SUPPORTING INFORMATION

Synthesis and Biological Activity of 7-Phenyl-6,9-dihydro-3*H*-pyrrolo[3,2-*f*]quinoline-9-ones: a New Class of Antimitotic Agents Devoid of Aromatase Activity

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Contents: procedures for the synthesis of compounds **1a-f, 2, 5, 6-15**, yields, chemical-physical properties (Rf, mp), and spectroscopic data of all synthesized compounds (IR, ^{1}H NMR, ^{13}C NMR), HRMS, the table of elemental analyses of all target compounds **16-26,** and the biological experimental section.

Instruments and starting materials

Melting points were determined on a Gallenkamp MFB 595 010M/B capillary melting point apparatus, and are not corrected. Infrared spectra were recorded on a Perkin-Elmer 1760 FTIR spectrometer using potassium bromide pressed disks; all values are expressed in cm^{-1}. UV-vis spectra were recorded on a Perkin-Elmer Lambda UV/VIS spectrometer. ^{1}H NMR spectra were recorded on a Bruker (300 MHz) spectrometer, using the indicated solvents; chemical shifts are reported in δ (ppm) downfield from tetramethylsilane as internal reference. Coupling constants are given in Hertz. In the case of multiplets, chemical shift was measured starting from the approximate center. Integrals were satisfactorily in line with those expected on the basis of compound structure. Elemental analyses were performed in the Microanalytical Laboratory, Department of Pharmaceutical Sciences, University of Padova, using a Perkin-Elmer elemental analyzer model 240B; results fell in the range of calculated values ± 0.4%. The analytical data are presented in detail for each final compound. Mass spectra were obtained on a Mat 112 Varian Mat Bremen (70Ev) mass spectrometer and Applied Biosystems Mariner System 5220 LC/Ms (nozzle potential 250.00). Column flash chromatography was performed on Merck silica gels (250-400 mesh ASTM); chemical reactions were monitored by analytical thin-layer chromatography (TLC) using Merck silica gel 60 F-254 glass plates with a 9:1 dichloromethane/methanol mixture as eluant, unless otherwise specified.

Solutions were concentrated in a rotary evaporator under reduced pressure. Starting materials were purchased from Aldrich Chimica, and Fluka Riedel-de Haen ($TiCl_3$, 15% HCl solution) and solvents from Carlo Erba, Fluka and Lab-Scan. DMSO was made anhydrous by distillation under vacuum and stored on molecular sieves.

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General procedure for synthesis of ethyl substituted-benzoyl-acetates[24] **(1a-f).** A 250-mL two-necked, round-bottomed flask equipped with a magnetic stirrer was fitted with a 100 mL pressure-equalizing constant-rate dropping funnel and a condenser, the top of which was connected to a mineral oil trap, to prevent air from entering the vessel during reactions and to monitor gas development. In the flask were subsequently placed 3 g (75 mmol) of sodium hydride (60% in mineral oil), which were then washed 4 times with 20-mL aliquots of benzene. Following the addition of 100 mL of fresh benzene and then 6.1 mL (d = 0.975, 50 mmol) of anhydrous diethyl carbonate, the mixture was heated under stirring. When it reached boiling point, a solution of commercial phenylethanones (3.4-5.6 g, 25 mmol) in benzene was added drop-wise, slowly (1 hour). When this addition was complete, the reaction mixture was refluxed until all hydrogen gas formation stopped. On cooling, a pasty, pink solid product separated; this was dissolved by adding 15 mL of glacial acetic acid drop-wise, followed by 45 mL ice-cold water. Once the organic layer was separated, the aqueous one was extracted with benzene, and the organic extract washed with cold water, dried with anhydrous Na_2SO_4 and evaporated. Distillation under vacuum was needed in order to remove completely the un-reacted diethyl carbonate and retrieve the desired product.

Ethyl 3-methoxy-benzoyl-acetate[2] **(1a)**. Dense yellow liquid, yield 60%; Rf 0.49 (n-hexane/ ethyl acetate 8:2); IR (NaCl) 1741 (CO), 1686 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.17 (t, 3H, J = 7.06 Hz, CH_3), 3.82 (s, 3H, OCH_3), 4.12 (q, 2H, J = 7.12 Hz, OCH_2), 4.18 (s, 2H, CH_2), 7.24 (dd, 1H, $J_{4',5'}$ = 8.29 Hz , $J_{4',6'}$ and $J_{4',2'}$ = 2.38 Hz, H-4'), 7.45 (m, 2H, H-2' and H-5') and (t, 1H, $J_{5',4'}$ e $J_{5',6'}$ = 7,25 Hz, H-5'), 7.55 (d, 1H, $J_{6',5'}$ = 7,82 Hz, H-6'); HR MS [MH^+] 223.245.

Ethyl 3-methyl-benzoyl-acetate (1b). Dark orange liquid, yield 96%; Rf 0.69 (eluant n-hexane/ ethyl acetate 8:2); IR (NaCl) 1737 (CO), 1665 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.17 (t, 3H, J = 7.91 Hz, CH_3), 2.37 (s, 3H, CH_3), 4.12 (q, 2H, J = 7.44 Hz, CH_2), 4.16 (s, 2H, CH_2), 7.42 (t, 1H, $J_{5,4}$ = $J_{5,6}$ = 7.25 Hz, H-5), 7.48 (dt, 1H, $J_{5,4}$ = 7.63 Hz, H-4), 7.76 (m, 2H, $J_{6,5}$ = 8.39 Hz, H-6 e H-2); HR MS [MH^+] 207.247.

Ethyl 3-bromo-benzoyl-acetate (1c). Dark orange liquid, yield 99%; Rf 0.56 (eluant n-hexane/ ethyl acetate 8:2); IR (NaCl) 1741 (CO), 1623 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.17 (t, 3H, J = 7.06 Hz, CH_3), 4.12 (q, 2H, J = 7.06 Hz, CH_2), 4.22 (s, 2H, CH_2), 7.52 (d, 1H, $J_{5,4}$ = $J_{5,6}$ = 8.01 Hz, H-5), 7.88 (ddd, 1H, $J_{6,5}$ = 8.01 Hz, $J_{6,4}$ = $J_{6,2}$ = 1.95 Hz, H-6), 7.95 (d, 1H, $J_{4,5}$ = 8.01 Hz, H-4), 8.10 (dd, 1H, $J_{2,4}$ = $J_{2,6}$ = 1.95 Hz, H-2); HR MS [MH^+] 272.118.

Ethyl 3-cyano-benzoyl-acetate (1d). Pale yellow solid, yield 20%; mp 75-77°C; Rf 0.37 (eluant n-hexane/ethyl acetate 8:2); IR (NaCl) 2225 (CN), 1739 (CO), 1665 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.17 (t, 3H, J = 7.06 Hz, CH_3), 4.12 (q, 2H, J = 7.06 Hz, CH_2), 4.27 (s, 2H, CH_2), 7.68 (t, 1H, $J_{5,4}$ = $J_{5,6}$ = 8,05 Hz, H-5), 8.24 (ddd, 1H, $J_{6,5}$ = 8.05 Hz, H-6), 7.91 (ddd, 1H, $J_{4,5}$ = 8.05 Hz, $J_{4,6}$ = $J_{4,2}$ = 1.91 Hz, H-4), 8.10 (dd, 1H, $J_{2,4}$ = $J_{2,6}$ = 1.91 Hz, H-2); HR MS [MH^+] 218.235.

Ethyl 4-cyano-benzoyl-acetate (1e). Yellow solid, yield 32%; mp 40-46°C; Rf 0.41 (eluant n-hexane/ ethyl acetate 8:2); IR (NaCl) 2219 (CN), 1743 (CO), 1665 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.17 (t, 3H, J = 7.06 Hz, CH_3), 4.12 (q, 2H, J = 7.06 Hz, CH_2), 4.27 (s, 2H, CH_2), 8.04 (d, 2H, $J_{3,2}$ = $J_{5,6}$ = 8.39 Hz, H-3 and H-5), 8.10 (d, 2H, $J_{2,3}$ = $J_{6,5}$= 8.39 Hz, H-2 and H-6); HR MS [MH^+] 218.233.

Ethyl thienyl-acetacetate[2] **(1f)**. Distillate at 130°C, 0.6 mm Hg; dense, pale orange liquid, yield 89%; Rf 0.38 (eluant n-hexane/ethyl acetate 8:2); IR (NaCl) 1738 (CO), 1625 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.18 (t, 3H, J = 6.99 Hz, CH_3), 4.12 (q and s, 4H, J = 6.99 Hz, CH_2 and OCH_2), 7.27 (dd, 1H, $J_{4,3}$ and $J_{4,5}$ = 4.04 Hz, H-4), 7.99 (dd, 1H, $J_{5,4}$ = 3.81 Hz and $J_{5,3}$ = 1.14 Hz, H-5), 8.07 (dd, 1H, $J_{3,4}$ = 4.96 Hz and $J_{3,5}$ = 1,14 Hz, H-3); HR MS [MH^+] 199.235.

Synthesis of 1-(2-diethylamino-ethyl)-5-nitro-1*H*-indole (2). A solution of commercial 5-nitro-indole (1g, 6.2 mmol) in 20 mL acetone was added to powdered KOH (1.728g, 30.8 mmol) and then an excess of chloroethyl-diethylamine hydrochloride (2.123g, 12.3 mmol). The stirred mixture was heated at 40°C for 3 h. After cooling at room temperature, 200 mL toluene was added and the suspension was left under stirring for 30 minutes. The insoluble solid was then filtered off and the

filtrate was washed several times with NaCl-saturated water, dried with anhydrous Na_2SO_4 and evaporated, yielding an oily reddish-orange product. Yield 90%; Rf 0.17 (chloroform/ethyl acetate 1:1); ^{1}H NMR (DMSO-d_6) δ 0.81 (t, 6H, J = 7.06 Hz, 2 CH_3), 2.44 (q, 4H, J = 7.06 Hz, 2 CH_2), 2.72 (t, 2H, J = 6.00 Hz, CH_2), 4.28 (t, 2H, J = 6.00 Hz, CH_2), 6.72 (d, 1H, $J_{3,2}$ = 3.05 Hz, H-3), 7.64 (d, 1H, $J_{2,3}$ = 3.35 Hz, H-2), 7.67 (d, 1H, $J_{7,6}$ = 9.35 Hz, H-7), (dd, 1H, $J_{6,7}$ = 9.35 Hz and $J_{6,4}$ = 2.48 Hz, H-6), 8.54 (d, 1H, $J_{4,6}$ = 2.48 Hz, H-4); HR MS [MH^+] 262.315.

Synthesis of 1-(2-diethylamino-ethyl)-5-amino-indole (5). A solution of nitroindole **2** (2.3 g, 9.9 mmol) in 150 mL absolute ethanol was dropped into a suspension of 10% Pd/C (125 mg) saturated with H_2 in ethanol 100 mL. The mixture was stirred at a temperature of 50°C and hydrogen at atmospheric pressure for 2-3 h. The catalyst was filtered off and the solution evaporated under reduced pressure to give the corresponding amino-indole as an oily orange substance. Yield 88%; Rf 0.26 (ethyl acetate/methanol 9:1); IR (NaCl) 3442 and 3358 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.89 (t, 6H, J = 7.25 Hz, 2 CH_3), 2.47 (q, 4H, J = 7.25 Hz, 2 CH_2), 2.66 (t, 2H, J = 6.96 Hz, CH_2), 4.06 (t, 2H, J = 6.77 Hz, CH_2), 4.43 (bs, 2H, NH_2), 6.09 (d, 1H, $J_{3,2}$ = 3.24 Hz, HC-3), 6.52 (dd, 1H, $J_{6,7}$ = 8.48 Hz and $J_{6,4}$ = 2.00 Hz, HC-6), 6.67 (d, 1H, $J_{4,7}$ = 2.10 Hz, HC-4), 7.11 (d, 1H, $J_{7,6}$ = 8.58 Hz, HC-7), 7.14 (d, 1H, $J_{2,3}$ = 3.05 Hz, HC-2); HR MS [MH^+] 232.299.

General procedure for synthesis of ethyl 3-substituted-(1*H*-indol-5-ylamino)-acrylates (6-15). In a 50-mL round-bottomed flask, 3-4 mmol of aminoindole **3-5** in 10-20 mL absolute ethanol were condensed with an equimolar quantity of β-keto-ester (prepared **1a-f,** or commercial benzoyl-acetacetate **1g** and 3-nitro-benzoylacetacetate **1h**) and with 1 mL glacial acetic acid and 100 mg drierite. The mixture was refluxed for about 24 h, the reaction being monitored by TLC analysis (dichloromethane/ethyl acetate 9:1). As the reaction did not come to completion, after 24 h the mixture was cooled and filtered to remove the drierite; the resulting solution was evaporated to dryness under vacuum and the residue purified by flash chromatography and re-crystallization from a suitable solvent.

Ethyl 3-phenyl-3-(indol-5-ylamino)-acrylate (6). Yellow crystalline solid, yield 43%; mp 97-100°C; Rf 0.83 (eluant dichloromethane/ethyl acetate 9/1); IR (KBr) 3444 (NH), 1625 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.23 (t, 3H, J = 7.06 Hz, CH3), 4.13 (q, 2H, J = 7.12 Hz, CH_2), 4.82 (s, 1H, CH), 6.22 (m, 1H, H-3), 6.57, (dd, 1H, $J_{6,7}$ = 8.39 Hz, $J_{6,4}$ = 2.10 Hz, H-6), 6.97 (d, 1H, $J_{4,6}$ = 1.72 Hz, H-4), 7.14 (d, 1H, $J_{7,6}$ = 8.39 Hz, H-7), 7.29 (m, 6H, H-2, H-2', H-3', H-4', H-5', H-6'), 10.24 (bs, 1H, NH), 10.99 (bs, 1H, NH); HR Ms [MH^+] 307.325.

Ethyl 3-(m-methoxy-phenyl)-3-(indol-5-ylamino)-acrylate(7). Dark red vitreous solid, yield 43%; mp 54-60°C; Rf 0.87 (eluant dichloromethane/ethyl acetate 9/1); IR (KBr) 3439 (NH), 1625 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.24 (t, 3H, J = 7.34 Hz, CH_3), 3.64 (s, 3H, OCH_3), 4.16 (q, 2H, J = 6.93 Hz, CH_2), 4.99 (s, 1H, CH), 6.01 (d, 1H, $J_{5,6}$ = 7.82 Hz, H-5), 6.51 (m, 1H, H-3), 6.74 (t, 1H, $J_{6,5}$ = 5.34 Hz and $J_{6,7}$ = 8.01 Hz, H-6), 6.91 (m, 3H, H-2', H-6' and H-4'), 7.03 (d, 1H, $J_{7,6}$ = 8.58 Hz, H-7), 7.20 (td, 1H, $J_{5',4'}$ and $J_{5',6'}$ = 8.20 Hz, H-5'), 7.33 (t, 1H, $J_{2,3}$ = 3.91 Hz, H-2), 10.43 (s, 1H, amine NH), 11.19 (s, 1H, indole NH); HR Ms [MH^+] 337.413.

Ethyl 3-(m-methyl-phenyl)-3-(indol-5-ylamino)-acrylate (8). Orange solid, yield 65%; mp 52°-54°C; Rf 0.73 (eluant dichloromethane/ethyl acetate 9/1); IR (KBr) 3442 (NH), 1625 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.22 (t, 3H, J = 6.80 Hz, CH_3), 2.22 (s, 3H, CH_3), 4.12 (q, 2H, J = 7.19 Hz, CH_2), 4.81 (s, 1H, CH), 6.24 (bs, 1H, H-3), 6.57 (dd, 1H, $J_{6,7}$ = 8.67 Hz, $J_{6,4}$ = 1.81 Hz, H-6), 6.99 (d, 1H, $J_{4,6}$ = 1.34 Hz, H-4), 7.14 (m, aryl 5H, H-7, H-2', H-4', H-5', H-6'), 7.26 (dd, 1H, $J_{2,3}$ = $J_{2,1}$ = 3.63 Hz, H-2), 10.23 (bs, 1H, NH), 10.98 (bs, 1H, NH); HR Ms [MH^+] 322.285.

Ethyl 3-(m-bromo-phenyl)-3-(indol-5-ylamino)-acrylate (9). Reddish-orange solid, yield 91%; mp 62°-64°C; Rf 0.90 (eluant dichloromethane/ethyl acetate 9/1); IR (KBr) 3412 (CO), 1625 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.23 (t, 3H, J = 7.25 Hz, CH_3), 4.13 (q, 2H, J = 7.06 Hz, CH_2), 4.85 (s, 1H, CH), 6.26 (m, 1H, H-3), 6.59 (dd, 1H, $J_{6,7}$ = 8.77 Hz, $J_{6,4}$ = 1.91 Hz, H-6), 7.02 (d, 1H, $J_{4,6}$ = 1.72 Hz, H-4), 7.18 (d, 1H, $J_{7,6}$ = 8.77 Hz, H-7), 7.20 (t, 1H, $J_{5',6'}$ = $J_{5,4}$ = 7.25 Hz, H-5'), 7.28 (dd,

1H, $J_{2,1}$ = $J_{2,3}$ = 2.86 Hz, H-2), 7.31 (dd, 1H, $J_{6',5'}$ = 8.39 Hz, H-6'), 7.50 (dd, 1H, $J_{4',5'}$ = 7.28 Hz, $J_{4',6'}$ = 0.57 Hz, H-4'), 7.54 (bs, 1H, H-2'), 10.17 (bs, 1H, NH), 11.02 (bs, 1H, NH); HR Ms [MH^+] 387.383.

Ethyl 3-(m-cyano-phenyl)-3-(indol-5-ylamino)-acrylate (10). Yellow crystalline solid, yield 45%; mp 62-65°C; Rf 0.75 (eluant dichloromethane/ethyl acetate 9/1); IR (KBr) 3439 (NH), 2239 (CN), 1623 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.24 (t, 3H, J = 7.06 Hz, CH_3), 4.14 (q, 2H, J = 7.06 Hz, CH_2), 4.91 (s, 1H, CH), 6.26 (m, 1H, H-3), 6.59 (dd, 1H, $J_{6,7}$ = 8.58 Hz, $J_{6,4}$ = 1.91 Hz, H-6), 7.02 (d, 1H, $J_{4,6}$ = 1.34 Hz, H-4), 7.17 (d, 1H, $J_{7,6}$ = 8.58 Hz, H-7), 7.28 (dd, 1H, $J_{2,3}$ = $J_{2,1}$ = 2.67 Hz, H-2), 7.43 (t, 1H, $J_{5',4'}$ = $J_{5',6'}$ = 7.73 Hz, H-5'), 7.59 (d, 1H, $J_{6',5'}$ = 7.82 Hz, H-6'), 7.76 (d, 1H, $J_{4',5'}$ = 7.82 Hz, H-4'), 7.85 (bs, 1H, H-2'), 10.17 (bs, 1H, NH), 11.04 (bs, 1H, NH); HR Ms [MH^+] 317.283.

Ethyl 3-(p-cyano-phenyl)-3-(indol-5-ylamino)-acrylate (11). Pale yellow solid, yield 18%; mp 85°-87°C; Rf 0.40 (eluant dichloromethane/ethyl acetate 9/1); IR (KBr) 3438 (NH), 2227 (CN), 1621 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.24 (t, 3H, J = 7.06 Hz, CH_3), 4.14 (q, 2H, J = 7.06 Hz, CH_2), 4.89 (s, 1H, CH), 6.25 (m, 1H, H-3), 6.58 (dd, 1H, $J_{6,7}$ = 8.58 Hz, $J_{6,4}$ = 2.10 Hz, H-6), 7.01 (d, 1H, $J_{4,6}$ = 1.53 Hz, H-4), 7.16 (d, 1H, $J_{7,6}$ = 8.39 Hz, H-7), 7.28 (dd, 1H, $J_{2,3}$ = $J_{2,1}$ = 2.76 Hz, H-2), 7.52 (d, 2H, $J_{2',3'}$ = $J_{6',5'}$ = 8.20 Hz, H-2' and H-6'), 7.74 (d, 2H, $J_{3',2'}$ = $J_{5',6'}$ = 8.20 Hz, H-3' and H-5'), 10,17 (bs, 1H, NH), 11.03 (bs, 1H, NH); HR Ms [MH^+] 317.287.

Ethyl 3-(m-nitro-phenyl)-3-(indol-5-ylamino)-acrylate (12). Dark orange solid, yield 48%; mp 105°-107°C; Rf 0.68 (eluant n-esane/ethyl acetate 6/4); IR (KBr) 3438 (NH), 1627 CO), 1346 and 1530 (NO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.25 (t, 3H, J = 7.06 Hz, CH_3), 4.15 (q, 2H, J = 6.99 Hz, CH_2), 4.97 (s, 1H, CH), 6.25 (m, 1H, H-3), 6.63 (dd, 1H, $J_{6,7}$ = 8.77 Hz, $J_{6,4}$ = 1.91 Hz, H-6), 7.05 (d, 1H, $J_{4,6}$ = 2.10 Hz, H-4), 7.17 (d, 1H, $J_{7,6}$ = 8.58 Hz, H-7), 7.27 (dd, 1H, $J_{2,1}$ = $J_{2,3}$ = 2.86 Hz, H-2), 7.54 (t, 1H, $J_{5',4'}$ = $J_{5',6'}$ = 7.44 Hz, H-5'), 7.74 (d, 1H, $J_{6',5'}$ = 7.82 Hz, H-6'), 8.15 (m, 2H, H-2' and H-4'), 10.19 (bs, 1H, NH), 11.02 (bs, 1H, NH); HR Ms [MH^+] 353.282.

Ethyl 3-thienyl-3-(indol-5-ylamino)-acrylate (13). Solid, yield 39%; mp 95°-97°C; Rf 0.90 (eluant chloroform/methanol 9/1); IR (KBr) 3441 (NH), 1624 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.23 (t, 3H, J = 7.25 Hz, CH_3), 4.12 (q, 2H, J = 7.25 Hz, CH_2), 5.05 (s, 1H, CH), 6.31 (t, 1H, $J_{3,2}$ = 2.20 Hz, H-3), 6.70 (dd, 1H, $J_{6,7}$ = 8.58 Hz, $J_{6,4}$ = 1.91 Hz, H-6), 6.94 (dd, 1H, $J_{4',5'}$ = $J_{4',3'}$ = 3.81 Hz, H-4'), 7.13 (dd, 1H, $J_{3',4'}$ = 3.81 Hz, $J_{3',5'}$ = 1.14 Hz, H-3'), 7.15 (d, 1H, $J_{4,6}$ = 1.91 Hz, H-4), 7.23 (d, 1H, $J_{7,6}$ = 8.39 Hz, H-7), 7.30 (t, 1H, $J_{2,3}$ = 2.20 Hz, H-2), 7.55 (dd, 1H, $J_{5',4'}$ = 4.96 Hz, $J_{5',3'}$ = 1.14 Hz, H-5'), 10.11 (bs, 1H, NH), 11.06 (bs, 1H, NH); HR Ms [MH^+] 313.341.

Ethyl 3-phenyl-3-(2-carboxymethyl-4-methoxy-indol-5-ylamino)-acrylate (14). Solid yellow product, yield 38%; mp 155-162°C; Rf 0.83 (eluant dichloromethane/ethyl acetate 9/1); IR (KBr) 3437 (NH), 1625 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.24 (t, 3H, J = 7,06 Hz, CH_3), 3.63 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 4.13 (q, 2H, J = 7.06 Hz, CH_2), 4.87 (s, 1H, CH), 6.28 (d, 1H, J = 1.14 Hz, H-6), 6.61 (d, 1H, J = 1.72 Hz, H-4), 6.90 (d, 1H, J = 2.10 Hz, H-3), 7.34 (m, 5H, aryl), 10.25 (bs, 1H, enamine NH), 11.83 (bs, 1H, indole NH); HR Ms [MH^+] 363.327.

Ethyl 3-phenyl-3-[1-(2-diethylamino-ethyl)-1*H*-indol-5-ylamino]-acrylate (15). Oily pinkish-orange product, yield 30%; Rf 0.51 ethylacetate/methanol 9:1); IR (KBr) 1623 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.82 (t, 6H, J = 7.16 Hz, 2 CH_3), 1.23 (t, 3H, J = 6.96 Hz, CH_3), 2.42 (q, 4H, J = 6.67 Hz, 2 CH_2), 2.63 (t, 2H, J = 6.48 Hz, CH_2), 4.12 (m, 4H, 2 CH_2), 4.83 (s, 1H, CH), 6.20 (d, 1H, $J_{3,2}$ = 3.43 Hz, HC-3), 6.60 (dd, 1H, $J_{6,7}$ = 8.77 Hz and $J_{6,4}$ = 2.10 Hz, HC-6), 6.96 (d, 1H, $J_{4,6}$ = 1.72 Hz, HC-4), 7.22 (d, 1H, $J_{7,6}$ = 8.45 Hz, HC-7), 7.30 (m, 6H, HC-2 and aryl-H), 10.25 (bs, 1H, NH); HR Ms [MH^+] 406.488.

7-Phenyl-6,9-dihydro-3*H*-pyrrolo[3,2-*f*]quinolin-9-one (16). IR (KBr) 3407 (NH), 3217 (NH, 1633 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.38 (s, 1H, H-8) 7.45 (t, 1H, $J_{2,1}$ = $J_{2,3}$ = 2.76 Hz, H-2), 7.56 (m, 5H, aryl 5H), 7.76 (d, 1H, $J_{4,5}$ = 8.77 Hz, HC-4), 7.85 (m, 2H, aryl 2H), 11.48 (bs, 1H, NH), 11,63 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 186 (C=O); HR MS [MH^+] 261.095.

7-(3'-Methoxy-phenyl)-6,9-dihydro-3*H*-pyrrolo[3,2-*f*]quinolin-9-one (17). IR (KBr) 3407 (NH), 3205 (NH), 1611 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.88 (s, 3H, OCH_3), 6.39 (s, 1H, H-8), 7.12 (dd, 1H, $J_{4',5'}$ = 8.20 Hz, H-4'), 7.46 (m, 6H, H-1, H-2, H-5, H-2', H-5', H-6'), 7.76 (d, 1H, $J_{4,5}$ = 8.77 Hz, H-4), 11.48 (bs, 1H, NH), 11.57 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 55.689 (CH_3), 177.63 (C=O); HR MS [MH^+] 291.112.

7-(3'-Methyl-phenyl)-6,9-dihydro-3*H*-pyrrolo[3,2-*f*]quinolin-9-one (18). IR (KBr) 3310 (NH), 3179 (NH), 2935 (CH_3), 1607 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.44 (s, 3H, CH_3), 6.39 (bs, 1H, H-8), 7.36 (d, 1H, J = 7.82 Hz, H-4'), 7.46 (m, 2H, aryl H), 7.54 (d, 1H, J = 8.77 Hz, H-4 and m, 1H, aryl H), 7.65 (m, 2H, aryl H), 7.75 (d, 1H, J = 8.99 Hz, H-5), 11.48 (bs, 1H, NH), 11.60 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 21 (CH_3), 185 (CO); HR MS [MH^+] 275.112.

7-(3'-Bromo-phenyl)-6,9-dihydro-3*H*-pyrrolo[3,2-*f*]quinolin-9-one (19). IR (KBr) 3422 (NH), 3199 (NH), 1618 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.39 (s, 1H, H-8), 7.46 (dd, 1H, J = 2.57 Hz, H-2), 7.53 (m, 3H, H-1, HC-4, H-5'), 7.76 (dd, 1H, $J_{4',5'}$ = 8.01 Hz, H-4'), 7.77 (d, 1H, $J_{5,4}$ = 8.58 Hz, H-5), 7.85 (dd, 1H, $J_{6',5'}$ = 7.82 Hz, H-6'), 8.05 (bt, 1H, H-2'), 11.50 (bs, 1H, NH), 11.65 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 188 (CO); HR MS [MH^+] 339.011.

7-(3'-Cyano-phenyl)-6,9-dihydro-3*H*-pyrrolo[3,2-*f*]quinolin-9-one (20). IR (KBr) 3437 (NH), 3203 (NH), 2241 (CN), 1608 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.64 (bs, 1H, H-8), 7.46 (dd, 1H, J = 2.65 Hz, H-2), 7.51 (bs, 1H, H-1), 7.55 (d, 1H, $J_{5,4}$ = 8.96 Hz, H-5), 7.77 (t, 1H, $J_{5',6'}$ = $J_{5',4'}$ = 8.10 Hz, H-5' and d, 1H, $J_{4,5}$ = 9.15 Hz, H-4), 7.99 (d, 1H, $J_{4',5'}$ = 7.82 Hz, H-4'), 8.23 (d, 1H, $J_{6',5'}$ = 8.01 Hz, H-6'), 8.39 (bs, 1H, H-2'), 11.55 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 124 (CN), 187 (CO); HR MS [MH^+] 286.093.

7-(4'-Cyano-phenyl)-6,9-dihydro-3*H*-pyrrolo[3,2-*f*]quinolin-9-one (21). IR (KBr) 3439 (NH), 3259 (NH), 2222 (CN), 1607 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.55 (bs, 1H, H-8), 7.31 (d, 1H, $J_{1,2}$ = 2.86 Hz, H-1), 7.36 (d, 1H, $J_{5,4}$ = 8.77 Hz, H-5), 7.49 (dd, 1H, $J_{2,1}$ = 2.86 Hz, H-2), 7.70 (dd, 1H, $J_{4,5}$ = 8.77 Hz, H-4), 7.78 (dd, 2H, $J_{2',3'}$ = $J_{6',5'}$ = 6.48 Hz, $J_{2',6'}$ = $J_{6',2'}$ = 2.10 Hz, H-2' and H-6'), 7.84 (dd, 2H, $J_{3',2'}$ = $J_{5',6'}$ = 6.39 Hz, $J_{3',5'}$ = $J_{5',3'}$ = 2.00 Hz, H-3' and H-5');); ^{13}C NMR (DMSO-d_6) δ 123 (CN); 187 (C=O); HR MS [MH^+] 286.122.

7-(3'-Nitro-phenyl)-6,9-dihydro-3*H*-pyrrolo[3,2-*f*]quinolin-9-one (22). Yellow solid, yield 75%; mp >300°C; Rf 0,54 (eluant ethylacetate/methanol 9:1); IR (KBr) 3403 (NH), 3328 (NH), 1614 (CO), 1349 and 1532 (NO_2) cm^{-1}; ^1H NMR (CD_3OD) δ 6.62 (s, 1H, HC-8), 7.32 (d, 1H, $J_{2,1}$ = 3.05 Hz, HC-2), 7.40 (d, 1H, $J_{5,4}$ = 9.15 Hz, HC-5), 7.50 (dd, 1H, $J_{1,2}$ = 3.05 Hz and $J_{1,4}$ = 0.76 Hz, HC-1), 7.70 (dd, 1H, $J_{5',4'}$ = $J_{5',6'}$ = 8.20 Hz, HC-5'), 7.73 (dd, 1H, $J_{4,5}$ = 8.96 Hz and $J_{4,1}$= 0.76 Hz, HC-4), 8.08 (ddd, 1H, $J_{6',5'}$ = 7.63 Hz, $J_{6',4'}$ = $J_{6',2'}$ =0.85 Hz, Hc-6'), 8.28 (ddd, 1H, $J_{4',5'}$ = 8.02 Hz and $J_{4',6'}$ = $J_{4',2'}$ = 0.85 Hz, HC-4'), 8.56 (dd, 1H, $J_{2',4'}$ = $J_{2',6'}$ = 1.90 Hz, HC-2'); ^{13}C NMR (DMSO-d_6) δ 186 (C=O); HR MS [MH^+] 306.235.

7-(3'-Amino)-phenyl-6,9-dihydro-3*H*-pyrrolo[3,2-*f*]quinolin-9-one (23). IR (KBr) 3403, 3328, 3215 (NH and NH_2), 1619 (CO), cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.36 (bs, 2H, NH_2), 6.22 (d, 1H, J = 1.53 Hz, H-8), 6.73 (dd, 1H, $J_{4',5'}$ = 8.11 Hz, $J_{4',6'}$ and $J_{4',2'}$ = 1.44 Hz, H-4'), 6.91 (d, 1H, $J_{6',5'}$ = 7.82 Hz, H-6'), 6.95 (dd, 1H, $J_{2',4'}$ and $J_{2',6'}$ = 2.38 Hz, H-2'), 7.20 (t, 1H, $J_{5',4',6'}$ = 7.73 HZ, H-5'), 7.43 (t, 1H, J = *3,2*4 Hz, H-2), 7.52 (d, 1H, $J_{4,5}$ = 9.92 Hz, H-4), 7.54 (d, 1H, $J_{1,2}$ = 2.29 Hz, H-1), 7.73 (d, 1H, $J_{5,4}$ = 8.58 Hz, H-5), 11.44 (bs, 1H, NH), 11.51 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 185 (CO); HR MS [MH^+] 276,125.

7-Thienyl-6,9-dihydro-3*H*-pyrrolo[3,2-*f*]quinolin-9-one (24). IR (KBr) 3447 (NH), 3273 (NH), 1605 (CO) cm^{-1}; ^1H NMR (CD_3OD) δ 6.55 (bs, 1H, H-8), 7.13 (dd, 1H, J = 3.6 and 5.3 Hz, H-4'), 7.29 (d, 1H, J = 2.8 Hz, H-2), 7.37 (d, 1H, J = 8.9 Hz, H-4), 7.47 (bd, 1H, J = 2.8 Hz, H-1), 7.53 (dd, 1H, J = 5.3 and 0.9 Hz, H-3'), 7.65 (dd, 1H, J = 3.6 and 0.9 Hz, H-5'), 7.69 (dd, 1H, J = 8.9 and 0.9 Hz, H-5); ^{13}C NMR (CD_3OD) δ 187 (C=O); HR MS [MH^+] 267.066.

7-Phenyl-3-(diethylamino-ethyl)-6,9-dihydro-pyrrolo[3,2-*f*]quinolin-9-one (25). IR (KBr) 3253 (NH), 1606 (CO) cm^{-1}; δ 0.85 (t, 6H, J = 7.14 Hz, 2 CH_3), 2.46 (q, 4H, J = 7.14 Hz, 2 CH_2), 2.73 (t, 2H, J = 6.38 Hz, CH_2), 4.30 (t, 2H, J = 6.38 Hz, CH_2), 6.35 (d, 1H, J = 1.71 Hz, HC-8), 7.48 (d, 1H, $J_{1,2}$ = 2.86 Hz, HC-1), 7.52 (d, 1H, $J_{2,1}$ = 2.86 Hz, HC-2), 7.57 (m, 4H, HC-5, HC-3', HC-4', HC-

5'), 7.84 (m, 3H, HC-4, HC-2', HC-6'), 11.60 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 12.3 (2 CH_3), 45.1 (CH_2), 47.1 (3 CH_2), 53.1 (CH_2), 178 (C=O); HR MS [MH^+] 360.525.

2-Carboxymethyl-4-methoxy-7-phenyl-6,9-dihydro-3*H*-pyrrolo[3,2-*f*]quinolin-9-one (26). IR (KBr) 3423 (NH), 3328 (NH), 1717 (CO), 1613 (CO), cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.87 (s, 3H, $COOCH_3$), 4.04 (s, 1H, OCH_3), 6.41 (s, 1H, H-8), 7.19 (s, 1H, H-5), 7.57 (m, 3H, aryl 3H), 7.85 (m, 2H, aryl 2H), 8.16 (s, 1H, H-1), 11.64 (bs, 1H, NH), 12.41 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 60.59 (CH_3), 72.54 (CH_3), 178,29 (C=O); HR MS [MH^+] 349,111.

Cell lines and Culture Conditions

Human cell lines - thyroid carcinoma (Aro), colon carcinoma (HT-29), pancreas adenocarcinoma (PT-45), hepatocellular carcinoma (Hep G2), ovary carcinoma (Ovcar-3), breast cancer (MCF-7), adrenocortical carcinoma (NCI-H295R), lung carcinoma (A549), uteri cervix carcinoma (Hela) and two mouse cell lines (BNL 1ME A.7R.1 and 4T1, from liver and breast cancer respectively) - were grown at 37 °C in a humidified incubator with 5% CO_2.

PT-45 and Ovcar-3 cell lines were cultured in RMPI medium supplemented with 10% heat-inactivated fetal bovine serum (FBS, Invitrogen), 100 U/mL penicillin G and 10 μg/mL streptomycin; NCI-H295R were grown in RPMI supplemented with 2% FBS, 100 U/mL penicillin G and 10 μg/mL streptomycin, 1% Insulin, Transferrin, Selenium-A (Gibco); Aro, HT-29, Hep G2, MCF-7, A549, Hela, BNL 1ME A.7R.1 and 4T1 were grown in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin G and 10 μg/mL streptomycin. All cell lines were purchased from the American Type Culture Collection. 4T1 cells were kindly donated by Prof. Mario Colombo of the Istituto Nazionale Tumori, Milano, Italy.

***In vitro* Cytotoxicity Assay**

The cytotoxic activity of 13 7-phenyl-3,6-dihydro-pyrrolo[3,2-*f*]quinoline-9-ones was determined using a standard 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazodium bromide (MTT)-based colorimetric assay (Sigma), using vincristine and taxol as reference drugs. Briefly, cell lines were seeded at a density of $7*10^3$ cells/well in 96-well microtiter plates (Costar). After 24 h, exponentially growing cells were exposed to the indicated compounds at final concentrations ranging from 0.1 to 50 μM. After 72 h, cell survival was determined by the addition of an MTT solution (10 μl of 5 mg/mL MTT in PBS).

After 4 h, 100 μl of 10% SDS in 0.01 N HCl were added, and the plates were incubated at 37 °C for a further 18 h; optical absorbance was measured at 550 nm by a LX300 Epson Diagnostic microplate reader. Survival ratios are expressed in percentages with respect to untreated cells. IC_{50} values were determined from replicates of 6-8 wells from at least two independent experiments.

Flow Activating Cell Sorting Analysis (FACS)

Ovcar-3 and Hep-G2 cells were cultured for 24 hours in a drug-free medium or supplemented with compounds **16-26** (5-10 μM). As previously described[37,38], cells were trypsinized with a cell scraper, washed twice with PBS, and fixed in 70% cold ethanol (30 min at –20 °C). Cells (10^6) were then washed once in citrate phosphate buffer (0.2 N Na_2HPO_4 and 0.1 M citric acid, 24:1), followed by PBS, and finally incubated in a RNAse solution (100 μg/mL in PBS). After 30 min at 37 °C, the cells were incubated in a propidium iodide solution (PI, Sigma, 100 μg/mL in PBS) at room temperature for a further 30 minutes. To determine the effects of compounds on cell cycle dynamics, DNA fluorescence was measured by flow cytometry, examining at least 15000 events with Lysis II software (Becton, Dickinson) at 488/525 nm (excitation/emission wavelengths).

All experiments were repeated 3-4 times and DNA content analysis was carried out using both logarithmic and linear scales. Results were comparable, irrespective of the scale used, and are shown on a logarithmic scale.

Confocal Microscopy Analysis

Ovcar-3 cells ($5*10^5$/well) were seeded on sterile microscope cover slips placed in 6-well plates. After 24 h, compound **16** (5 μM) and vincristine (5 μM) were added to the culture medium and cells were incubated for a further 24 hours. As described previously[39,40], cells were fixed with 4% formaldehyde in PBS at 22 °C, washed three times with PBS, permeabilized in 0.2 % Triton X-100 in PBS for 10 minutes at 22 °C, and placed in methanol at –20 °C for 30 minutes. They were then washed with PBS, incubated for 1 h at 37 °C with 2% Bovine Serum Albumin (BSA) and subsequently with a mouse monoclonal anti-β-tubulin antibody (diluted 1:400) for 1 h at 37 °C. Slides were washed three times with PBS and incubated with a Tetramethyl Rhodamine Isothiocyanate (TRITC)-conjugated rat anti-mouse IgG (diluted 1:200 in a 2% BSA solution in PBS) for another hour at 37 °C. Slides were then washed repeatedly with PBS, mounted with mounting medium and analyzed by confocal microscopy (SP-2, Leyca) under red light.

Tubulin polymerization assay

The effects of compound **16** (0.625, 1.25, 2.5, 5 μM) on the polymerisation of microtubule protein isolated from porcine brain were analysed using a microtubule polymerization assay kit (Cytoskeleton) following the recommended procedure. Polymerization was followed by fluorescent enhancement due to a fluorescent reporter in microtubules as polymerization occurred. The assay was carried out in 96-well microtiter plates and the reaction was initiated with the addition of tubulin. The plates were incubated at 37 °C in a fluorescence microplate reader (Fluoroskan Ascent FL Labsystems) and fluorescence (λ_{ex} 355 nm; λ_{em} 450 nm) was measured every minute for 60 min. Taxol and vincristine 3 μM were used as reference compounds.

Aromatase Activity Assay

Aromatase activity in subconfluent H295R cells was measured by Tritiated Water Release Assay[32], with 0.5 μM [1β-^{3}H(N)]-androst-4-ene-3,17-dione (25.3 Ci/mmol; DuPont NEN, Boston, MA, USA) as substrate. Briefly, H295R cells were seeded at 10^6 cells/well on 6-well plates and, after 48 h, were cultured for 24 h in DMEM-F12 in the absence or presence of FSK (25 μM), a substance which up-regulates CYP19 activity and gene transcripts by adenylate cyclase activation[41]. One hour before addition of the substrate, compounds **16**, **17**, **19** (5-10-15 μM) and Letrozole (5 μM) were added. After a further 2 h exposure at 37 °C, 750 μl of incubation media were mixed with 5 volumes of chloroform and vortexed for 60 sec to extract unconverted substrate. The aqueous phase was increased to 1.5 mL with distilled water and centrifuged at 800g for 5 min. An aliquot of ^3H_2O (1 mL) was placed in tubes containing 1 mL of 5% charcoal and 0.5 % dextran T-70, vortexed for 60 seconds and centrifuged at 9000 g for 30 min. After centrifugation, 1 mL of the ^3H_2O phase was quantified by counting in 5-mL Picofluor 15 premixed cocktail in a liquid scintillation counter. The results are expressed as pmoles [^{3}H]H_2O released per hour and normalized for mg protein (pmol/h/mg protein). Protein yield was determined by the Bradford method[42].

***In vivo* Antitumor Activity**

The *in vivo* cytotoxic activity of compound **16** was investigated using a syngenic hepatocellular carcinoma model (BNL 1ME A.7R.1) in Balb/c mice. Male mice, 8 weeks old, were purchased from Charles-River (Calco, Lecco, Italy) and tumors were induced by a subcutaneous injection at both flanks of 10^7 BNL 1ME A.7R.1 cells in 200 μl sterile PBS. Animals were randomLy divided in two groups, and starting on the second day, they were daily dosed intraperitoneally (i.p.) with 500 μl of free vehicle [0.9% NaCl containing 5% polyethylene glycol and 0.5% Tween 80] or compound **16** (40 mg/kg body weight). Ten days later, animals were sacrificed and the tumor sizes were measured[43].

To measure the perpendicular diameter of each mass, calipers were used. In particular, the tumor volume (V) was calculated by the rotational ellipsoid formula: $V = A \times B^2/2$, where A is the longer diameter (axial) and B is the shorter diameter (rotational). All experimental procedures were

accomplished following guidelines recommended by the Institutional Animal Care and Use Committee of Padua University.

Statistical analysis

Results are reported as means ± standard error (M ± S.E.). Statistical analysis was performed by one-way analysis of variance or Student's *t*-test, as appropriate. A *P* value of less than 0.05 was considered statistically significant.

Table: elemental analyses

Compound	**Formula**	**Calculated**				**Obtained**			
		%C	**%H**	**%N**	**%S**	**%C**	**%H**	**%N**	**%S**
16	$C_{17}H_{12}N_2O$	78.44	4.65	10.76		78.39	4.46	10.37	
17	$C_{18}H_{14}N_2O_2$	74.45	4.86	9.65		74.24	4.61	9.32	
18	$C_{18}H_{14}N_2O$	78.81	5.14	10.21		79.07	4.88	9.92	
19	$C_{18}H_{14}N_2O$	78.81	5.14	10.21		79.07	4.88	9.92	
20	$C_{18}H_{11}N_3O$	75.78	3.89	14.73		75.40	3.69	14.92	
21	$C_{18}H_{11}N_3O$	75.78	3.89	14.73		75.45	3.61	14.58	
22	$C_{17}H_{11}N_3O_3$	66.88	3.63	13.76		66.69	3.87	13.61	
23	$C_{17}H_{13}N_3O$	74.17	4.76	15.26		74.01	4.40	15.41	
24	$C_{15}H_{10}N_2OS$	67.65	3.78	10.52	12.04	67.39	3.58	10.25	11.97
25	$C_{23}H_{25}N_3O$	76.85	7.01	11.69		76.75	7.10	11.62	
26	$C_{20}H_{16}N_2O_4$	68.96	4.63	8.04		69.57	4.35	7.92	