Progesterone utility in the synthesis of steroidal heterocyclic compounds with antitumor activity

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Abstract

One-pot and efficient method for the synthesis of progesteronopyridine 5a-c, 6a-c and 7a,b and/or progesteronopyran derivatives 9a-c and 10a,b by condensation reaction of progesterone 1 with different aldehydes and active methylene compounds in the presence of ammonium acetate or piperidine. New progesteronopyrimidine derivatives 12a-d and 13a, b were synthesized via interaction of progesterone 1 with urea or thiourea and/or guanidine reagents and aldehyde. Progesterone 1 was examined to synthesize heterocyclic compound 16 containing γ-Lactone chiral carbon via the reaction of hydrazone derivative 14 with phenyl isothiocyanate followed by boiling with chloroacetic acid in benzene. The biological activity of compounds 5a, 5b, 6b, 7a, 9b, 9c, 12a, 12c, and 13a were evaluated as growth inhibitors of the liver and the breast carcinoma human cell line (HEPG2 & MCF7). Compounds 13a, 12a and 7a showed a higher potency than the standard.

Key Words: Progesterone, MCR’s (multicomponents reaction), (pyridine, pyran, pyrimidine, γ-Lactone) derivatives, HEPG2 & MCF7.

1. Introduction:

Progesterone is a female sex hormone that plays an important physiological role to regularize and rebuild the changes caused to the body by estrogen in the luteal phase of the menstrual cycle.1 Progesterone is a steroid hormone consists chemically of four fused hydrocarbons containing the functional groups of ketone, acetyl and two methyl groups. It is a precursor of all steroid hormones and an intermediate in the biosynthesis of androgens, estrogens and the corticoid.2 Synthetic compounds with progesterone are used in the prevention of miscarriage, treatment of menstrual disorders and development in the cosintuent of some contraceptives.3 Progesterone like pregnenlone and dehydroepiandrosterone, belongs to the group of neurosteroids that are found in high concentrations in certain areas in the brain and are synthesized there. Neurosteroids affect synaptic functioning is neuroprotective and also affect myelinisation. They are being investigated for their potential to improve memory and cognitive ability.4, 5 Progesterone plays an important role in other systems. For example it raises epidermal growth factor-1 levels 6 reduces spasm and increases core temperature during ovulation.7 In addition, it relaxes smooth muscles, acts as an anti-inflammatory agent8 and regulates the immune response, normalizes blood cloting and vascular tone.9 Moreover it assists the thyroid function in bone building and prevents endometrial cancer by regulating the effects of estrogen.10 On the other hand; pyridine and pyrimidine nucleuses are prevalent in numerous natural products and are extremely important in chemistry of biologically systems.11, 12 They play a key role catalyzing both biological and chemical systems. In many enzymes of living organisms, it is the prosthetic pyridine nucleotide that is involved in various oxidation-reduction processes. The pyridine ring exists also in the important vitamins niacin and pyridoxine (vitamin B6) and in the highly toxic alkaloids such as nicotine.13-15 Moreover, substituted pyridines are reported as leukotriene B-4 antagonists.16, 17 The literature survey have also revealed the important of pyran nucleus as a
privileged structure because many of its derivative possess useful pharmacological and anticancer activities. Poly functionalized pyrans and their derivatives have been the subject of significant interest from the synthetic community and have been widely recognized as versatile scaffolds with diverse biological activity. Upon all the previous information and in continuation of our previous work for synthesis of different bioactive fused heterocyclic derivatives using MCR’s as useful tool for synthesis. This report explains the building of poly functionally heterocyclic rings over progesterone skeleton to improve its physiological activity. Examination the activity of some newly synthesized products as antitumor agents have been carried out.

Experimental:

Progesterone was purchased from Sigma Company, USA. The appropriate precautions in handling moisture sensitive compounds were undertaken. Melting points were determined on an electro thermal apparatus (Buchi 535, Switzerland) in an open capillary tube and are uncorrected. IR spectra expressed in cm⁻¹ were recorded in KBr pellets on a PA-9721 IR spectrophotometer. ¹H and ¹³C-NMR spectra were recorded in [D₆] DMSO as solvent on a Jeol 270&/or 500 MHz spectrometer and the chemical shifts were recorded in 6 values (ppm) relative to TMS as internal reference. Mass spectra were recorded on Kratos (75 eV) Ms equipment. Elemental analysis was carried out by the micro-analytical unit at the National Research Centre, Giza, Egypt. All reactions were monitored by thin layer chromatography, carried out on 0.2 mm silica gel 60 F-254 (Merck) plates using UV light (245 and 365 nm) for detection.

General Procedure:

Synthesis of 5a-c, 6a-c and 7a,b

To a solution of progesterone (0.134 g, 0.001 mol), added aldehyde (0.001 mol), and malononitrile (0.066 g, 0.001 mol) or α-cyano-acetophenone (0.113 g, 0.001 mol) and/or ethyl cyanoacetate (0.130 g, 0.001 mol) in (30 ml) ethanol containing ammonium acetate (0.980 g, 2% excess). The reaction mixture was heated under reflux for 3-5 h., until all starting materials had disappeared indicated by TLC. The solvent was evaporated under reduced pressure and the remaining solids were crystallized from the proper solvent.

2-Amino-6-(3-oxo-androst-4-ene-17-yl)-4-phenyl-nicotinonitrile 5a

Yellow crystals from benzene. Yield (86%), mp. 90 ºC. Ms (EI) m/z (%): 465 [M⁺, 80.2%]; IR (KBr, cm⁻¹): 3329 (NH₂); 3126 (C-H, aromatic); 2862 (CH₃); 2217 (CN); 1991 (C=O); 1696 (C=O); 1640 (C=C); 1603 (C=N). ¹H-NMR (500 MHz, DMSO-d₆, TMS): δ 0.81 (s, 3H, CH₃-19); 1.13 (s, 3H, -CH₂-18); 5.63 (s, 1H, CH-4 of progesterone); 6.61 (s, 2H, NH₂, D₂O exchangeable); 7.17-7.94 (m, 6H, 5 aromatic protons+1H pyridine ring). ¹³C-NMR (125 MHz, DMSO-d₆, TMS): δ 19.6 (C-18), 20.0 (C-19), 20.8 (C-15), 20.9 (C-11), 25.8 (C-16), 28.7 (C-8), 30.4 (C-1), 30.8 (C-6), 30.9 (C-7), 31.1 (C-12), 38.5 (C-2), 38.5 (C-10), 40.0 (C-13), 43.2 (C-9), 46.7 (C-14), 47.2 (C-17), 118.0 (CN), 119.3 (C-4), 126.7, 129.1, 138.1 (C-phenyl), 91.7, 110.5, 155.2, 164.3, 166.1(C-pyridine ring), 162.6 (C-5), 197.6 (C-3). Anal. Calcd. For C₃₁H₃₂N₂O: C, 79.71%; H, 7.58%; N, 9.02%; Found: C, 79.40%; H, 7.50%; N, 9.00%.

2-Amino-4-(4-chloro-phenyl)-6-(3-oxo-androstene-4-ene-17-yl)-nicotinonitrile 5b

Pale yellow crystals from EtOH. Yield (82%), mp. 120 ºC. Ms (EI) m/z (%): 501[M⁺², 5.6%]; 499[M⁺,16.3%]; 362[M⁺(Ar+CN),65.4%]; IR (KBr, cm⁻¹): 3305 (NH₂); 3126 (C-H, aromatic); 2962 (CH₃); 2216 (CN); 1700 (C=O); 1645 (C=C); 1602 (C=N). ¹H-NMR (270 MHz, DMSO-d₆, TMS): δ 0.88 (s, 3H, CH₃-19); 1.14(s, 3H, -CH₂-18); 5.58 (s, 1H, CH-4, of progesterone); 6.81(s, 2H, NH₂, D₂O exchangeable); 7.15(s,1H,pyridine proton); 7.30 (dd,2H, aromatic protons J₁H₁=8Hz); 7.44 (dd,2H, aromatic protons J₁H₂=8Hz). ¹³C-NMR (125 MHz, DMSO-d₆, TMS): δ 19.3 (C-18), 20.1 (C-19), 20.8 (C-15), 20.9 (C-11), 25.6 (C-16), 28.3 (C-8), 30.2 (C-1), 29.9 (C-7), 30.8 (C-6), 31.1 (C-12), 38.0 (C-2), 38.1 (C-10), 39.1 (C-13), 42.5 (C-9), 47.7 (C-17), 47.4 (C-14), 118.0 (CN), 119.8 (C-4), 128.1, 129.3, 134.5, 136.4 (C- aromatic), 91.7, 111.2, 155.8, 164.3, 166.4 (C-pyridine ring), 160.6 (C-5), 198.4 (C-3). Anal. Calcd. For C₃₁H₂₁ClN₂O: C, 74.50%; H, 6.85%; N, 8.40%; Cl, 7.09%. Found: C, 74.20%; H, 6.71%; N, 8.35%; Cl, 7.00%.
2-Amino-4-(2, 5-dimethoxy-phenyl)-6-(3-oxo-androst-4-ene-17-yl)-nicotinonitrile 5c

Yellow crystals from EtOH. Yield (73%), mp. 110 °C. Ms (EI) m/z (%): 525 [M]+, 30.2%; IR (KBr, cm−1): 3315 (NH); 3126 (C-H, aromatic); 2845 (CH2); 2212 (CN); 1700 (C=O); 1644 (C=C); 1604 (C=N). 1H-NMR (270 MHz, DMSO-d6, TMS): δ 0.78 (s, 3H, CH3-19); 1.13 (s, 3H, CH3-18); 3.64 (s, 3H, OCH3); 3.71 (s, 3H, OCH3); 5.65 (s, 1H, CH-4 of progesterone); 6.52 (s, 2H, NH2, D2O exchangeable); 6.63(d,1H,aromatic proton); 7.61(d, 1H, aromatic proton); 6.73(s, 1H, aromatic proton); 7.41(s,1H, pyridine proton). Anal. Calcd. For C37H38N2O: C, 75.40%; H, 7.51%; N, 8.00%; Found: C, 75.20%; H, 7.32%; N, 7.95%.

6-(3-oxo-androst-4-ene-17-yl)-2, 4-diphenyl-nicotinonitrile 6a

Yellow crystals from EtOH. Yield (71%), mp. 145 °C. Ms (EI) m/z (%): 526 [M]+, 89.7%; IR (cm−1): 3126 (C-H, aromatic); 2862 (CH2); 2220 (CN); 1699 (C=O); 1640 (C=C); 1606 (C=N). 1H-NMR (270 MHz, DMSO-d6, TMS): δ 0.97 (s, 3H, CH3-19); 1.14 (s, 3H, CH3-18); 5.63 (s, 1H, CH-4 of progesterone); 7.26-7.97 (m, 11H, 5H phenyl+1H pyridine proton). 13C-NMR (125 MHz, DMSO-d6, TMS): δ 19.7 (C-18), 20.5 (C-19), 20.8 (C-15), 20.9 (C-11), 25.1 (C-16), 29.7 (C-8), 30.4 (C-1), 30.9 (C-7), 32.6(C-6), 34.5 (C-12), 37.9 (C-2), 39.1 (C-10), 42.5 (C-13), 42.9 (C-9), 45.7 (C-14), 47.1 (C-17), 118.9 (CN), 119.1 (C-4), 127.1,127.6, 129.1,129.5, 136.0, 137.4 (C-phenyl), 105.8, 106.4, 154.8, 163.3, 167.7 (C-pyridine ring), 162.6 (C-5), 197.6 (C-3). Anal. Calcd. For C37H38N2O: C, 84.4%; H, 7.30%; N, 5.30%; Found: C, 84.30 %; H, 7.22%; N, 5.12%.

4-(4-Chloro-phenyl)-6-(3-oxo-androst-4-ene-17-yl)-2-phenyl-nicotinonitrile 6b

Orange crystals from EtOH. Yield (83%), mp. 105 °C. Ms (EI) m/z (%): 562[M]+, 8.2%; 560[M-24.6%]; 424[M-2(Ar+CN), 54.5%]; IR (cm−1): 3126 (C-H, aromatic); 2862 (CH2); 2210 (CN); 1695 (C=O); 1640 (C=C); 1614 (C=N). 1H-NMR (270 MHz, DMSO-d6, TMS): δ 0.97 (s, 3H, CH3-19); 1.14 (s, 3H, CH3-18); 5.63 (s, 1H, CH-4 of progesterone); 7.32-8.06 (m, 10H, 5Hphenyl+4H aromatic +1H pyridine proton). 13C-NMR (125 MHz, DMSO-d6, TMS): δ 19.3 (C-18), 20.0 (C-19), 20.8 (C-15), 20.9 (C-11), 25.8 (C-16), 29.7 (C-8), 30.4 (C-1), 30.9 (C-7), 32.8 (C-6), 34.2 (C-12), 38.5 (C-2), 39.0 (C-10), 42.8 (C-13), 42.9 (C-9), 45.7 (C-14), 47.3 (C-17), 118.0 (CN), 119.8 (C-4), 127.4, 127.6, 129.4, 136.2 (C-aromatic), 128.8, 129.3, 134.8, 136.1 (C- phenyl), 105.9, 106.2, 154.5, 163.6, 167.4 (C-pyridine ring), 162.6 (C-5), 197.6 (C-3). Anal. Calcd. For C37H37ClN2O: C, 79.20%; H, 6.65%; N, 4.99%; Cl, 6.32. Found: C, 79.12%; H, 6.62%; N, 4.85%; Cl, 6.28%.

4-(2, 5-Dimethoxyphenyl)-6-(3-oxo-androst-4-ene-17-yl)-2-phenyl-nicotinonitrile 6c

Orange crystals from EtOH. Yield (68%), mp. 95 °C. Ms (EI) m/z (%): 586[M]+, 40.0%; IR (cm−1): 3126 (C-H, aromatic); 2862 (CH2); 2210 (CN); 1699 (C=O); 1641 (C=C); 1610 (C=N). 1H-NMR (270 MHz, DMSO-d6, TMS): δ 0.71 (s, 3H, CH3-19); 1.09 (s, 3H, CH3-18); 3.62(s, 3H, OCH3); 3.69 (s, 3H, OCH3); 5.31 (s, 1H, CH-4 of progesterone); 6.42(d,1H, aromatic proton); 6.60(d,1H, aromatic proton); 6.81 (s,1H, aromatic proton); 7.25-7.99 (m, 6H, 5H phenyl+1H pyridine proton). Anal. Calcd. For C30H28O3N2: C, 79.80%; H, 7.21%; N, 4.77%; Found: C, 79.72%; H, 7.12%; N, 4.65%.

4-phenyl-6-(3-Oxo-androst-4-ene-17-yl)-2-oxo-1,2-dihydro-pyridine-3-carbonitile 7a

Yellow crystals from EtOH. Yield (81%), mp. 132 °C. Ms (EI)m/z, (%): 466 [M]+, 37.1%; IR (KBr, cm−1): 3150 (NH), 3026 (C-H, aromatic); 2860 (CH2); 2219 (CN); 1700 (C=O); 1689 (C=O); 1646 (C=C). 1H-NMR (500 MHz, DMSO-d6, TMS): δ 0.88 (s, 3H, CH3-19); 1.18 (s, 3H, CH3-18); 5.21 (s, 1H, CH-4 of progesterone) 5.70(s,1H, pyridine proton); 7.70-7.43 (m, 5H, 5 aromatic protons); 8.10 (s,1H,NH, D2O exchangeable). 13C-NMR (125 MHz, DMSO-d6, TMS): δ 18.4 (C-16); 20.0(C-18); 20.5(C-19); 20.9(C-15); 21.0 (C-15); 28.8 (C-1); 29.1(C-8); 30.6 (C-7); 31.4 (C-12); 32.5 (C-6); 37.5 (C-13); 38.2 (C-2); 39.0 (C-10); 42.2 (C-9); 46.0 (C-14); 50.2 (C-17); 95.5, 102.2, 141.4, 162.7,169.2 (C-pyridine); 117.2 (CN); 119.8 (C-4); 126.5, 127.1, 128.9, 134.7 (C-phenyl); 162.3 (C-5); 197.5 (C-3). Anal. Calcd. For C30H26N2O2: C, 79.80%; H, 7.34%; N, 6.00%; Found: C, 79.73%; H, 7.22%; N, 5.93%.
NMR (270 MHz, DMSO-d$_6$) proton): 4.49 (s, 1H, CH-5 of pyran proton); 5.70 (s, 1H, CH-4 of progesterone); 6.61 (s, 2H, NH);

Orange crystals from EtOH. Yield (73%), mp. 110 ºC. Ms (EI) m/z (%): 468 [M$^+$, 83.2%]; IR (KBr, cm$^{-1}$): 3347 (NH$_2$), 3026 (C-H, aromatic); 2962 (CH$_3$); 3016 (NH$_2$); 2219 (CN); 1698 (C=O); 1640 (C=C), 1222 (O-C). $^1$H-NMR (270 MHz, DMSO-d$_6$, TMS): δ 0.88 (s, 3H, CH$_3$-19); 1.18 (s, 3H, CH$_3$-18); 3.91 (s,1H,CH-4 of pyran proton ); 4.42 (s,1H,CH-5 of pyran proton); 5.77 (s, 1H, CH-4 of progesterone); 6.82 (s, 2H, NH$_2$), D$_2$O exchangeable), 7.14-7.55 (5H, 5 aromatic protons).

2-Amino-4-(4-chloro-phenyl)-6-(3-oxo-androst-4-ene-17-yl)-4-H- pyran-3-carbonitrile 9b

Orange crystals from EtOH. Yield (73%), mp. 110 ºC. Ms (EI) m/z (%): 468 [M$^+$, 83.2%]; IR (KBr, cm$^{-1}$): 3347 (NH$_2$), 3026 (C-H, aromatic); 2962 (CH$_3$); 3016 (NH$_2$); 2219 (CN); 1698 (C=O); 1640 (C=C), 1222 (O-C). $^1$H-NMR (270 MHz, DMSO-d$_6$, TMS): δ 0.88 (s, 3H, CH$_3$-19); 1.18 (s, 3H, CH$_3$-18); 4.01 (s,1H,CH-4 of pyran proton); 4.49 (s,1H,CH-5 of pyran proton); 5.77 (s, 1H, CH-4 of progesterone); 6.82 (s, 2H, NH$_2$), D$_2$O exchangeable), 6.90 (dd, 2H, aromatic protons); 7.03 (dd, 2H, aromatic protons). $^{13}$C-NMR (125 MHz, DMSO-d$_6$, TMS): δ 16.1 (C-16), 20.2 (C-18), 20.6 (C-19), 21.9 (C-15), 22.1 (C-11), 28.4 (C-1), 28.9 (C-8), 30.4 (C-7), 32.2 (C-6), 31.9 (C-12), 38.9 (C-2), 37.1 (C-13), 39.7 (C-10), 42.9 (C-9), 46.5 (C-14), 49.8 (C-17), 117.6 (C(4), 119.7 (C-4), 125.6, 127.9, 128.4, 142.4 (C-phenyl), 29.4, 58.1, 119.9, 158.4, 159.9 (C- pyran ring), 161.2 (C-5), 196.6 (C-3). Anal. Calcd. For C$_{31}$H$_{30}$N$_2$O$_2$: C, 79.50%; H, 7.70%; N, 5.90%; Found: C, 79.42%; H, 7.62%; N, 5.81%.

2-Amino-4-(2,5-dimethoxyphenyl)-6-(3-oxo-androst-4-ene-17-yl)-4-H- pyran-3-carbonitrile 9c

Yellow crystals from Benzene. Yield (68%), mp. 78 ºC. Ms (EI) m/z (%): 528 [M$^+$, 65.0%]; IR (cm$^{-1}$): 3340 (NH$_2$), 3016 (C-H, aromatic); 2870 (CH$_3$); 2210 (CN); 1698 (C=O); 1645 (C=C), 1225 (O-C). $^1$H-NMR (270 MHz, DMSO-d$_6$, TMS): δ 0.78 (s, 3H, CH$_3$-19); 1.14 (s, 3H, CH$_3$-18); 3.76 (s, 6H, 2OCH$_3$); 3.89 (s,1H,CH$_3$-pyran proton); 4.41 (s,1H,CH$_5$-pyran proton); 5.65 (s, 1H, CH-4 of progesterone); 6.62 (s, 2H, NH$_2$),D$_2$O exchangeable), 6.77-7.05 (m, 3H, 3 aromatic protons). Anal. Calcd. For C$_{35}$H$_{30}$N$_2$O$_4$: C, 74.91%; H, 7.60%; N, 5.30%; Found: C, 74.82%; H, 7.52%; N, 5.22%.

4-(4-chloro-phenyl)-6-(3-oxo-androst-4-ene-17-yl)-2-phenyl-4-H- pyran-3-carbonitrile 10a

Yellow crystals from EtOH. Yield (69%), mp. 144 ºC. Ms (EI) m/z (%):565[M$^+$, 3.6%]; 563[M$^+$,10.5%]; IR (KBr, cm$^{-1}$): 3016 (C-H, aromatic); 2950 (CH$_3$); 2221 (CN); 1700 (C=O); 1641 (C=C), 1214 (O-C). $^1$H-NMR (270 MHz, DMSO-d$_6$, TMS): δ 0.84 (s, 3H, Me-19); 1.08 (s, 3H, -CH$_3$-18); 3.87(s,1H,CH-4 of pyran proton); 4.34( s, 1H, CH-5
of pyran proton; 5.60 (s, 1H, CH-4 of progesterone); 7.12-7.21 (m, 4H, 4 aromatic protons); 7.38-7.49 (m, 5H, phenyl protons). Anal. Calcd. For C_{37}H_{35}ClNO2: C, 78.80%; H, 6.80%; N, 2.50%; Cl, 6.28%. Found: C, 78.72%; H, 6.75%; N, 2.43%; Cl, 6.12%.

4-(2, 5-Dimethoxyphenyl)-6-(3-oxo-androst-4-ene-17-yl)-2-phenyl-4H-pyran-3-carbonitrile 10b

Brown crystals from Benzene. Yield (62%), mp. 70 ºC. Ms (EI) m/z, (%): 494 [M-H] -17-[6-(4-Chlorophenyl)-2-thioxo-1, 2-dihydro-pyrimidin-4-yl]-3-oxo-androst-4-ene 12d

4-(2, 5-Dimethoxyphenyl)-6-(3-oxo-androst-4-ene-17-yl)-2-phenyl-4H-pyran-3-carbonitrile 10b

Brown crystals from Benzene. Yield (62%), mp. 70 ºC. Ms (EI) m/z, (%): 494 [M-H] -17-[6-(4-Chlorophenyl)-2-thioxo-1, 2-dihydro-pyrimidin-4-yl]-3-oxo-androst-4-ene 12d

By a suspension of progesterone I (0.314g, 0.001mmol) in freshly prepared Sodium ethoxide, in absolute ethanol the aldehyde (0.001mmol), urea, thiourea and guanidine (0.001mmol) were added. The reaction mixture was heated under reflux for 3-5 h., until the starting materials had disappeared as indicated by TLC, and then poured into ice-water. The solid product so formed dried and crystallized from the appropriate solvent.

6-(4-Chloro-phenyl)-4-(3-oxo-androst-4-ene-17yl)-1H-pyrimidin-2-one 12a

Yellow crystals from Benzene. Yield (82%), m.p. 80 ºC. Ms (EI) m/z, (%): 476 [M-H] -17-[6-(4-Chlorophenyl)-2-thioxo-1, 2-dihydro-pyrimidin-4-yl]-3-oxo-androst-4-ene 12d

6-(4-Chloro-phenyl)-4-(3-oxo-androst-4-ene-17yl)-1H-pyrimidin-2-one 12a

Yellow crystals from MeOH. Yield (77%), m.p. 170 ºC. Ms (m/z, %): 432 [M-H] -17-[6-(4-Chlorophenyl)-2-thioxo-1, 2-dihydro-pyrimidin-4-yl]-3-oxo-androst-4-ene 12d

4-(3-oxo-androst-4-ene-17yl)-6-furan-2-yl-1H-pyrimidin-2-one 12b

Yellow crystals from MeOH. Yield (76%), m.p. 148 ºC. Ms (m/z, %): 494 [M-H] -17-[6-(4-Chlorophenyl)-2-thioxo-1, 2-dihydro-pyrimidin-4-yl]-3-oxo-androst-4-ene 12d

17-[6-(4-Chlorophenyl)-2-thioxo-1, 2-dihydro-pyrimidin-4-yl]-3-oxo-androst-4-ene 12c

Yellow crystals from MeOH. Yield (76%), m.p. 148 ºC. Ms (m/z, %): 494 [M-H] -17-[6-(4-Chlorophenyl)-2-thioxo-1, 2-dihydro-pyrimidin-4-yl]-3-oxo-androst-4-ene 12d

17-(6-Furan-2-yl-2-thioxo-1, 2-dihydro-pyrimidin-4-yl)-3-oxo-androst-4-ene 12d

General procedure: Synthesis of progesterone pyrimidine derivatives of 12a-d and 13a,b

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Pale yellow crystals from MeOH. Yield (63%), m.p. 160 °C. Ms (m/z, %): 448 [M$$^+$$], 23.7%], 372 [M$$^-$$-furan, 46%], IR (KBr, cm$$^{-1}$$): 3130 (NH), 3090 (C-H, aromatic); 2924 (CH$$_3$$); 1698 (C=O); 1645 (C=C); 1603 (C=N); 1195 (C=S). 1H-NMR (500 MHz, DMSO-d$$_6$$, TMS): δ 0.78 (s, 3H, CH$$-$$19); 1.18 (s, 3H, CH$$-$$18); 5.62 (s, 1H, CH-4, of progesterone), 6.62 (1H, pyrimidine ring); 6.95 (d, 1H, furan proton), 7.35 (t, 1H, furan proton), 7.83 (d, 1H, furan proton), 10.01 (s, 1H, NH, D$$O$$ exchangeable). Anal. Calcd. For C$$_{27}$$H$$_{22}$$N$$O$$S: C, 72.29%; H, 7.19%; N, 6.24%.

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17-[2-Amino-6-(4-chlorophenyl)-pyrimidin-4-yl] - 3-oxo-androst-4-ene 13a

White crystals from MeOH. Yield (70%), m.p. 118 °C. Ms (m/z, %): 477[M$$^+$$], 6.3%]; 475[M$$^-$$, 18.2%]; 364[M$$^-$$-P\text-CIC}_2H_4, 47.1%]; IR (KBr, cm$$^{-1}$$): 3363 (NH$$_2$$), 3085 (C-H, aromatic); 2954 (CH$$_3$$); 1700 (C=O); 1645 (C=C); 1609 (C=N). 1H-NMR (500 MHz, DMSO-d$$_6$$, TMS): δ 0.87 (s, 3H, CH$$-$$19); 1.05 (s, 3H, CH$$-$$18); 5.59 (s, 1H, CH-4, of progesterone), 6.52 (s, 1H, NH$$_2$$, D$$O$$ exchangeable ), 6.54 (s, 1H, pyrimidine ring), 7.46 (d, 2H, aromatic protons); 7.71 (d, 2H, aromatic protons). 13C-NMR (125 MHz, DMSO-d$$_6$$, TMS): δ 20.0 (C-18); 22.5 (C-11); 22.8 (C-19); 25.0(C-16); 26.6 (C-15); 31.3 (C-7); 32.3 (C-6); 34.0 (C-2); 35.0 (C-1); 35.5 (C-8); 36.2 (C-12); 37.2 (C-10); 42.1 (C-13); 50.1 (C-9); 51.2 (C-17); 55.5 (C$$-$$14); 123.8 (C-4); 128.8, 129.0 131.4, 134.7 (C-aromatic); 98. 6, 159.9, 162.4, 165.0 (C-pyrimidine); 170.0 (C$$-$$5), 198.7 (C-3). Anal. Calcd. For C$$_{29}$$H$$_{23}$$ClN$$O$$: C, 73.17%; H, 7.20%; N, 8.83%; Cl, 7.45%. Found: C, 73.05%; H, 7.02%; N, 8.71%; Cl, 7.30%.

17-(2-Amino-6-furan-2-yl-pyrimidin-4-yl)-3-oxo-androst-4-ene 13b

Yellow crystals from Benzene. Yield (77%), m.p. 190 °C. Ms (m/z, %): 431 [M$$^+$$, 39.0%]; IR (KBr, cm$$^{-1}$$): 3351 (NH$$_2$$), 3092 (C-H, aromatic); 2924 (CH$$_3$$); 1699 (C=O); 1645 (C=C); 1611 (C=N). 1H-NMR (500 MHz, DMSO-d$$_6$$, TMS): δ 0.89 (s, 3H, Me-19); 1.14 (s, 3H, -CH$$-$$18); 5.62 (s, 1H, CH-4, of progesterone), 6.30 (s, 2H, NH$$_2$$, D$$O$$ exchangeable ), 6.62 (s, 1H, pyrimidine ring); 6.92 (t, 1H, furan ring), 7.31 (d, 1H, furan ring), 7.83 (d, 1H, furan ring). Anal. Calcd. For C$$_{27}$$H$$_{22}$$N$$O$$: C, 75.14%; H, 7.71 %; N, 9.74%; Found: C, 75.01 %; H, 7.15 %; N, 9.22%.

17-(1-Hydrazonoethyl)-10-(androst-4-ene-3-ylidene) hydrzone 14

To a solution of progesterone 1 (0.314 g, 0.001 mol) added hydrazine hydrate 98% (0.05 g, 0.001 mol) in ethanol and refluxed for 1 h, the solvent was evaporated under reduced pressure and the residue was purified with the proper solvent.

White crystals from EtOH. Yield (90%), mp. 180 °C. Ms (m/z, %): 342 [M$$^+$$, 34.5%]; IR (KBr, cm$$^{-1}$$): 3343 (NH$$_2$$), 2872 (CH$$_3$$), 1647 (C=C), 1043(C=N). 1H-NMR (270 MHz, DMSO-d$$_6$$, TMS): δ 0.81 (s, 3H, Me-19); 0.90 (s,3H, H$$_3$$C=NNH$$_2$$); 1.09 (s, 3H, -CH$$-$$18); 5.86 (s, 2H, NH$$_2$$, D$$O$$ exchangeable); 5.61 (s, 1H, CH-4, of progesterone). Anal. Calcd. For C$$_{27}$$H$$_{22}$$N$$O$$: C, 73.60%; H, 10.10%; N, 16.40%; Found: C, 73.55%; H, 10.02%; N, 16.36%.

17-(1-(2-phenylcarbamothioyl)hydrazono)ethyl)androst-4-ene-3-ylidine–N-phenylhydrazine-carbothioamide 15

To a solution of 14 (0.150 g, 0.001 mol) in ethanol (20 ml) phenyl isocyanate (0.135 g, 0.001 mol) was added. The reaction mixture was heated under reflux for about 4 h, then cooling it to the room temperature and filters the product which crystallized from (EtOH).

Yellow crystals from EtOH. Yield, 71%, mp. 139 °C. Ms (m/z, %): 612 [M$$^+$$, 25.0%]; IR (KBr, cm$$^{-1}$$): 3279 (br,NH), 3016 (C-H, Aromatic), 2922 (CH$$_3$$); 1595 (C=N), 1177 (C=S). 1H-NMR (270 MHz, DMSO-d$$_6$$, TMS): δ 0.86 (s, 3H, Me-19); 0.91(s, 3H, CH$$_3$$C=NNH); 1.11 (s, 3H, -CH$$-$$18); 5.40 (s, 1H, CH-4, of progesterone); 6.64-7.11 (m, 10H, phenyl protons); 9.31 (s,1H, NNH, D$$O$$ exchangeable), 9.36 (s, 1H, NH$$_2$$, D$$O$$ Exchangeable), 9.76(s, 1H, NH$$Ph$$, D$$O$$ Exchangeable), 10.01 (s, 1H, NH$$Ph$$, D$$O$$ Exchangeable). Anal. Calcd. For C$$_{37}$$H$$_{34}$$N$$O$$S$$_2$$: C, 68.60%; H, 7.20%; N, 13.70%; Found: C, 68.52%; H, 7.03%; N, 13.61 %.

Cyclopent[a]phenanthren-3-hydrazinyl-2-(phenylamino)-1, 3-oxathiolan-5-one 16
To a solution of 15 (0.600 g, 0.001 mol) in benzene (20 ml), chloroacetic acid (0.094 g, 0.001 mol) was added. The reaction mixture was heated under reflux for about 3 h. Then cooled to the room temperature and filtered the product was crystallized from benzene.

Yellow crystal from benzene. Yield 67%, mp. 162 º C. Ms (m/z, %): 728 [M+], 45.0%; IR (KBr, cm⁻¹): 3426 (br, NH); 3016 (C-H, Aromatic), 2922 (CH₃); 2849 (CH₂); 1726 (C=O, lactone), 1645 (C=C); 1600 (C=N); 1459 (CH₂ bending).

1H-NMR (270 MHz, DMSO-d₆, TMS): δ 0.88 (s, 3H, Me-19); 0.91 (s, 3H, CH₃-CH=N); 1.09 (s, 3H, -CH₃-18); 4.04 (d, 4H, 2CH₂-C=O); 4.28 (s, 2H, 2NH-Ph, D₂O exchangeable); 5.83 (s, 1H, CH-4, progesterone); 7.31-7.64 (m, 12H, 10 aromatic protons +2 NH lactone).

13C-NMR (125 MHz, DMSO-d₆, TMS): δ 20.5 (C-18), 22.2 (C-11), 22.7 (C-19), 23.4 (C-16), 26.6 (C-2), 27.1 (C-15), 28.4 (CH₂-lactone), 31.4 (C-7), 33.0 (C-6), 35.1 (C-8), 37.1 (C-12), 37.5 (C-17), 38.5 (C-10), 39.1 (C-1), 42.0 (C-13), 50.1 (C-9), 56.0 (C-14), 111.5 (C-4), 113.2, 117.2, 129.0, 147.6 (C-phenyl), 128.3 (C-lactone), 155.3 (C-3), 164.6 (CH₃-CH=N), 166.4 (C-5), 172.3 (C=O- lactone).

Bioassay

Cytotoxic effect on human cell lines (HEPG2 & MCF7)

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide] to purple formazan²⁶.

Procedure:

All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, and Sanford, ME, USA). Cells were batch cultured for 10 days, then seeded at concentration of 10x10³ cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37 ºC for 24 h under 5% CO₂ using a water jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of sample to give a final concentration of (100.00 - 50.00 - 25.00 - 12.50 - 6.25 - 3.13 - 0.78 and 1.56 µg/ml). Cells were suspended in RPMI 1640 medium (for HePG2 and MCF7), 1% antibiotic-antimycotic mixture (10,000 µg/ml Potassium Penicillin, 10,000 µg/ml Streptomycin Sulphate and 25 µg/ml Amphotericin B) and 1% L-glutamine in 96-well flat bottom micro plate at 37 ºC under 5% CO₂. After 48 h of incubation, medium was aspirated, 40 µl MTT salt (2.5 μg/ml) were added to each well and incubated for further four hours at 37ºC under 5% CO₂. To stop the reaction and dissolving the formed crystals, 200 μL of 10% Sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37°C. A positive control composed of 100 µg/ml was used as a known cytotoxic natural agent gives 100% lethality under the same conditions.²⁷

The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595nm and a reference wavelength of 620 nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts and its final concentration on the cells was less than 0.2%.

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The percentage of change in viability was calculated according to the formula:

\[ \text{((Reading of extract / Reading of negative control) -1) x 10.} \]

A probit analysis was carried for LC₅₀ and LC₉₀ determination using SPSS 11 program.

2. Results and discussion:

A simple one pot multicomponent reaction technique was utilized²⁴, for the synthesis of novel biologically active heterocyclic steroids. A mixture of progesterone 1, aromatic aldehyde 2 and malononitrile 3 were heated in absolute ethanol containing ammonium acetate under reflux to afford the corresponding aminoprogestanopyridine derivatives 5a-e (Scheme 1).
The formation of adducts 5a-c can be rationalized by initial formation of arylidene malononitrile 4 as fleeting intermediate via standard Knoevenagel condensation. This is followed by Michael type addition of progesterone 1 to the activated double bond of the arylidene that furnished the adduct product to cyclize via intramolecular rearrangement yielding the desired product 5 (Scheme 2).
The most important feature to confirm the aromatic structure of compound 5a was the absence of hydrogen proton in 1H-NMR spectrum of C-4 and NH of pyridine ring. It showed D₂O exchangeable of NH₂ at δ 6.61 ppm. All the other analytical and spectroscopic data were in accordance with the suggested structure 5 (cf. exp. section).

To generalize such methodology and synthesize other different pyridine derivatives, the previous reactions were conducted using other methylene reagents like ω-cyano-acetophenone and ethylcyanoacetate to form the corresponding pyridine derivatives 6a-c and 7a, b respectively (Scheme 3).

The structures of these products were assigned based on the elemental analysis and other spectroscopic data. The IR spectra showed the presence of the nitrile group at 2219 cm⁻¹ and the pyridinone carbonyl group ν 1689 cm⁻¹, respectively in 7a, as an example. On the other hand the 1H-NMR revealed the absence of the characteristic signals of the ester ethyl and the amino groups (c.f.exp. section).

It is believed that compound 8 was not formed due to steric effect because ester group is more bulky than cyano group, so the formation of 7 is more stable.

Carrying out the previous reaction in the presence of piperidine instead of ammonium acetate led to the formation of the aminoprogesteno pyran derivatives 9 and 10 respectively (Scheme 4). All the micro-analysis and spectroscopic data were in accordance with the suggested structures 9a-c and 10a, b (c.f. exp. section).
For more utility of the previous phenomena to synthesize different active heterocyclic steroids; urea, thiourea and guanidine were used. A mixture of progesterone $1$, aldehydes and nitrogen reagents $11$ and guanidine were allowed to react in freshly prepared sodium methoxide to form the corresponding pyrimidine derivatives $12a-d$ and $13a, b$ respectively (Scheme 5).

Structure elucidation of $12a$ was derived from its spectral data; IR spectrum reflected characteristic bands to the pyrimidine carbonyl group at $\nu$ 1659 cm$^{-1}$ and NH band at $\nu$ 3158 cm$^{-1}$. The $^1$H-NMR showed a singlet at $\delta$ 6.97 ppm of pyrimidine proton and D$_2$O exchangeable signals at $\delta$ 7.91 ppm for the NH group.

Furthermore, this study was extended to include the behaviour of progesterone $1$ towards hydrazine hydrate to produce the hydrazone derivative $14$ which was confirmed with analytical and spectroscopic data. Compound $14$ was allowed to react with phenyl isothiocyanate to produce the corresponding acyclic structure $15$. (Scheme 6)

The $^1$H-NMR of adduct $15$ reflect the existence of the aromatic protons beside 4NH D$_2$O exchangeable signals at $\delta$ 9.31, 9.36, 9.76, 10.01, respectively (c.f., exp. Section).
The thiobenzoyl adduct 15 was allowed to react with chloroacetic acid in boiling benzene afforded the lactone 16 adduct via SN2 mechanism on alkyl moiety rather than carboxyl group (chlorine atom is good leaving group) followed by cyclization on the activated C=N yielded the desired product 16 (Scheme 7).
The IR spectrum of compound 16 exhibits strong absorption bands at ν 1600, 1726 cm⁻¹ due to C=N and C=O of γ-lactone⁶ (the lower frequency of carbonyl of lactone is due to transannular effect of sulphur atom through the ring). The ¹H-NMR spectrum of compound 16 when run in DMSO (d₆) showed the two singlets appearing at 4.04 & 4.28 ppm respectively attributed to CH₂ lactone and NHPh. The ¹³C-NMR explains the chiral carbon at δ128.3 ppm (c.f., exp. Section).

**Bioactivity**

**Antitumor activity**

Cytotoxicity of Pyridine, Pyran, and Pyrimidine derivatives 5a, 5b, 6b, 7a, 9b, 9c, 12a, 12c, and 13a were bioassayed against two human tumour cell lines namely hepatocellular carcinoma cell line (HEPG2) and Caucasian...
breast adenocarcinoma (MCF7) at Bioassay-Cell Culture Laboratory, National Research Centre, Egypt. LC$_{50}$ of promising compounds were calculated after 48 h of continuous drug exposure as shown in Table (1).

Table (1): Cytotoxic activity of some new pyridine, pyran, and pyrimidine derivatives against HEPG2 and MCF7

<table>
<thead>
<tr>
<th>Code sample</th>
<th>LC$_{50}$ (µg/mL)</th>
<th>HEPG2</th>
<th>MCF7</th>
</tr>
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<tbody>
<tr>
<td>Standard (Doxorubicin)</td>
<td></td>
<td>22.6</td>
<td>42.5</td>
</tr>
<tr>
<td>5a</td>
<td>55.2</td>
<td>49.8</td>
<td></td>
</tr>
<tr>
<td>5b</td>
<td>58.7</td>
<td>51.4</td>
<td></td>
</tr>
<tr>
<td>6b</td>
<td>-----</td>
<td>78.8</td>
<td></td>
</tr>
<tr>
<td>7a</td>
<td>19.6</td>
<td>22.3</td>
<td></td>
</tr>
<tr>
<td>9b</td>
<td>71.8</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>9c</td>
<td>-----</td>
<td>84.5</td>
<td></td>
</tr>
<tr>
<td>12a</td>
<td>12.7</td>
<td>22.6</td>
<td></td>
</tr>
<tr>
<td>12c</td>
<td>-----</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>13a</td>
<td>12.2</td>
<td>8.5</td>
<td></td>
</tr>
</tbody>
</table>

The results displayed in Table (1), the values of LC50 of the compounds 13a, 12a and 7a have high cytotoxic effect more than doxorubicin the chosen anticancer drug in both cell lines. Regarding HEPG2 cell line, LC$_{50}$ values are nearly half that of the standard and their LC$_{50}$ are 12.2 and 12.7 µg/ml for 13a, 12a respectively and 19.6 µg/ml for 7a. While compounds 9b, 5a and 5b have moderate exhibit activity against same cell line with LC$_{50}$ are 71.8, 55.2 µg/ml and 58.7 µg/ml, respectively. Also, the results indicate that compounds 6b, 9c and 12c have no activity against liver anticancer cells (Fig. 1). Bio-assayed antitumor activity of the tested compounds against breast cancer cell lines MCF7. The results indicated that compounds 13a, 7a, 12a have highest anticancer activity in comparison with that of the standard drug since its LC$_{50}$ is 8.5, 22.3, and 22.6 µg/ml, respectively, while that of the standard equal to 42.5 µg/ml. On the other hand, compounds 5a, 6b, and 9c showed very low breast anticancer activity, while the 12c and 9b compounds are of no cytotoxicity (Fig. 2). Therefore, it could be concluded that compounds 13a, 12a, and 7a have high cytotoxic activity against both types of liver and breast cancer cells subjected in this study. The above mentioned results revealed that the materials under investigation have killing potency to the cancer cells with low concentrations and can be arranged in the following order: progesterone pyrimidine > pyridine > pyran. This may be attributable to the presence of nitrogen moiety in the ring.
References:


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