676, 7.967 (m, 3H, phenyl group), 8.549 (s, 2H, NH_2, D_2O exchangeable), 8.975 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 425[M^+, 100], 144 (5); Calc. for $C_{28}H_{31}N_3O$ (425.58): C, 79.02; H, 7.34; N, 9.87%; found: C, 78.97; H, 7.31; N, 9.81%

2.1.13. General procedure for synthesis of compounds 15a, 15b and 15c

To a solution of **14** (0.1057 g, 4 mmol) in absolute ethanol (30 mL), formic acid (0.0115 g, 4 mmol), acetic acid (0.015 g, 4 mmol) or chloroacetate (0.0236 g, 4 mmol) was added. The reaction mixture in each case was heated under reflux for 4–6 hrs until the starting materials were disappeared as indicated by TLC. The reaction mixture was treated with ice/water mixture and neutralized by KOH, drop by drop in to ice water. The formed solid product was collected by filtration and crystallized from absolute ethanol

4-hydroxy-8a-methyl-14-phenyl-1,2,6a,7,8,8a,9,12,14,15,15a,15b-dodecahydro-13H-naphtho[2″,1″:4′,5′]indeno[2′,1′:5,6]pyrido[2,3-d]pyrimidin-13-one (15a)

Dark gray powder from absolute ethanol, yield 0.06 g (53%): mp 208–210 ^{0}C; IR (KBr, cm^{-1}): ν 3321 (NH), 3024 (OH), 2931 (CH_3), 2864 (CH_2), 1719 (C═O), 1622 (C═N), 1597 (C═C);; ^{1}H NMR (DMSO – d_6, ppm): δ = 0.824 (s, 3H, 18-CH_3), 0.824–2.762 (m, estrane moiety), 1.327 (m, 1H, 14-CH), 2.505 (d, 2H, 15-CH_2), 4.433 (s, 1H, 26-CH), 6.53 (s, 1H, NH, D_2O exchangeable), 7.031 (m, 1H, phenyl group), 7.503 (d, 2H, phenyl group), 7.509 (m, 2H, phenyl group), 7.562 (s, 1H, NH, D_2O exchangeable), 8.977 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 451[M^+, 40], 408 (15), 396 (23), 342 (2), 324 (15); Calc. for $C_{29}H_{29}N_3O_2$ (451.57): C, 77.14; H, 6.47; N, 9.31; O, 7.09%; found: C, 77.10; H, 6.43; N, 9.31%; found: C, 77.10; H, 6.43; N, 9.28%

4-hydroxy-8a,11-dimethyl-14-phenyl-1,2,6a,7,8,8a,9,12,14,15,15a,15b-dodecahydro-13H-naphtho[2″,1″:4′,5′] indeno[2′,1′:5,6]pyrido[2,3-d]pyrimidin-13-one (15b)

Gray powder from absolute ethanol, yield 0.11 g (94%): mp 204–206 °C; IR (KBr, cm^{-1}): ν 3751 (NH), 3412 (OH), 2929 (CH_3), 2868 (CH_2), 1721 (C═O), 1631 (C═N), 1598 (C═C); ^{1}H NMR (DMSO – d_6, ppm): δ = 0.804 (s, 3H, 18-CH_3), 0.804–2.732 (m, estrane moiety), 1.292 (m, 1H, 14-CH), 2.499 (s, 3H, 22-CH_3), 2.732 (d, 2H, 15-CH_2), 4.203 (s, 1H, 26-CH), 6.653 (s, 1H, NH, D_2O exchangeable), 7.031 (m, 1H, phenyl group), 7.045 (d, 2H, phenyl group), 7.059 (m, 2H, phenyl group), 7.552 (s, 1H, NH, D_2O exchangeable), 8.990 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 465[M^+, 33], 462 (67), 428 (100), 423 (5), 355 (28), 339, (67); Calc. for $C_{30}H_{31}N_3O_2$ (465.24): C, 77.39; H,

6.71; N, 9.03%; found: C, 77.34; H, 6.68; N, 8.97%

11-(chloromethyl)-4-hydroxy-8a-methyl-14-phenyl-1,2,6a,7,8,8a,9,12,14,15,15a,15b-dodecahydro-13H-naphtho[2″,1″:4′,5′]indeno[2′,1′:5,6]pyrido[2,3-d]pyrimidin-13-one (15c)

Orange powder from absolute ethanol, yield 0.11 g (88%): mp 210–212 °C; IR (KBr, cm^{-1}): ν 3751 (NH), 3316 (OH), 2928 (CH_3), 2867 (CH_2), 1718 (C═O), 1619 (C═N), 1597 (C═C); ^{1}H NMR (DMSO – d_6, ppm): δ = 0.811 (s, 3H, 18-CH_3), 0.811–2.740 (m, estrane moiety), 1.341 (m, 1H, 14-CH), 2.490 (d, 2H, 15-CH_2), 4.302 (s, 2H, 27-CH2, chloromethyl), 4.621 (s, 1H, 26-CH), 7.424 (s, 1H, NH, D_2O exchangeable), 7.031 (m, 1H, phenyl group), 7.045 (d, 2H, phenyl group), 7.059 (m, 2H, phenyl group), 8.057 (s, 1H, NH, D_2O exchangeable), 9.013 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 500[M^+, 25%], 282 (12); Calc. for $C_{30}H_{30}ClN_3O_2$ (500.04): C, 72.06; H, 6.05; Cl, 7.09; N, 8.40%; found: C, 72.09; H, 6.09; Cl, 7.04; N, 8.35%

2.1.14. General procedure for synthesis of compounds 16a and 16b

To a solution of **14** (0.105 g, 4 mmol) in (ethanol: 10% potassium hydroxide), carbon disulphide was added in the rate of (1:1) (0.105 g, 4 mmol:0.028 g, 4 mmol) or (1:2) (0.105 g, 4 mmol : 0.038 g , 2 mmol). The reaction mixture was heated under reflux for 2–4 hrs until all the starting materials were disappeared as indicated by TLC. The reaction mixture was treated with ice/water. The formed solid of each product was collected by filtration and crystallized from absolute ethanol.

4-hydroxy-8a-methyl-14-phenyl-11-thio-1,2,6a,7,8,8a,9,10,11,12,14,15,15a,15b-tetradecahydro-13H-naphtho[2″,1″:4′,5′]indeno[2′,1′:5,6]pyrido[2,3-d]pyrimidin-13-one (16a)

White powder from absolute ethanol, yield 0.067 g (55.8%): mp 224–226 °C; IR (KBr, cm^{-1}): ν 3855–3751 (3NH), 3309 (OH), 2930 (CH_3), 2867 (CH_2), 1718 (C═S), 1620 (C═N), 1598 (C═C); ^{1}H NMR (DMSO – d_6 ppm): δ = 0.824 (s, 3H, 18-CH_3), 0.824–2.751 (m, estrane moiety), 1.328 (m, 1H, 14-CH), 2.353 (d,2H, 15-CH), 4.43 (s, 1H, 26-CH), 5.83 (s, 1H, NH, D_2O exchangeable), 6.526 (s, 1H, NH, D_2O exchangeable), 7.029 (m, 1H, phenyl group), 7.057 (d, 2H, phenyl group), 7.324 (m, 2H, phenyl group), 7.442 (s, 1H, NH, D_2O exchangeable), 9.008 (S, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 483[M^+, 10%], 422 (48), 402 (20), 321 (7); Calc. for $C_{29}H_{29}N_3O_2S$ (483.63): C, 72.02; H, 6.04; N, 8.69; O, 6.62; S, 6.63%; found: C, 71.98; H, 6.01; N, 8.64; O, 6.57; S, 6.60%

4-hydroxy-8a-methyl-14-phenyl-1,2,6a,7,8,8a,9,10, 14,15,15a,15b-dodecahydro-11H-naphtho[2″,1″:4′,5′]indeno[2′,1′:5,6]pyrido[2,3-d]pyrimidin-11,13(12H)-dithione (16b)

White powder from absolute ethanol, yield 0.053 g (64.12%): mp 220–222 °C; IR (KBr, cm^{-1}): ν 3855–3758 (3NH), 3022 (OH), 2929 (CH_3), 2867 (CH_2), 1717 (C═S), 1618 (C═N), 1599(C═C); ^{1}H NMR (DMSO – d_6, ppm): δ = 0.822 (s, 3H, 18-, CH_3), 0.824–2.751 (m, estrane moiety), 1.329 (m, 1H, 14-CH), 2.730 (d,2H, 15-CH_2), 4.321 (s, 1H, 26-CH), 5.681 (s, 1H, NH, D_2O exchangeable), 6.526 (s, 1H, NH, D_2O exchangeable), 7.028 (m, 1H, phenyl group), 7.056 (d, 2H, phenyl group), 7.132 (m, 2H, phenyl group), 8.979 (s, 1H, NH, D_2O exchangeable), 9.071 (S, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (%): 422[M^+, 5], 422 (5), 284 (7); Calc. for $C_{29}H_{29}N_3OS_2$ (499.69): C, 69.71; H, 5.85; N, 8.41; O, 3.20; S, 12.83%; found: C, 69.66; H, 5.81; N, 8.37; O, 3.17; S, 12.79%

2.1.15. Synthesis of (z)-N-(11-cyano-4-hydroxy-8a-methyl-12-phenyl-2,6b,7,8,8a,9,12,13,13a,13b-decahydro-1H-naphtho[2′,1′:4,5]indeno [1,2-b]pyridin-10-yl)-N,N-dimethylformimidamide (17)

To a solution of **14** (0.1057 g, 4 mmol) and DMF-DMA (0.04 g, 4 mmol) in absolute ethanol in the presence of acetonitrile (0.01 g, 4 mmol), The reaction mixture was heated under reflux at 70 ^{0}C for 2 hrs until all the starting materials were disappeared as indicated by TLC. The reaction mixture was treated with ice/water mixture. The precipitate was filtered off and crystallized from absolute ethanol as Pale brown powder. yield 0.1 g (83.68%); mp 230–232 °C; IR (KBr, cm^{-1}): ν 3393 (NH), 3311 (OH), 2929 (CH_3), 2868 (CH_2), 2372 (C═N), 1718 (C═N), 1622 (C═C); ^{1}H NMR (DMSO – d_6 ppm): δ = 0.820 (s, 3H, 18-CH_3), 0.820–2.780 (m, estrane moiety), 1.326 (m, 1H, 14-CH), 2.730 (d, 2H, 15-CH), 3.300 (s, 6H, 2Me), 4.321 (s, 1H, 22-CH), 7.028 (m, 1H, phenyl group), 7.056 (d, 2H, phenyl group), 7.134 (m, 2H, phenyl group), 8.012 (S, 1H, 24-CH), 8.537 (S, 1H, NH, D_2O exchangeable), 8.975 (s, 1H, C-3, OH, D_2O exchangeable); MS (EI) *m/z* (%): 478[M^+, 5], 422 (15), 385 (13), 341 (12), 325 (65); Calc for $C_{31}H_{34}N_4O$ (478.64): C, 77.79; H, 7.16; N, 11.71%; found: C, 77.74; H, 7.12; N, 11.67%

2.1.16. Synthesis of ethyl (z)-N-(11-cyano-4-hydroxy-8a-methyl-12-phenyl-2,6b,7,8,8a,9,12,13,13a,13b-decahydro-1H-naphtho[2′,1′:4,5] indeno[1,2-b]pyridin-10-yl)formimidate (18)

To a solution of **14** (0.1057 g, 4 mmol) and triorthoformate (0.037 g, 4 mmol) in absolute ethanol in the presence of acetic anhydride, The reaction mixture was heated under refluxed for 5–7 hrs until all the starting materials were disappeared as indicated by TLC. The reaction mixture was treated with ice/water mixture. The precipitate was filtered off and crystallized from absolute ethanol as off white powder. yield0.05 g (41.84%); mp 240–242 °C; IR (KBr, cm^{-1}): ν 3751 (NH), 3309 (OH), 2928 (CH_3), 2864 (CH_2), 2210 (C≡N), 1719 (C═N), 1618 (C═C), 1246 (C—O); ^{1}H NMR (DMSO – d_6, ppm): δ = 0.811 (s, 3H, 18-CH_3), 0.811–2.739 (m, estrane moiety), 1.359 (m, 1H, 14-CH), 1.323 (t, 3H, 26-CH_3), 2.490 (d, 2H, 15-CH_2), 3.323 (q, 2H, 25-CH_2), 4.321 (s, 1H, 22-CH), 7.028 (m, 1H, phenyl group), 7.458 (d, 2H, phenyl group), 7.473 (m, 2H, phenyl group), 7.498 (S, 1H, 24-CH), 7.511 (S, 1H, NH, D_2O exchangeable), 9.007 (s, 1H, C-3, OH, D_2O exchangeable); MS (EI) *m/z* (%): 479[M^{+2}, 3], 403 (5); Calc for $C_{31}H_{33}N_4O_2$ (479.62): C, 77.63; H, 6.94; N, 8.76%; found: C, 77.59; H, 6.40; N, 8.72%

2.1.17. Synthesis of 12-amino-13-imino-8a-methyl-14-phenyl-2,6b,7,8,8a,9,12,13,14,15,15a,15b- dodecahydro-1H-naphtho[2″,1″:4′,5′]indeno[2′,1′:5,6]pyrido[2,3-d]pyrimidin-4-ol (19)

To a solution of **18** (0.119 g, 4 mmol) in absolute ethanol hydrazine hydrate (0.008 g, 4 mmol) was added, The reaction mixture was heated under reflux for 2–3 hrs until all the starting materials were disappeared as indicated by TLC. The reaction mixture was treated with ice/water mixture. The formed solid was collected by filtration and crystallized from absolute ethanol as beige powder, yield 0.1 g (86.2%); mp 220–222 °C; IR (KBr, cm^{-1}): ν 3743 (NH_2), 3302 (NH), 3114 (OH), 2928 (CH_3), 2859 (CH_2), 1717 (C═N), 1618 (C═C); MS (EI) *m/z* (%): 465 [M^{+1}, 47], 424 (70), 400 (65), 375 (5); Calc. for $C_{29}H_{31}N_5O$ (465.6): C, 74.81; H, 6.71; N, 15.04%; found: C, 74.78; H, 6.66; N, 15.01%

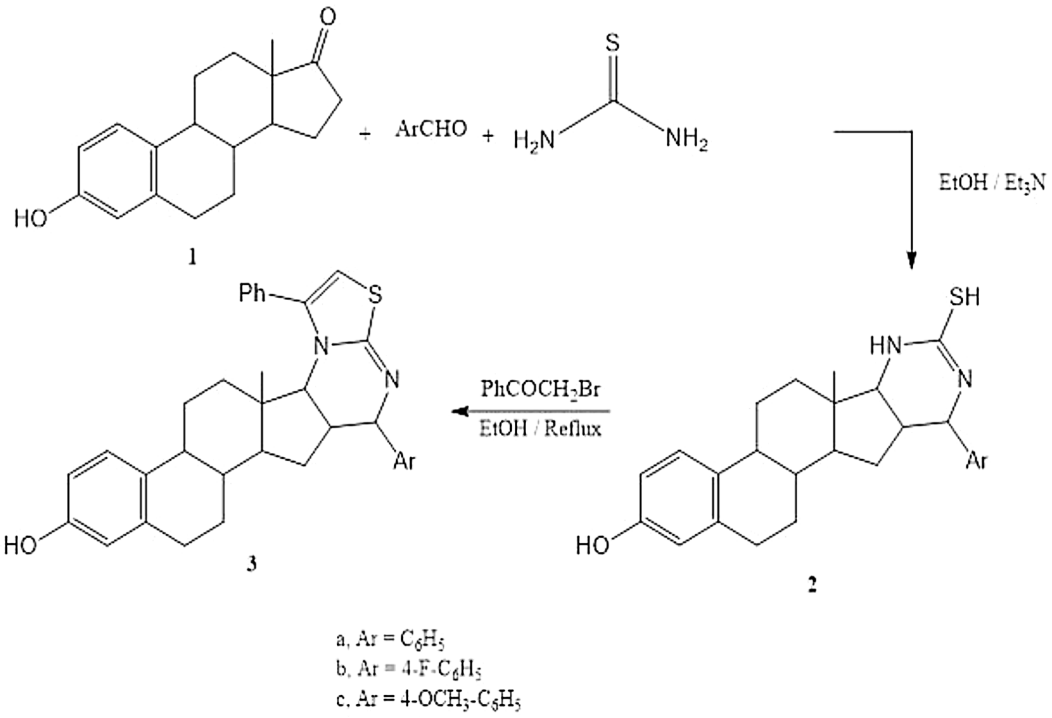
2.2. In vitro cytotoxic assay

2.2.1. Cell propagation and maintenance

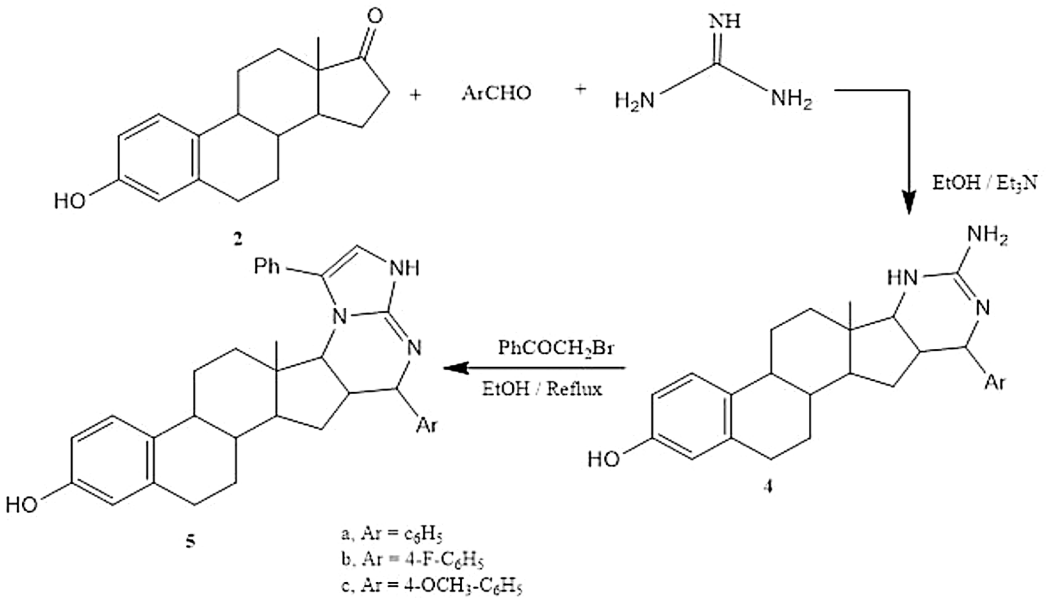
MCF-7 human breast cancer cells, PC3 human prostate cancer cells, and HepG2 human liver cancer cells were obtained from VACSERA (The holding company for biological products & vaccines) and maintained in the proper conditions. The cells were cultured in RPMI (Roswell Park Memorial Institute) medium supplemented by 2% serum (maintenance medium) at 37C in a humidified incubator with 5% CO2. The cells harvested after trypsinization (0.025% trypsin and 0.02% EDTA) and washed twice with Dulbecco's phosphate buffered saline (DPBS). When the cell density reached approximately 80%, cells were split for further culture. The experiments were made up when the cells were in the logarithmic growth phase.

2.2.2. Cytotoxicity assay

Cell viability was measured by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. MTT assay is based on the conversion of MTT into formazan crystals by living cells, which reflects cytotoxicity based on mitochondrial activity [15]. The cytotoxic impact of the tested compounds measured by MTT assay using MCF7, PC3 and HepG2 cells respectively. The cells were incubated with various concentrations of the test compounds (31.25, 62.5, 125, 250, 500, 1000 μg/



Scheme 1.

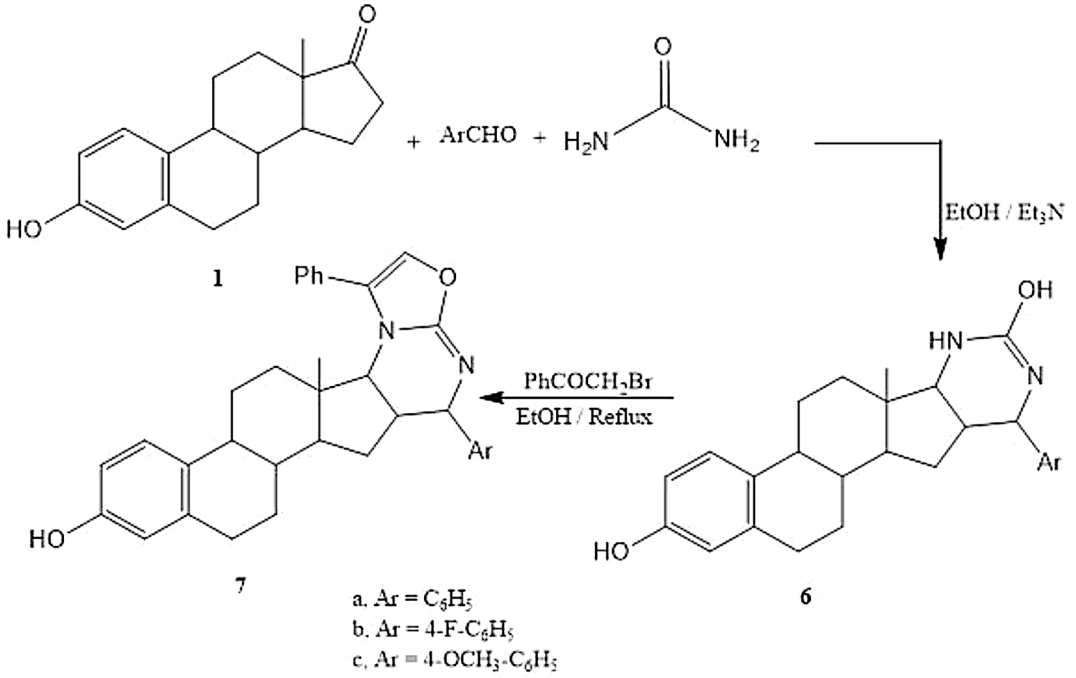


Scheme 2.

ml) for 24 h at a cell density of 104 cells/well of 96 well plate. After 24 h incubation, MTT dissolved in PBS was added to each well at a final concentration of 5 mg/ml, and the samples were incubated at 37 °C for 4 h. After 4 h, the medium was decanted and dimethyl sulfoxide (DMSO) was added to each well and left for 30 min to dissolve formazan crystals that formed during MTT cleavage in actively metabolizing cells. Absorbance of formazan in each plate was measured at 570 nm, using a microplate reader (Model 500; BIORad Instrument Inc., USA). Using the relation between the used concentrations and the mitochondrial activity (%), IC50 of the tested compound was calculated. For the untreated cells (negative control), medium was added instead of the test compound. A positive control Doxorubicin (Dox, Mr = 543.5) was used as a cytotoxic natural agent giving 100% inhibition. Dimethyl sulfoxide (DMSO) was the vehicle used for dissolution of tested compound and its final concentration on the cells was less than 0.2%. All tests and analyses were done in triplicate and the results were averaged.

2.3. Statistical analysis

All the data are expressed as Mean ± standard error mean. Data were calculated using Sigma plot ver. 125. Analysis of the data was done using student *t*-test to detect the significant difference among the studied compounds. Differences were considered to be statistically significant when P less than 0.05.



Scheme 3.

3. Results and discussion

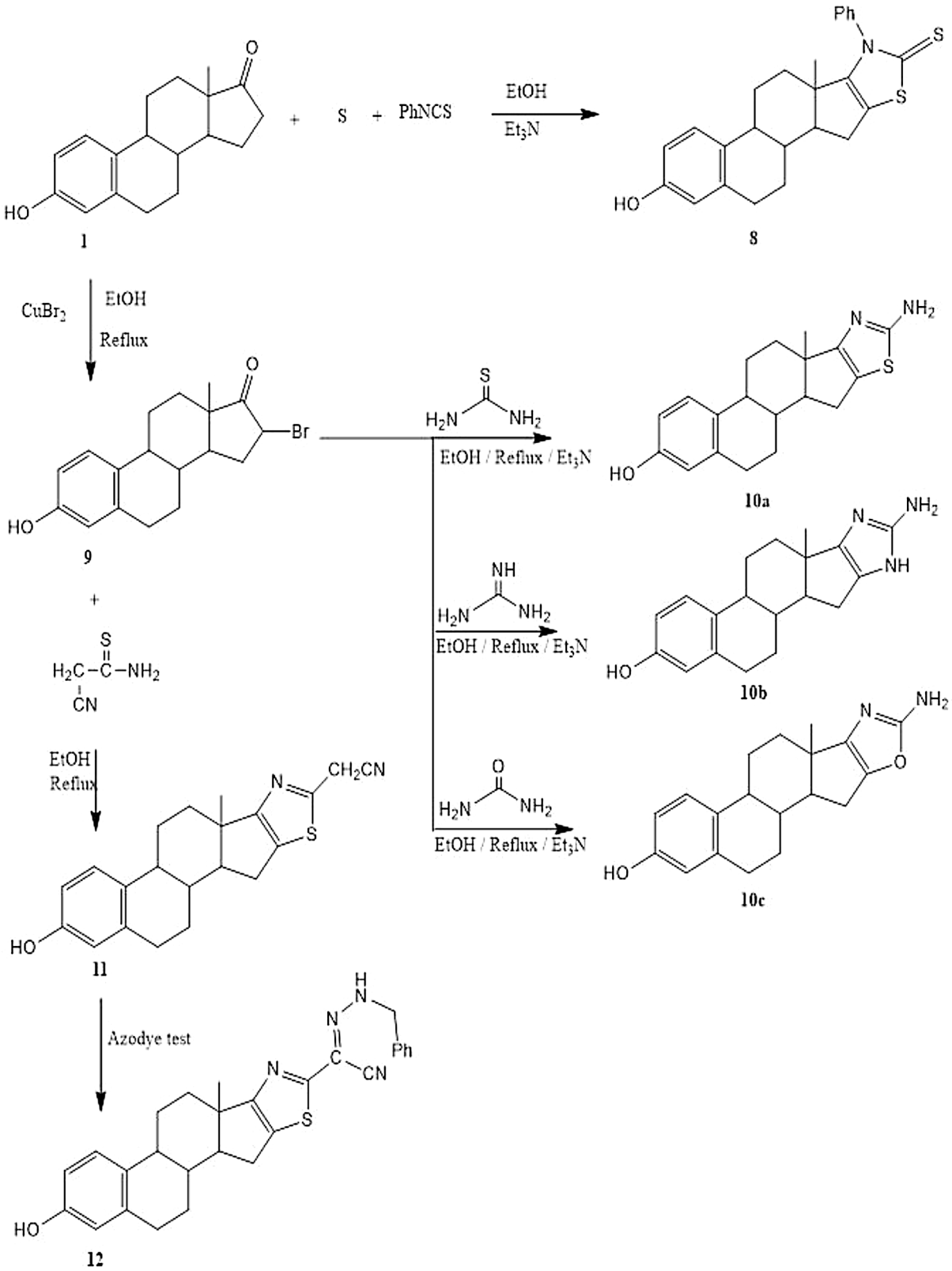
3.1. Chemistry

Pyrimidine, thiazole, imidazole, pyran, pyridine and pyridopyrimidine rings represent molecular frameworks that serve as a platform for developing pharmaceutical agents for various applications. Many derivatives of these rings proved as antitumor agents [10–14]. The goal of this research is to synthesis a variety of compounds in order to study their function as anticancer agents, by using estrone **(1)** as starting material. The development of the favored new compounds by the synthetic pathways followed is shown in the schemes 1-5. When estrone **(1)** was allowed to react with benzaldehyde, 4-fluorobenzaldehyde or 4-mothoxy benzaldehyde and thiourea in refluxing absolute ethanol afforded pyrimidinylestrane derivatives **2a, 2b** and **2c** respectively, which reacted with phenacylbromide in equimolar amount by using absolute ethanol as a solvent to afford thiazolopyrimidinylestrane derivatives **3a, 3b** and **3c** respectively (Scheme l). The previous compounds were determined by means of their IR, MS, ^{1}HNMR and analytical data. The IR spectra of the previous compounds revealed absorption band at 1717, 1718 (C═N) while compounds **2a, 2b** and **2c** revealed absorption bands at 3855, 3858 (SH) and 3343, 3751 (NH), ^{1}HNMR and EIMS agreed well with the assigned structure of experimental part (Scheme 1).

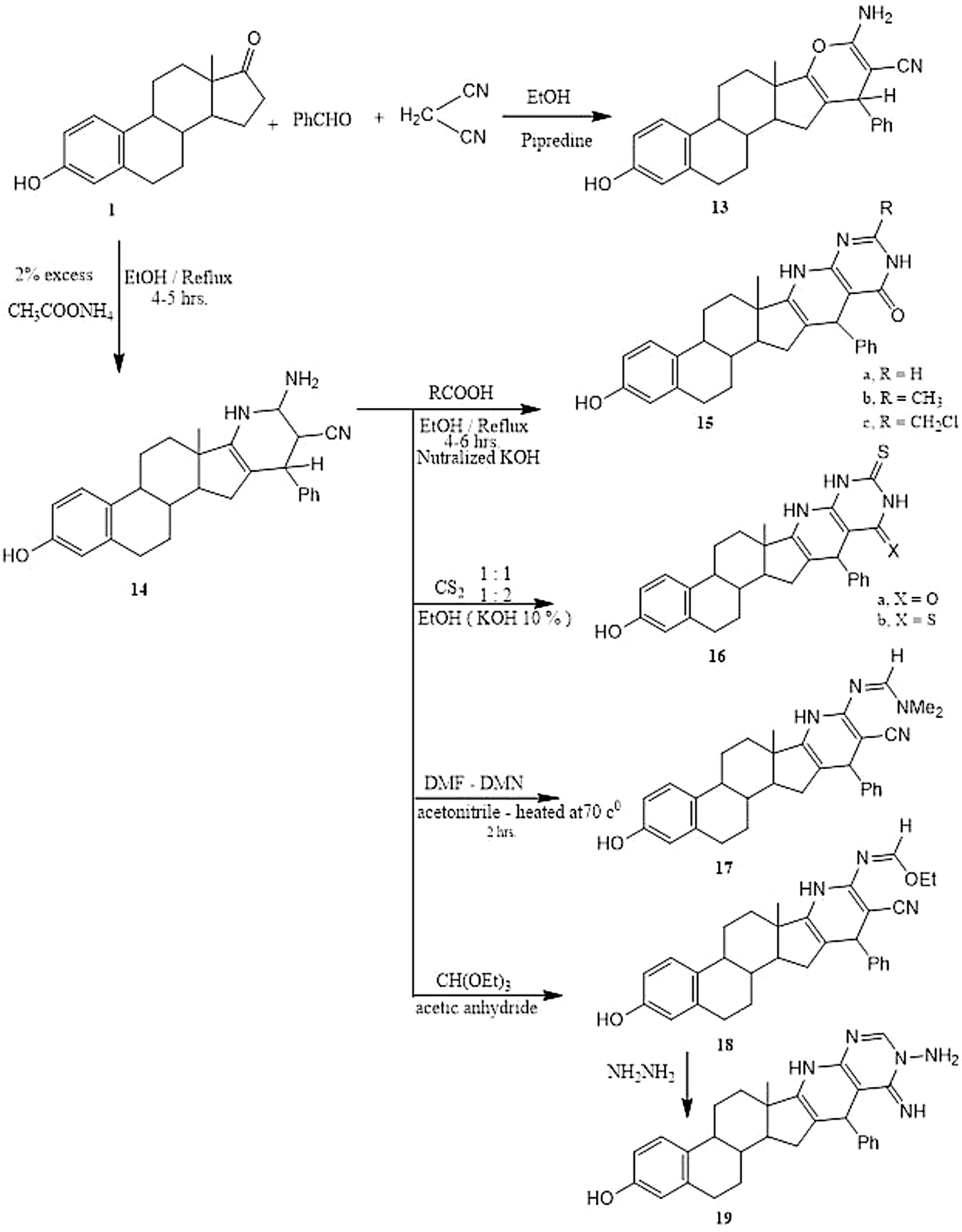
Similarly, the reaction of estrone **(1)** with benzaldehyde, 4 -fluorobenzaldehyde or 4- methoxy benzaldehyde and corresponding pyrimidinylestrane derivatives **4a, 4b** and **4c** which reacted with phenacyl bromide in the presence of boiling absolute ethanol to produce imidazolopyrimidinylestrane derivatives **5a, 5b** and **5c** respectively, (Scheme 2). The inference of the produced estrone derivatives **4a , 4b** and **4c** can be checked by IR, MS, ^{1}HNMR spectra, The IR spectra showed the appearance of the (NH_2) band at 3214 and 3751 while (NH) band at 3117, 3345, 3678 and the absence of C═O band in the IR spectra of compound **1**. The IR spectra of compounds **5a, 5b,** and **5c** also revealed the presence of characteristic absorption bands corresponding to NH group and the absence of NH_2 band. Also all the analytical and spectral data of these compounds are in accordance with the proposed structures (*c.f* experimental part, Scheme 2).

By the same way, the reaction of estrone **(1)** with benzaldehyde ,4-fluorobenzaldehyde or 4-methoxybenzaldehyde and urea in the presence of absolute ethanol as a solvent produced pyrimidinylestrane derivatives **6a, 6b** and **6c** respectively, which were reacted with phenacylbromide to produce oxazolopyrimidinylestrone derivatives **7a, 7b** and **7c** (Scheme 4). When estrone **(1)** was allowed to react with sulphur and phenyl isothiocyanate in refluxing absolute ethanol by adding dropwise of triethylamine afforded thiazoloestrane derivative **8** (Scheme 4). Furthermore, estrone **(1)** was allowed to react with cupper bromide in refluxing absolute ethanol to produce 16-bromo-estrone **9**, which allowed to react with thiourea, guanidine or urea in refluxing absolute ethanol by adding 2–3 drops of triethylamine to produce thiazolo, imidazolo and oxazoloestrane derivatives **10a, 10b** and **10c** respectively, The ^{1}HNMR and EIMS and all spectral data agreed well with the assigned structures of the produced compounds (*c.f* experimental part. When compound **9** was allowed to react with cyanothioacetamide in boiling absolute ethanol produced the thiazoloestrane derivative **11,** which allowed to react with sodium hydroxide, sodium nitrate and aniline hydrochloride (Azo dye test) to produce compound **12** (*c.f* experimental parts, Scheme 4).

In this study, one pot multi-component reactions were attempted as a straight forward method for the synthesis of heterocyclic estrane derivatives. The reaction of estrone **(1)** with benzaldehyde and malononitrile in refluxing absolute ethanol by adding piperidine or 20% excess of ammonium acetate afforded aminopyranoestrane derivative **13** and aminopyridinoestrane derivative **14** respectively (Scheme 5). The formation of these derivatives can be explained by the possible mechanism represented in our article *Abd-Elhalim et al* [16]. The IR spectra showed strong absorption band at 3303, 3316 (NH_2), 2180, 2221, (CN) respectively, and 3750 (NH) of compound **14**. The^{1}HNMR and EIMS agreed well with the assigned structure of experimental part. When compound **14** was allowed to react with formic acid, acetic acid or chloroacetate by refluxing absolute ethanol produced pyridopyrimidinylestrane derivatives **15a, 15b** and **15c,** respectively (*c.f* experimental part, Scheme 5). The reaction of compound **14** with equimolar amounts of carbon disulfide or double molar of carbon disulfide in the presence of 10% KOH with absolute ethanol produced compounds **16a** and **16b** respectively. When compound **14** was allowed to react with DMF-DMA in the


EtOH
Et3N
PhNCS
S
Ph
N
S
HO
1
8
CuBr2
EtOH
Reflux
Br
9
H2N
NH2
EtOH / Reflux / Et3N
10a
NH
10b
O
10c
CH2CN
11
Azodye test
CN
12

Scheme 4.


PhCHO
EtOH
Pipredine
2% excess
CH3COONH4
EtOH / Reflux
4-5 hrs.
RCOOH
EtOH / Reflux
4-6 hrs
Nutralized KOH
CS2 1 : 1 1 : 2
EtOH (KOH 10 %)
DMF - DMN
acetonitrile - heated at70 c0
2 hrs.
CH(OEt)3
acetic anhydride
NH2NH2
a, R = H
b. R = CH3
c, R = CH2Cl
a. X = O
b. X = S
1
13
14
15
16
17
18
19

Scheme 5.

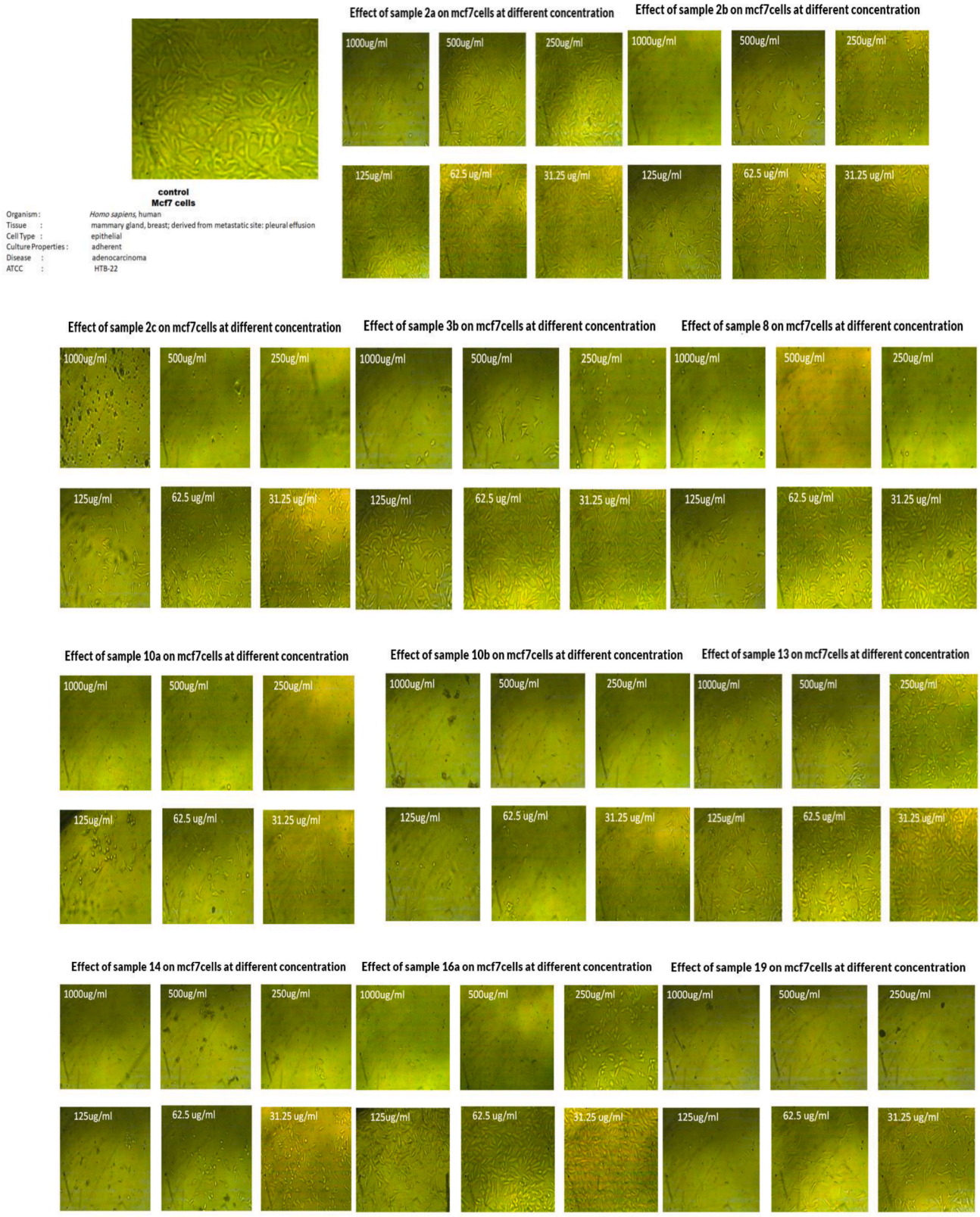


Fig. 1. Effect of the tested compounds on the morphology of MCF-7 cells at different concentrations.

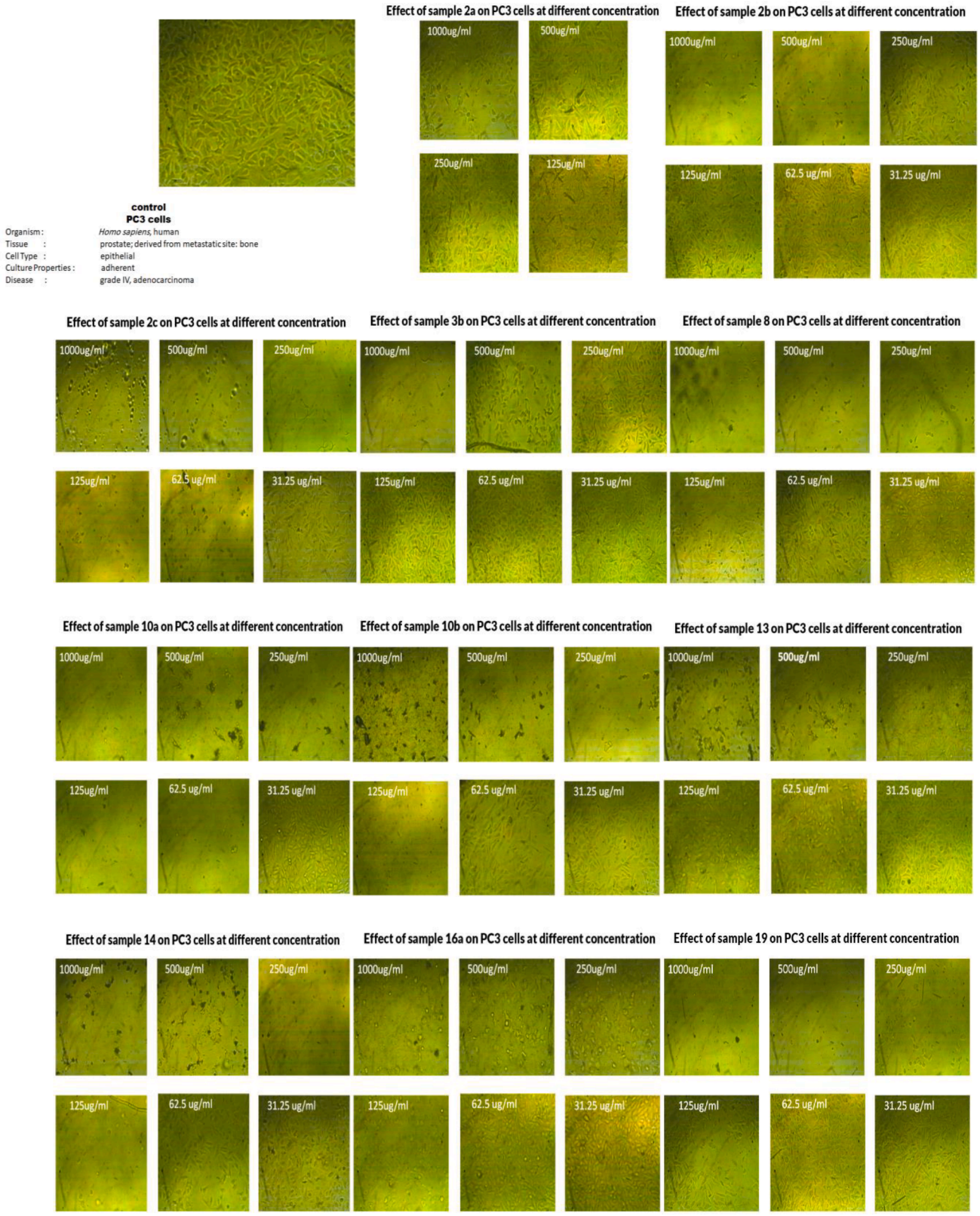


Fig. 2. Effect of the tested compounds on the morphology of PC3 cells at different concentrations.

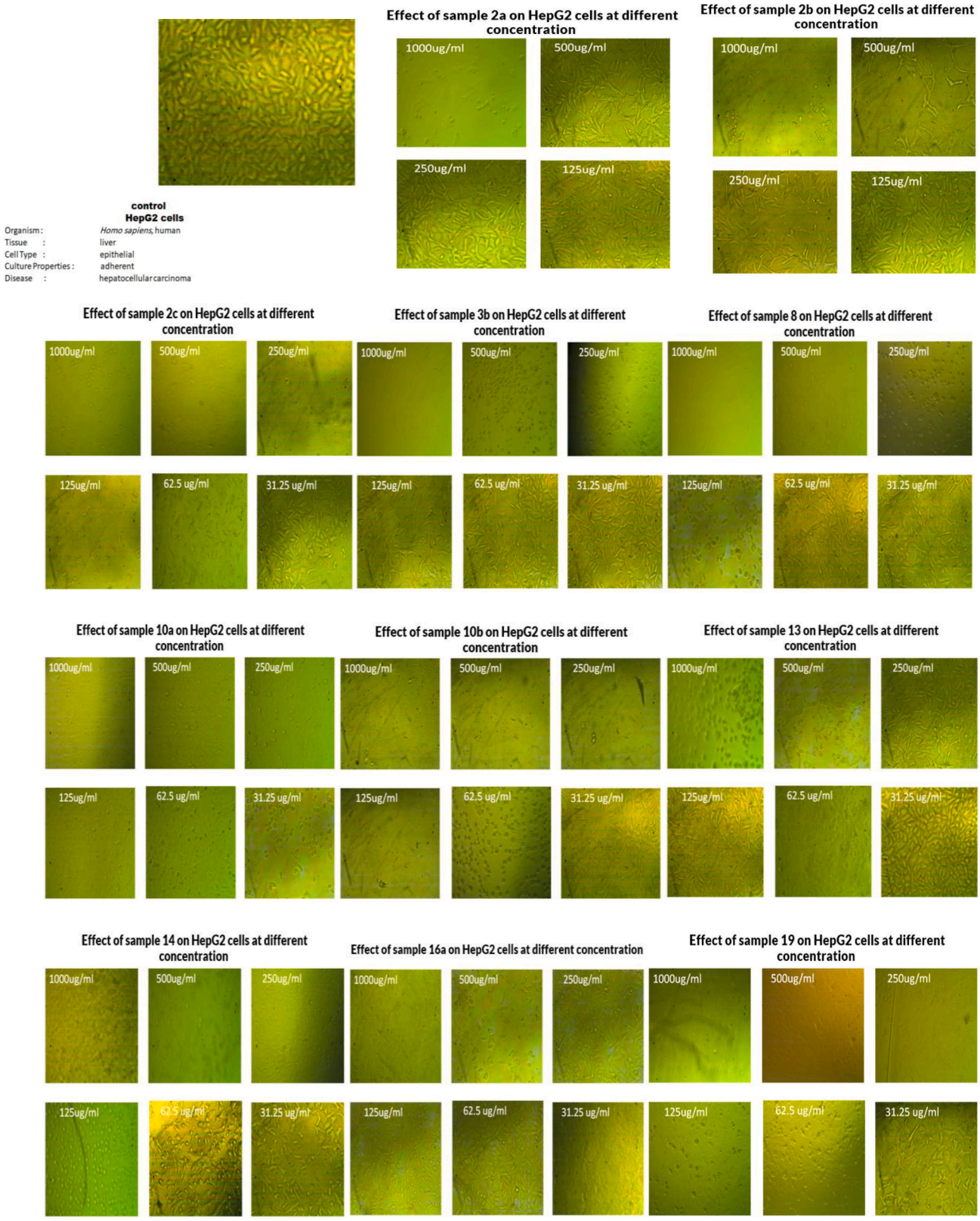


Fig. 3. Effect of the tested compounds on the morphology of HepG2 cells at different concentrations.

Table 3
The in vitro cytotoxic activity of synthesized compounds on HepG2 cancer cell line.

Compd.No	IC_{50} (μg/ml)	Compd.No	IC_{50} (μg/ml)
2a	479.83	**10b**	31.13
2b	357.08	**13**	223.43
2c	24.64	**14**	36.62
3b	91.28	**16a**	216.82
8	59.42	**19**	33.96
10a	19.07		
Doxorubicin	98.55	**Doxorubicin**	98.55

IC_{50}: Concentration required to inhibit cell viability by 50%.

presence of acetonitrile in refluxing absolute ethanol by heating at 70 ^{0}C, the compound **17** was afforded which established by means of their spectral and analytical data. The reaction of compound **14** with triorthoformate in absolute ethanol by refluxing in the presence of acetic anhydride afforded compound **18** which reacted with hydrazine hydrate in boiling absolute ethanol to produce pyridopyrimidinylestrane derivative **19**.

3.2. Biological assays

3.2.1. In vitro evaluation of cytotoxic activity

The cytotoxic activity of the tested compounds **2a, 2b, 2c, 3b, 8, 10a, 10b, 13, 14, 16a** and **19** were investigated individually as anticancer agents a against human breast cancer MCF7, human prostate cancer PC3 and human liver cancer HepG2 cells at different concentrations (31.25, 62.5, 125, 250, 500 and 1000 μg/ml) by using MTT assay which is based on the diversion of MTT into formazan crystals by living cells, that reflects cytotoxicity based on mitochondrial activity. Data explained in (Figs. 1–3) showed average percentage of the toxicity of cancer cells treated with different concentrations of these compounds after 24 h. Doxorubicin used as reference drug with IC50 values of 93.08 μg/ml, 72.39 μg/ml and 98.55 μg/ml against the three tested cell lines MCF7, PC3 and HepG2 respectively. The employment of DMSO as a solvent had insignificant effect on MCF7, PC3 and HepG2 cells viability when treated for 24 h. As compared to the control cells, all tested compounds affected significantly on cell growth inhibition. Data in table (1) and figures (4(A–C)) revealed that the cytotoxic activity of the tested compounds was in the descending order of compound **10a** > **10b** > **14** > **19** > **2c** > **8** > **16a** > **2b** > **3b** > **13** > **2a** against MCF-7 cancer cells. At 24 h, compounds **10a**, **10b**, **14**, **19**, **2c**, **8** and **16**a (18.26, 18.27, 24.15, 26.61, 54.21, 77.12 and 92.52 μg/ml respectively) showed more inhibitory effect than Dox (93.08 μg/ml). On the other hand, compounds **2b**, **3b**, **13** and **2a** revealed moderate to slight cell growth inhibition of MCF-7 with IC50 values of 141.65, 156.5, 172.07 and 616.69 μg/ml, respectively. Data in table (2) and figures (5(A–C)) showed that the cytotoxic activity from the highest to the lowest as follow compound **2c** > **10a** > **10b** > **14** > **8** > **19** > **13** > **16a** > **2b** > **3b** > **2a** contra PC3 cells. The treatment of PC3 cells with compounds **2c**, **10a**, **10b** and **14** (22.89, 31.75, 38.4, 48.11 μg/ml respectively) showed the highest cytotoxic activity as compared to Dox (72.39 μg/ml) after 24 h. Furthermore, the other compounds **8**, **19**, **13**, **16a**, **2b**, **3b** and **2a** with IC50 values of 92.77, 111.17, 164.61, 164.86, 202.5, 331.3 and 690.41 μg/ml respectively showed moderate to slight inhibition growth against PC3 cancer cells in comparison with control cells. From the data obtained (Table 3, Fig. 6(A–C)), the most cytotoxic compounds contra HepG2 cells was in the descending order of **10a** > **2c** > **10b** > **19** > **14** > **8** > **3b** > **16a** > **13** > **2b** > **2a**. Compounds **10a**, **2c**, **10b**, **19**, **14**, **8** and **3b** with IC50 values (19.07, 24.64, 31.13, 33.96, 36.62, 59.42 and 91.28 μg/ml respectively) revealed more cytotoxic than Dox values (98.55 μg/ml) when treated with HepG2 cells for 24 h. As compared to control cells, compounds **16a**, **13**, **2b** and **2a** showed moderate inhibition effect of HepG2 cell growth with IC50 of 216.82, 223.43, 357.08 and 479.83 μg/ml, respectively. Finally, these results showed that the

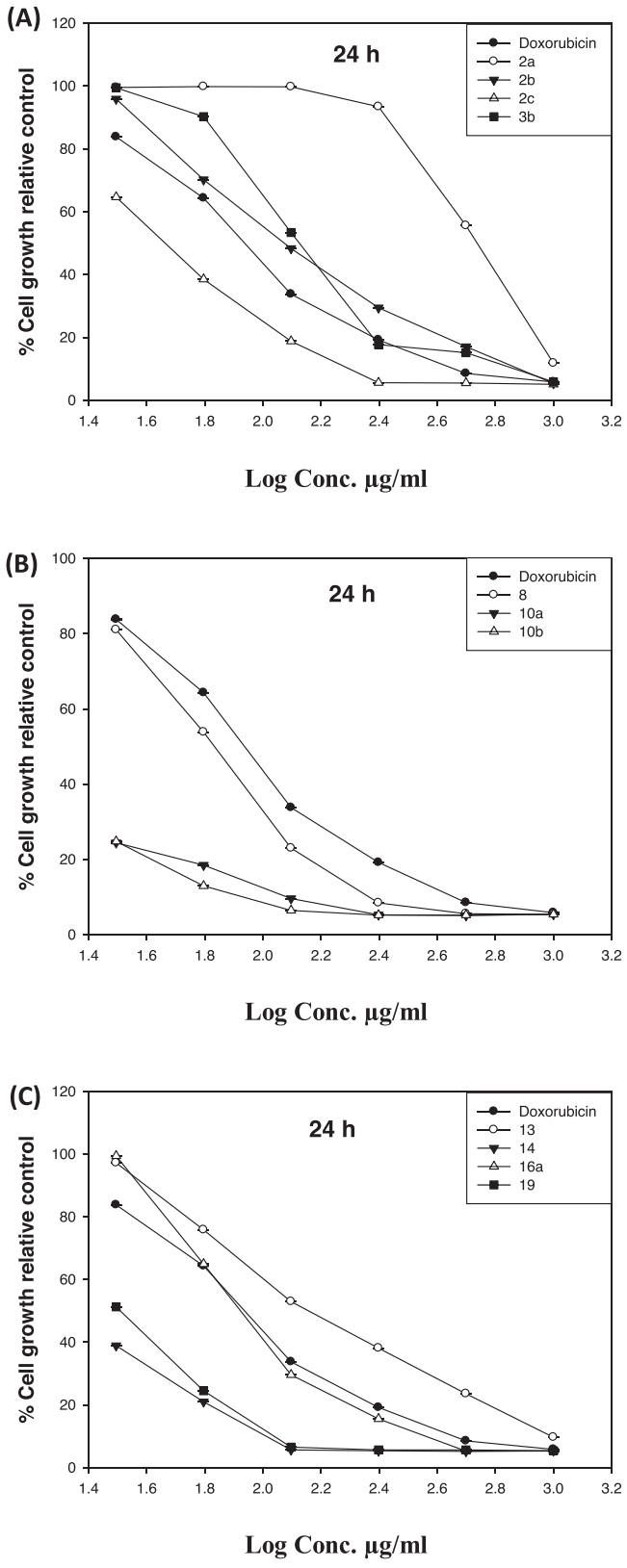
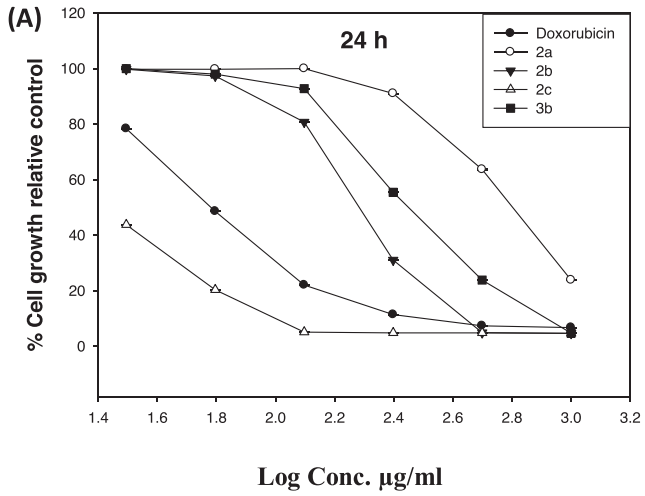
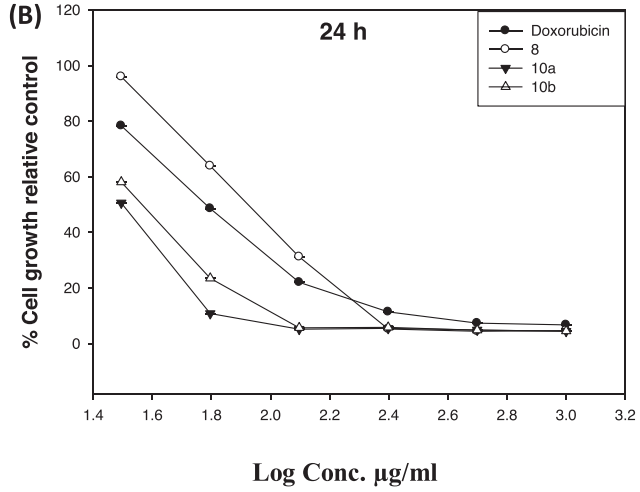


Fig. 4. (A-C): Effects of tested compounds on MCF-7 cells at 24 h.





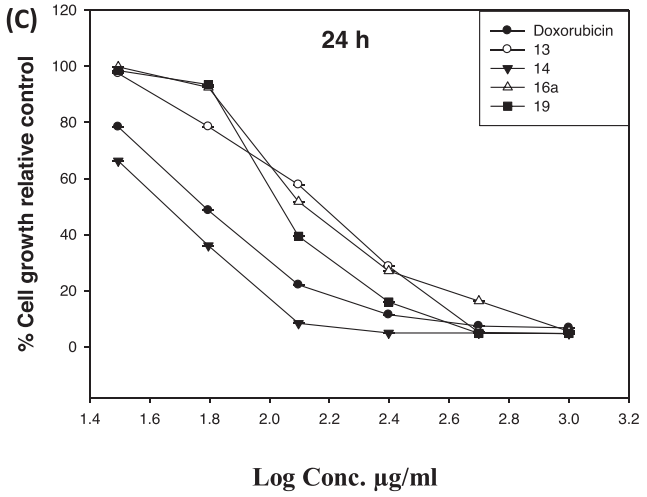
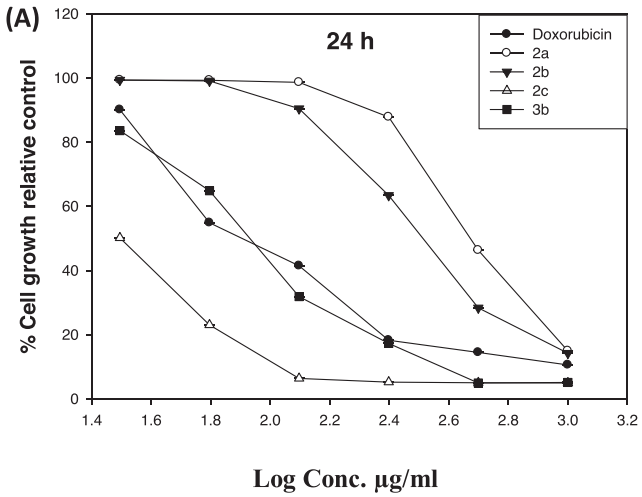
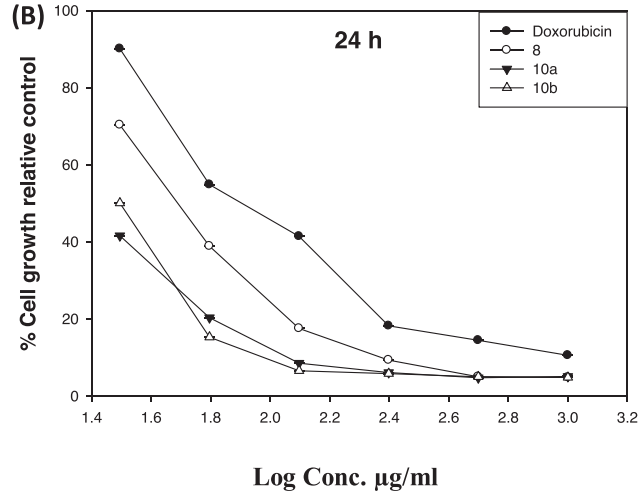


Fig. 5. (A-C): Effects of tested compounds on PC3 cells at 24 h.





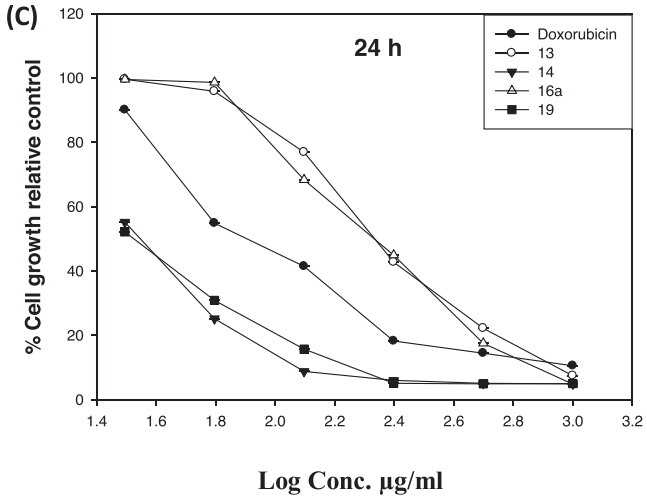


Fig. 6. (A-C): Effects of tested compounds on HepG2 cells at 24 h.

tested compounds **10a**, **10b**, **2c**, and **14** are better than Dox when treated with cancer cell lines (MCF7, PC3 and HepG2 cells) after 24 h incubation time.

3.2.1.1. Structure activity relationship.. The analysis of the structure activity relationships indicates that the combining (Figs. 4-6) of several heterocyclic rings to estrone moiety contributed to changes in the cytotoxic activity of these compounds. At 48 h incubation time amino thiazoloestrane moiety, compound **10a** is more cytotoxic compound followed by amino imidazoleestrane moiety, compound **10b** and phenyl thiazoloestrane moiety, compound **8** on MCF7, PC3 and HepG2 cells. The presence of methoxy phenyl group in compound **2c** is more effective than the presence of fluoro phenyl group in compound **2b** and phenyl group in compound **2a** and also the existence of phenyl thiazolo group attached to compound **2b** made no change in the cytotoxic activity of compound **3b** when treated with three cell lines. The addition of amino phenyl pyridine carbonitrile ring to estrone moiety to give compound **14** is more potent than the addition of amino phenyl pyran carbonitrile ring to estrone moiety to give compound **13** and also the addition of amino-imino phenyl pyrido pyrimidinyl ring to estrone moiety to give compound **19** is more effective than the addition of phenyl pyrido thiopyrimidinone ring to estrone moiety to give compound **16a** in all cell lines. These results established the importance of the existence of pyrimidine, thiazole, imidazole, pyran, pyridine and pyridopyrimidine rings as pharmacophores for the anticancer activity.

4. Conclusion

This study described a facile synthesis of newly synthesized hybrid estrane heterocyclic compounds and investigated also the importance of heterocyclic ring moiety incorporation into the estrane nucleus to form new promising anticancer agents. The tested compounds **10a**, **10b**, **2c**, and **14** showed the lowest IC_{50} values and the best cytotoxic effect against MCF7, PC3 and HepG2 cell lines respectively as compared to Dox values after 24 h incubation time. All results were confirmed by morphology study. Finally, we recommend these estrane heterocyclic compounds as target for extension studies before going through phase 1 of clinical trials.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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