Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications.

Supporting information

Contents

Synthesis of imidazo[2,1-b][1,3,4]thiadiazole-chalcones as apoptosis inducing anticancer agents

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Experimental Section

General

All chemicals were purchased from Spectrochem Pvt. Ltd (Mumbai, India), Aldrich (Sigma–Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 GF-254, and visualization on TLC was attained by UV light or iodine indicator. Column chromatography was performed with Merck 60–120 mesh silica gel. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker UXNMR/XWIN-NMR (300 M Hz) instruments. Chemical shifts are reported in parts per million (δ in ppm) relative to the peak for TMS (tetramethylsilane) as an intrernal standard, coupling constants are reported in hertz (Hz). ESI spectra were recorded on Micro mass, Quattro LC using ESI^{+} software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. High-resolution mass spectra were recorded on a QSTAR XL Hybrid MS–MS mass spectrometer. Melting points were determined with an Electro thermal melting point apparatus, and are uncorrected.

Chemistry

6-(4-Methoxyphenyl)-2-methylimidazo[2,1-b][1,3,4]thiadiazole

The appropriate 5-methyl-1,3,4-thiadiazol-2-amine (**2**) (1.5 g, 13 mmol) was dissolved in 80 mL of acetone and treated with the equivalent of the appropriate 2-bromo-1-(4-methoxyphenyl)ethanone (**1a**) (2.9 g, 13 mmol). The reaction mixture was refluxed for 4-5 h (according to a TLC test), the resulting salt (**3a**) was separated by filtration and without further purification, refluxed for 1 h with 200 mL of 6N HCl. Before complete cooling, the solution was cautiously basified by dropwise addition of 15% NH_4OH. The resulting base was collected by filtration and crystallized from ethanol to afford compound (**4a**) as a white

solid (2.9 g, 90%); mp 173-175 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.81 (s, 3H, **-CH_3**), 3.87 (s, 3H, **OCH_3**), 6.98 (d, 2H, *J*=8.7 Hz, **ArH**), 7.23-7.26 (m, 1H, **ArH**), 7.73 (d, 2H, *J*=8.7 Hz, **ArH**) ppm; MS (ESI): *m/z* 246 $(M+H)^+$

6-(4-Fluorophenyl)-2-methylimidazo[2,1-b][1,3,4]thiadiazole

This compound was prepared according to the method described for compound (**4a**), employing compound (**2**) (1.5 g, 13 mmol) and 2-bromo-1-(4-fluorophenyl)ethanone (**1b**) (2.8 g, 13 mmol) to obtain the pure product (**4b**) as a white solid (2.6 g, 87%); mp 146-148 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.82 (s, 3H, **-CH_3**), 7.11-7.17 (m, 2H, **ArH**), 7.76-7.84 (m, 3H, **ArH**) ppm; MS (ESI): *m/z* 234 $(M+H)^+$.

6-(4-Chlorophenyl)-2-methylimidazo[2,1-b][1,3,4]thiadiazole

This compound was prepared according to the method described for compound **4a**, employing compound (**2**) (1.5 g, 13 mmol) and 2-bromo-1-(4-chlorophenyl)ethanone (**1c**) (3.0 g, 20 mmol) to obtain the pure product (**4c**) as a white solid (2.9 g, 89%); mp 169-171 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.82 (s, 3H, **-CH_3**), 7.40-7.46 (m, 3H, **ArH**), 7.77 (d, 2H, *J*=8.6 Hz, **ArH**) ppm; MS (ESI): *m/z* 250 $(M+H)^+$.

6-Chloro-2-methylimidazo[2,1-b][1,3,4]thiadiazole

The appropriate 5-methyl-1,3,4-thiadiazol-2-amine (**2**) (2.0 g, 17 mmol) was dissolved in ethanol (30 ml) and treated with the equivalent of the appropriate 2-bromoacetic acid. (2.4 g, 17 mmol). The reaction mixture was refluxed for 7 h (according to a TLC test), the resulting salt (**3d**) was separated by filtration and without further purification, refluxed for 2 h with 15 mL of $POCl_3$. After completion of reaction, solvent was evapourated under vaccum and pour residue into ice. Further, this solution basified with 20% NH_4OH. The resulting excess base was collected by filtration and precipitate crystallized from ethanol to afford compound (**4d**) as a white solid (2.0 g, 69%); mp 129-131 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.82 (s, 3H, **-CH_3**), 7.26-7.30 (m, 1H, **ArH**) ppm; MS (ESI): *m/z* 174 $(M+H)^+$

6-(4-methoxyphenyl)-2-methylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde

The Vilsmeier reagent was prepared at 0-5 ^{o}C by dropping $POCl_3$ (10 eq) into a stirred solution of DMF (8 mL) in $CHCl_3$ (10 eq). The 6-(4-methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole (**4a**) (2.5 g, 10 mmol) was suspended in chloroform (20 mL).The mixture thus obtained was dropped into the Vilsmeier reagent while maintaining stirring and cooling. The reaction mixture was kept for 3 h at room temperature and under reflux for 8-12 h (according to a TLC test). Chloroform was removed under reduced pressure and the resulting oily liquid was poured onto ice. The crude aldehyde was collected by filtration and

crystallized from ethanol to obtain the pure product (**5a**) as a yellow solid (2.2 g, 78%). mp 146-148 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.84 (s, 3H, **-CH_3**), 3.88 (s, 3H, **OCH_3**), 7.03 (d, 2H, *J*=8.5 Hz, **ArH**), 7.80 (d, 2H, *J*=8.5 Hz, **ArH**), 9.99 (s, 1H, **CHO**) ppm; MS (ESI): *m/z* 274 $(M+H)^+$.

6-(4-fluorophenyl)-2-methylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde

This compound was prepared according to the method described for compound (**5a**), employing 6-(4-fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole (**4b**) (2.5 g, 11 mmol) to obtain the pure product (**5b**) as a white solid (2.07 g, 74%). mp 146-148 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.85 (s, 3H, **-CH_3**), 7.33 (d, 2H, *J*=8.1 Hz, **ArH**), 7.73 (d, 2H, *J*=8.0 Hz, **ArH**), 10.01 (s, 1H, **CHO**) ppm; MS (ESI): *m/z* 262 $(M+H)^+$.

6-(4-chlorophenyl)-2-methylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde

This compound was prepared according to the method described for compound (**5a**), employing 6-(4-chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole (**4c**) (2.5 g, 10 mmol) to obtain the pure product (**5c**) as a white solid (2.2 g, 80%). mp 151-153 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.83 (s, 3H, **-CH_3**), 7.31 (d, 2H, *J*=8.2 Hz, **ArH**), 7.69 (d, 2H, *J*=8.2 Hz, **ArH**), 10.03 (s, 1H, **CHO**) MS (ESI): *m/z* 278 $(M+H)^+$.

6-chloro-2-methylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde

This compound was prepared according to the method described for compound (**5a**), employing 6-chloro-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole (**4d**) (2 g, 12 mmol) to obtain the pure product (**5d**) as a white solid (1.6 g, 70%); mp 103-105 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.85 (s, 3H, **CH_3**), 9.91 (s, 1H, **CHO**) ppm MS (ESI): *m/z* 202 $(M+H)^+$.

1-(6-(4-Methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)ethanol (**8a**)

A solution of 6-(4-methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**5a**) (520 mg, 2 mmol) in dry tetrahydrofuran (THF) was added to stirred solution of methyl magnesium bromide in tetrahydrofuran (2 eq) (1.5 M) at 0 ^{o}C for and then stirred at room temperature for 5-6 h. After completion of reaction saturated aqueous ammonium chloride solution 5-10 ml was added, and the THF was removed in vaccum followed by ethyl acetate was added. The organic layer was extracted and washed with brine solution, dried over anhydrous Na_2SO_4 and evaporated in vaccum to obtain pure compound (**8a**) (390 mg, 71%) and used for next step without purification; mp 174-176 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$); δ 1.79 (d, 3H, *J*=6.1 Hz, CH_3), 2.76 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 5.31-5.42 (m, 1H, **-CH-**), 6.98 (d, 2H, *J*=8.3 Hz, **ArH**), 7.61 (d, 2H, *J*=8.3 Hz, ArH) ppm; MS (ESI) *m/z* 290 [M+H].

1-(6-(4-Fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)ethanol (**8b**)

This compound was prepared according to the method described for compound (**8a**), employing 6-(4-fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**5b**) (549 mg, 2 mmol) to obtain the pure product (**8b**) as a white solid (408 mg, 70%). mp 159-161 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.80 (d, 3H, *J*=6.7 Hz, CH_3), 2.75 (s, 3H, CH_3), 5.30-5.36 (q, 1H, *J*=6.7 Hz, **-CH-**), 7.10-7.15 (m, 2H, **ArH**), 7.57-7.62 (m, 2H, **ArH**) ppm; MS (ESI): *m/z* 278 $(M+H)^+$.

1-(6-(4-Chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)ethanol (**8c**)

This compound was prepared according to the method described for compound (**8a**), employing 6-(4-chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**5c**) (560 mg, 2 mmol) to obtain the pure product (**8c**) as a white solid (409 mg, 69%); mp 148-150 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.79 (d, 3H, *J*=6.7 Hz, **CH_3**), 2.74 (s, 3H, **CH_3**), 5.31-5.37 (q, 1H, *J*=6.7 Hz, **-CH-**), 7.39 (d, 2H, *J*=8.5 Hz, **ArH**), 7.56 (d, 2H, *J*=8.5 Hz, **ArH**) ppm; MS (ESI): *m/z* 294 $(M+H)^+$.

1-(6-Chloro-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)ethanol (**8d**)

This compound was prepared according to the method described for compound (**8a**), employing 6-chloro-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**5c**) (600 mg, 3 mmol) to obtain the pure product (**8d**) as a white solid (402 mg, 62%). mp 110-112 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.75 (d, 3H, *J*=6.8 Hz, **CH_3**), 2.74 (s, 3H, **CH_3**), 5.24-5.29 (q, 1H, *J*=6.8 Hz, **-CH-**) ppm; MS (ESI): *m/z* 218 $(M+H)^+$.

1-(6-(4-Methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)ethanone (**9a**)

A solution of 2-iodoxy benzoic acid (2 eq) and dimethyl sulfoxide (8 mL) (quantity) was stirred for 10 min at room temperature until homogeneous solution. A solution of 1-(6-(4-methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)ethanol (**8a**) (350 mg, 1.2 mmol) in dimethyl sulfoxide was added slowly, and it was stirred for 2-3 h. After completion of reaction, ice water was added to reaction mixture and the mixture was stirred for another 10 min. To this mixture ethyl acetate was added and filtered through celite. The organic layer was separated and washed with water subsequently saturated Na_2CO_3 solution and brine, after that dried over anhydrous Na_2SO_4 and evaporated in vacuum to attain the pure white solid compound (**9a**) (275 mg, 79%) and was used directly for next step; mp 157-159 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.43 (s, 3H, **CH_3**), 2.63 (s, 3H, **CH_3**), 3.91 (s, 3H, OCH_3), 7.21 (d, 2H, *J*=8.4 Hz, **ArH**), 7.49 (d, 2H, *J*=8.4 Hz, **ArH**) ppm; MS (ESI): *m/z* 288 $(M+H)^+$.

1-(6-(4-Fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)ethanone (**9b**)

This compound was prepared according to the method described for compound (**9a**), employing 1-(6-(4-fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)ethanol (**8b**) (320 mg, 1.2 mmol) to obtain the pure product (**9b**) as a white solid (257 mg, 81%); mp 141-143 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.57 (s, 3H, **CH$_3$**), 2.80 (s, 3H, **CH$_3$**), 7.23-7.29 (m, 2H, **ArH**), 7.85 (d, 2H, *J*=8.2 Hz, **ArH**) ppm; MS (ESI): *m/z* 276 $(M+H)^+$.

1-(6-(4-Chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)ethanone (**9c**)

This compound was prepared according to the method described for compound (**9a**), employing 1-(6-(4-chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)ethanol (**8c**) (350 mg, 1.2 mmol) to obtain the pure product (**9c**) as a white solid (295 mg, 85%); mp 155-157 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.49 (s, 3H, **CH$_3$**), 2.79 (s, 3H, **CH$_3$**), 7.31 (d, 2H, *J*=8.6 Hz, **ArH**), 7.53 (d, 2H, *J*=8.6 Hz, **ArH**) ppm; MS (ESI): *m/z* 292 $(M+H)^+$.

1-(6-Chloro-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)ethanone (**9d**)

This compound was prepared according to the method described for compound (**9a**), employing 1-(6-chloro-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)ethanol (**8d**) (369 mg, 1.7 mmol) to obtain the pure product (**9d**) as a white solid (260 mg, 71%); mp 106-108 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.43 (s, 3H, **CH$_3$**), 2.76 (s, 3H, **CH$_3$**) ppm; MS (ESI): *m/z* 216 $(M+H)^+$.

(*E*)-3-(6-(4-Methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**7a**)

A mixture of 1-(3,4,5-trimethoxyphenyl)ethanone (**6a**) (210 mg, 1 mmol) and 6-(4-methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**5a**) (273 mg, 1 mmol) was dissolved in 10 mL ethanol. To this mixture, pottasium hydroxide (40%, 1 mL) was added at 0-5 ^{o}C. The reaction mixture was stirred at room temperature for 1-2 h. Then this reaction mixture was poured over crushed ice and acidified with dil HCl. The light yellow solid thus obtained was filtered, washed with water and dried. The residue was purified on column chromatography (silica gel with 50% ethyl acetate in hexane) to afford compound (**7a**) as a yellow solid (372 mg, 80%). mp 176-178 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.93 (s, 3H, **CH$_3$**), 3.88 (s, 3H, **OCH$_3$**), 3.95 (s, 3H, **OCH$_3$**), 3.97 (s, 6H, **CH$_3$**), 7.04 (d, 2H, *J*=9.1 Hz, **ArH**), 7.34 (s, 2H, **ArH**), 7.67 (d, 2H, *J*=9.1 Hz, **ArH**), 8.04 (d, 1H, *J*=15.9 Hz, **C=C**), 8.16 (d, 1H, *J*=15.9 Hz, **C=C**); ^{13}C NMR (300 MHz, $CDCl_3$): δ 189.9, 160.45, 160.11, 159.81, 153.0, 133.8, 129.9, 129.0, 125.9, 120.9, 118.8, 114.3, 105.9, 60.9, 56.2, 55.4, 18.0 ppm; MS (ESI): *m/z* 466 $(M+H)^+$ HRMS (ESI): calcd for $C_{24}H_{24}N_3O_5S$

$(M+H)^+$ 466.1431; found: 466.1429; IR (KBr) (ν_{max}/cm^-): 2926, 2850, 2359, 1645, 1599, 1567, 1515, 1497, 1415, 1403, 1262, 1205, 1161, 1023, 847.

(*E*)-3-(6-(4-Fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**7b**)

This compound was prepared according to the method described for compound (**7a**), employing 6-(4-fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**5b**) (261 mg, 1 mmol) and 1-(3,4,5-trimethoxyphenyl)ethanone (**6a**) (210 mg, 1 mmol) to obtain the pure product (**7b**) as a yellow solid (353 mg, 78%); mp 219-221 ^oC; ^1H NMR (500 MHz, $CDCl_3$): δ 2.84 (s, 3H, **CH_3**), 3.95 (s, 3H, **OCH_3**), 3.97 (s, 6H, **OCH_3**), 7.34 (s, 2H, **ArH**), 7.47 (d, 2H, *J*=8.6 Hz, **ArH**), 7.69 (d, 2H, *J*=8.6 Hz, **ArH**), 7.99 (d, 1H, *J*=15.5 Hz, **C=C**), 8.19 (d, 1H, *J*=15.5 Hz, **C=C**); ^{13}C NMR (400 MHz, $CDCl_3$): δ 188.8, 160.2, 153.1, 149.6, 149.0, 142.5, 133.7, 130.5, 129.6, 128.4, 121.3, 119.5, 116.0, 106.1, 60.9, 56.3, 18.1ppm; MS (ESI): *m/z* 454 $(M+H)^+$ HRMS (ESI): calcd for $C_{23}H_{21}FN_3O_4S$ $(M+H)^+$ 454.1237; found: 466.1232; IR (KBr) (ν_{max}/cm^-): 2924, 2853, 1744, 1649, 1594, 1508, 1464, 1402, 1354, 1330, 1275, 1135, 998, 836.

(*E*)-3-(6-(4-Chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (7**c**)

This compound was prepared according to the method described for compound (**7a**), employing 6-(4-chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**5c**) (277 mg, 1 mmol) and 1-(3,4,5-trimethoxyphenyl)ethanone (**6a**) (210 mg, 1 mmol) to obtain the pure product (**7c**) as a yellow solid (398 mg, 85%); mp 168-170 ^oC; ^1H NMR (300 MHz, $CDCl_3$): δ 2.84 (s, 3H, **CH_3**), 3.95 (s, 3H, **OCH_3**), 3.97 (s, 6H, **OCH_3**), 7.16-7.22 (m, 2H, **ArH**), 7.35 (s, 2H, **ArH**), 7.70-7.74 (m, 2H, **ArH**), 8.00 (d, 1H, *J*=15.8 Hz, **C=C**), 8.19 (d, 1H, *J*=15.8 Hz, **C=C**); ^{13}C NMR (300 MHz, $CDCl_3$): δ 189.9, 153.5, 149.3, 137.7, 134.5, 129.5, 129.4, 126.6, 122.8, 121.3, 118.8, 114.3, 104.9, 61.0, 56.5,18.0 ppm ppm; MS (ESI): *m/z* 470 $(M+H)^+$ HRMS (ESI): calcd for $C_{23}H_{21}ClN_3O_4S$ $(M+H)^+$ 470.0941; found: 466.0931.

(*E*)-3-(6-Chloro-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1-(3,4,5-trimethoxy phenyl)prop-2-en-1-one (**7d**)

This compound was prepared according to the method described for compound (**7a**), employing 6-chloro-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**5d**) (201 mg, 1 mmol) and 1-(3,4,5-trimethoxyphenyl)ethanone (**6a**) (210 mg, 1 mmol) to obtain the pure product (**7d**) as a yellow solid (322 mg, 82%); mp 170-172 ^oC; ^1H NMR (300 MHz, $CDCl_3$):

δ 2.82 (s, 3H, **CH$_3$**), 3.95 (s, 3H, **OCH$_3$**), 3.98 (s, 6H, **OCH$_3$**), 7.32 (s, 2H, **ArH**), 7.86 (d, 1H, *J*=15.4 Hz, **C=C**), 8.01 (d, 1H, *J*=15.4 Hz, **C=C**); ^{13}C NMR (400 MHz, $CDCl_3$): δ 189.8, 153.6, 137.8, 134.7,129.9, 128.7, 122.9, 121.9, 119.9, 105.2, 61.0, 56.6, 18.0 ppm; MS (ESI): *m/z* 394 $(M+H)^+$ HRMS (ESI): calcd for $C_{17}H_{17}ClN_3O_4S$ $(M+H)^+$ 394.0628; found: 394.0622; IR (KBr) (ν_{max}/cm^{-}): 2932, 2837, 1735, 1658, 1577, 1507, 1441, 1412, 1343, 1254, 1229, 1164, 1128, 990, 966, 828.

(*E*)-3-(6-(4-Methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1-(6-(3,4,5-trimethoxyphenyl)pyridin-2-yl)prop-2-en-1-one (**7e**)

This compound was prepared according to the method described for compound (**7a**), employing 6-(4-methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**5a**) (273 mg, 1 mmol) and 1-(6-(3,4,5-trimethoxyphenyl)pyridin-2-yl)ethanone (**6d**) (287 mg, 1 mmol) to obtain the pure product (**7e**) as a yellow solid (428 mg, 79%); mp 225-227 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.80 (s, 3H, **CH$_3$**), 3.86 (s, 3H, **OCH$_3$**), 3.94 (s, 3H, **OCH$_3$**), 3.98 (s, 6H, **OCH$_3$**), 6.98 (d, 2H, *J*=9.1 Hz, **ArH**), 7.45 (s, 2H, **ArH**), 7.69 (d, 2H, *J*=9.1 Hz, **ArH**), 7.87-7.96 (m, 2H, **ArH**), 8.08-8.12 (dd, 1H, *J*=1.5 Hz, *J*=8.3 Hz, **ArH**), 8.22 (d, 1H, *J*=15.8 Hz, **C=C**), 8.86 (d, 1H, *J*=15.8 Hz, **C=C**); MS (ESI): *m/z* 543 $(M+H)^+$; IR (KBr) (ν_{max}/cm^{-}): 2924, 2853, 2360, 1742, 1664, 1579, 1520, 1402, 1347, 1263, 1213, 1159, 1008, 834.

(*E*)-3-(6-(4-Fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1-(6-(3,4,5-trimethoxyphenyl)pyridin-2-yl)prop-2-en-1-one (**7f**)

This compound was prepared according to the method described for compound (**7a)**, employing 6-(4-fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**5b**) (261 mg, 1 mmol) and 1-(6-(3,4,5-trimethoxyphenyl)pyridin-2-yl)ethanone (**6d**) (287 mg, 1 mmol) to obtain the pure product (**7f**) as a yellow solid (376 mg, 71%); mp 210-212 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.78 (s, 3H, **CH$_3$**), 3.94 (s, 6H, **OCH$_3$**), 3.99 (s, 3H, **OCH$_3$**), 7.35-7.41 (m, 4H, **ArH**), 7.59 (d, 2H, *J*=8.6 Hz, **ArH**), 7.68-7.74 (m, 2H, **ArH**), 7.92 (dd, 1H, *J*=7.9 Hz, *J*=1.6 Hz, **ArH**), 8.12 (d, 1H, *J*=15.9 Hz, **C=C**), 8.62 (d, 1H, *J*=15.9 Hz, **C=C**); MS (ESI): *m/z* 531 $(M+H)^+$; IR (KBr) (ν_{max}/cm^{-}): 2925, 2853, 2357, 1744, 1650, 1577, 1504, 1459, 1413, 1311, 1270, 1160, 1126, 1003, 952, 824.

(*E*)-3-(6-(4-Chlorophenyl)-2-methylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1-(6-(3,4,5-trimethoxyphenyl)pyridin-2-yl)prop-2-en-1-one (**7g**)

This compound was prepared according to the method described for compound (**7a)**, employing 6-(4-chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**5c**)

(277 mg, 1 mmol) and 1-(6-(3,4,5-trimethoxyphenyl)pyridin-2-yl)ethanone (**6d**) (287 mg, 1 mmol) to obtain the pure product (**7g**) as a yellow solid (453 mg, 83%); mp 238-240 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.81 (s, 3H, **CH_3**), 3.94 (s, 3H, **OCH_3**), 3.98 (s, 6H, **OCH_3**), 7.42 (d, 2H, *J*=8.3 Hz, **ArH**), 7.44 (s, 2H, **ArH**), 7.69 (d, 2H, *J*=8.3 Hz, **ArH**), 7.87-7.97 (m, 2H, **ArH**), 8.10 (dd, 1H, *J*=7.5 Hz, *J*=1.5 Hz, **ArH**), 8.16 (d, 1H, *J*=15.8 Hz, **C=C**), 8.90 (d, 1H, *J*=15.8 Hz, **C=C**); MS (ESI): *m/z* 547 $(M+H)^+$; IR (KBr) (ν_{max}/cm^{-}): 2924, 2853, 1742, 1664, 1603, 1506, 1475, 1378, 1308, 1258, 1212, 1155, 1048, 835.

(*E*)-3-(6-Chloro-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1-(6-(3,4,5-trimethoxyphenyl)pyridin-2-yl)prop-2-en-1-one (**7h**)

This compound was prepared according to the method described for compound (**7a**), employing 6-chloro-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**5d**) (201 mg, 1 mmol) and 1-(6-(3,4,5-trimethoxyphenyl)pyridin-2-yl)ethanone (**6d**) (287 mg, 1 mmol) to obtain the pure product (**7h**) as a yellow solid (376 mg, 80%); mp 195-197 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.84 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 3.91 (s, 6H, OCH_3), 7.01 (d, 1H, *J*=9.1 Hz, **ArH**), 7.47 (s, 2H, **ArH**), 8.01-8.11 (m, 4H, **ArH**, **C=C**) ppm; MS (ESI): *m/z* 471 $(M+H)^+$; IR (KBr) (ν_{max}/cm^{-}): 2926, 2852, 1741, 1650, 1572, 1509, 1465, 1405, 1355, 1332, 1246, 1228, 1166, 1136, 996, 842.

(*E*)-1-(3,4-Dimethoxyphenyl)-3-(6-(4-methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)prop-2-en-1-one (**7i**)

This compound was prepared according to the method described for compound (**7a**), employing 6-(4-methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**5a**) (273 mg, 1 mmol) and 1-(3,4-dimethoxyphenyl)ethanone (**6b**) (180 mg, 1 mmol) to obtain the pure product (**7i**) as a yellow solid (326 mg, 75%); mp 179-181 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.85 (s, 3H, **CH_3**), 3.88 (s, 3H, **OCH_3**), 3.98 (s, 6H, **OCH_3**), 6.98-7.01 (dd, 2H, *J*=8.3 Hz, *J*=1.9 Hz, **ArH**), 7.04 (s, 1H, **ArH**), 7.67-7.70 (m, 3H, **ArH**), 7.76 (dd, 1H, *J*=8.5 Hz, *J*=1.6 Hz, **ArH**), 8.05 (d, 1H, *J*=15.3 Hz, **C=C**), 8.20 (d, 1H, *J*=15.3 Hz, **C=C**); ppm; MS (ESI): *m/z* 436 $(M+H)^+$; IR (KBr) (ν_{max}/cm^{-}): 2928, 2835, 1649, 1595, 1572, 1518, 1419, 1348, 1248, 1186, 1127, 1034, 1019, 922, 833.

(*E*)-1-(3,4-Dimethoxyphenyl)-3-(6-(4-fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4] thiadiazol-5-yl)prop-2-en-1-one (**7j**)

This compound was prepared according to the method described for compound (**7a**), employing 6-(4-fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**5b**) (261 mg, 1 mmol) and 1-(3,4-dimethoxyphenyl)ethanone (**6b**) (180 mg, 1 mmol) to obtain

the pure product (**7j**) as a yellow solid (330 mg, 78%); mp 166-168 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.87 (s, 3H, **CH₃**), 3.99 (s, 6H, **OCH₃**), 6.98 (d, 1H, *J*=8.4 Hz, **ArH**), 7.16-7.22 (m, 3H, **ArH**), 7.67-7.77 (m, 3H, **ArH**), 8.01 (d, 1H, *J*=15.1 Hz, **C=C**), 8.24 (d, 1H, *J*=15.1 Hz, **C=C**); ^{13}C NMR (400 MHz, $CDCl_3$): δ 188.2, 164.0, 162.0, 153.1, 149.3, 131.5, 130.4, 129.6, 127.8, 122.9, 119.4, 115.9, 110.7, 56.0, 18.1 ppm; MS (ESI): *m/z* 424 $(M+H)^+$; IR (KBr) (ν_{max}/cm^{-}): 2922, 2852, 1744, 1657, 1577, 1520, 1446, 1420, 1316, 1262, 1220, 1165, 1146, 1022, 963, 814.

(*E*)-3-(6-(4-Chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one (**7k**)

This compound was prepared according to the method described for compound (**7a**), employing 6-(4-chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**5c**) (277 mg, 1 mmol) and 1-(3,4-dimethoxyphenyl)ethanone (**6b**) (180 mg, 1 mmol) to obtain the pure product (**7k**) as a yellow solid (356 mg, 81%); mp 177-179 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.86 (s, 3H, **CH₃**), 3.99 (s, 6H, **OCH₃**), 6.98 (d, 1H, *J*=9.1 Hz, **ArH**), 7.47 (d, 2H, *J*=8.3 Hz, **ArH**), 7.67-7.70 (m, 3H, **ArH**), 7.75 (dd, 1H, *J*=9.1, *J*=2.3 Hz, **ArH**), 8.00 (d, 1H, *J*=15.1 Hz, **C=C**), 8.24 (d, 1H, *J*=15.0 Hz, **C=C**); ^{13}C NMR (300 MHz, $CDCl_3$): δ 188.2, 160.3, 153.2, 149.1, 134.6, 131.9, 129.8, 127.6, 122.9, 121.5, 119.6, 110.7, 109.9, 55.9, 18.1 ppm; MS (ESI): *m/z* 440 $(M+H)^+$; IR (KBr) (ν_{max}/cm^{-}): 2959, 2918, 1650, 1593, 1575, 1521, 1486, 1422, 1349, 1295, 1280, 1197, 1057, 1021, 917, 830.

(*E*)-3-(6-Chloro-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one (7l)

This compound was prepared according to the method described for compound (**7a**), employing 6-chloro-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**5d**) (201 mg, 1 mmol) and 1-(3,4-dimethoxyphenyl)ethanone (**6b**) (180 mg, 1 mmol) to obtain the pure product (**7l**) as a yellow solid (254 mg, 70%); mp 160-162 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.84 (s, 3H, **CH₃**), 3.98 (s, 6H, **OCH₃**), 6.97 (d, 1H, *J*=8.3 Hz, **ArH**), 7.66 (s, 1H, **ArH**), 7.72 (dd, 1H, *J*=8.3 Hz, *J*=1.9 Hz, **ArH**), 7.86 (d, 1H, *J*=15.4 Hz, **C=C**), 8.05 (d, 1H, *J*=15.4 Hz, **C=C**) ppm; MS (ESI): *m/z* 364 $(M+H)^+$; IR (KBr) (ν_{max}/cm^{-}): 2934, 2832, 1662, 1593, 1509, 1428, 1391, 1345, 1311, 1252, 1226, 1126, 1039, 990, 813.

(*E*)-1-(4-Fluorophenyl)-3-(6-(4-fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)prop-2-en-1-one (7m)

This compound was prepared according to the method described for compound (**7a**), employing 6-(4-fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**5b**)

(261 mg, 1 mmol) and 1-(4-fluorophenyl)ethanone (**6c**) (138 mg, 1 mmol) to obtain the pure product (**7m**) as a yellow solid (301 mg, 79%); mp 153-155 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.90 (s, 3H, **CH_3**), 7.13-7.22 (m, 2H, **ArH**), 7.57 (d, 1H, *J*=15.5 Hz, **C=C**), 7.87-7.97 (m, 2H, **ArH**), 8.02 (d, 1H, *J*=15.5 Hz, **C=C**), 8.10 d, 2H, *J*=9.2 Hz, **ArH**), 8.18 (d, 2H, *J*=8.5 Hz, **ArH**); ^{13}C NMR (400 MHz, $CDCl_3$): δ 187.6, 176.4, 131.1, 130.6, 129.9, 128.0, 120.1, 118.9, 116.8, 116.5, 115.7, 17.5 ppm; MS (ESI): *m/z* 382 $(M+H)^+$; IR (KBr) (ν_{max}/cm^{-}): 2928, 2865, 1667, 1592, 1507, 1488, 1455, 1426, 1350, 1308, 1249, 1123, 1089, 1012, 836.

(*E*)-1-(6-(4-Methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (11a)

A mixture of 1-(6-(4-methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)ethanone (**9a**) (144 mg, 0.5 mmol) and 3,4,5-trimethoxybenzaldehyde (**10**) (98 mg, 0.5 mmol) was dissolved in 10 mL ethanol. To this mixture, pottasium hydroxide (40%, 1 mL) was added at 0-5 ^{o}C. The reaction mixture was stirred at room temperature for 1-2 h. Then this reaction mixture was poured over crushed ice and acidified with dil HCl. The light yellow solid thus obtained was filtered, washed with water and dried. The residue was purified on column chromatography (silica gel with 50% ethyl acetate in hexane) to afford compound (**11a**) as a yellow solid (186 mg, 80%); mp 193-195 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.84 (s, 3H, **CH_3**), 3.79 (s, 6H, **OCH_3**), 3.86 (s, 6H, **CH_3**), 6.51 (s, 2H, **ArH**), 6.95 (d, 1H, *J*=15.1 Hz, **C=C**), 7.10 (dd, 2H, *J*=9.1 Hz, *J*=2.3 Hz, **ArH**), 7.65-7.71 (m, 3H, **ArH**, **C=C**), ^{13}C NMR (300 MHz, $CDCl_3$): δ 178.4, 160.9, 153.2, 152.2, 142.1, 131.6, 130.3, 126.7, 125.3, 123.7, 113.7, 105.1, 102.4, 60.9, 55.9, 55.2, 18.1 ppm; MS (ESI): *m/z* 466 $(M+H)^+$; HRMS (ESI): calcd for $C_{24}H_{24}N_3O_5S$ $(M+H)^+$ 466.1431; found: 466.1424; IR (KBr) (ν_{max}/cm^{-}): 2937, 2836, 2352, 1650, 1608, 1580, 1504, 1453, 1416, 1377, 1326, 1250, 1176, 1125, 1066, 1002, 837.

(*E*)-1-(6-(4-Fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (11b)

This compound was prepared according to the method described for compound (**11a**), employing 1-(6-(4-fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)ethanone (**9b**) (137 mg, 0.5 mmol) and 3,4,5-trimethoxybenzaldehyde (**10**) (98 mg, 0.5 mmol) to obtain the pure product (**11b**) as a yellow solid (172 mg, 76%); mp 171-173 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.85 (s, 3H, **CH_3**), 3.81 (s, 6H, **OCH_3**), 3.87 (s, 3H, **OCH_3**), 6.53 (s, 2H, **ArH**), 6.96 (d, 1H, *J*=15.9 Hz, **C=C**), 7.16-7.22 (m, 2H, **ArH**), 7.69-7.78 (m, 3H, **ArH**, **C=C**); ^{13}C NMR (300 MHz, $CDCl_3$): δ 178.3, 161.2, 153.3, 151.0, 142.8, 140.3, 139.9, 132.2, 130.5,

125.5, 123.4, 115.1, 105.3, 60.9, 56.0, 18.2 ppm; MS (ESI): *m/z* 454 $(M+H)^+$; HRMS (ESI): calcd for $C_{23}H_{21}FN_3O_4S$ $(M+H)^+$ 454.1237; found: 454.1231; IR (KBr) (ν_{max}/cm^-): 2935, 2836, 1653, 1595, 1527, 1505, 1485, 1419, 1390, 1326, 1243, 1156, 1127, 1066, 1004, 844.

(*E*)-1-(6-(4-Chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-3-(3,4,5-tri methoxyphenyl)prop-2-en-1-one (**11c**)

This compound was prepared according to the method described for compound (**11a**), employing 1-(6-(4-chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)ethanone (**9c**) (145 mg, 0.5 mmol) and 3,4,5-trimethoxybenzaldehyde (**10**) (98 mg, 0.5 mmol) to obtain the pure product (**11c**) as a yellow solid (169 mg, 72%); mp 189-191 ^oC; ^1H NMR (400 MHz, $CDCl_3$): δ 2.52 (s, 3H, **CH_3**), 3.83 (s, 3H, **OCH_3**), 3.87 (s, 6H, **OCH_3**), 6.59 (s, 2H, **ArH**), 6.71 (d, 1H, *J*=15.3 Hz, **C=C**), 7.41 (d, 2H, *J*=8.3 Hz, **ArH**), 7.61 (d, 2H, *J*=8.3 Hz, **ArH**), 7.90 (d, 1H, *J*=15.3 Hz, **C=C**) ppm; ^{13}C NMR (300 MHz, $CDCl_3$): δ 188.6, 160.4, 153.0, 149.1, 142.4, 134.7, 133.6, 129.9, 129.0, 128.1, 121.4, 119.7, 106.0, 60.9, 56.2, 18.1 ppm MS (ESI): *m/z* 470 $(M+H)^+$ HRMS (ESI): calcd for $C_{23}H_{21}ClN_3O_4S$ $(M+H)^+$ 470.0941; found: 470.0935; IR (KBr) (ν_{max}/cm^-): 2933, 2842, 1691, 1588, 1508, 1458, 1429, 1347, 1239, 1126, 1004, 843.

(*E*)-1-(6-Chloro-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**11d**)

This compound was prepared according to the method described for compound (**11a**), employing 1-(6-chloro-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)ethanone (**9d**) (108 mg, 0.5 mmol) and 3,4,5-trimethoxybenzaldehyde (**10**) (98 mg, 0.5 mmol) to obtain the pure product (**11d**) as a yellow solid (134 mg, 68%); mp 169-171 ^oC; ^1H NMR (300 MHz, $CDCl_3$): δ 2.84 (s, 3H, **CH_3**), 3.91 (s, 3H, **OCH_3**), 3.93 (s, 6H, **OCH_3**), 6.90 (s, 2H, **ArH**), 7.10 (d, 1H, *J*=15.9 Hz, **C=C**), 7.80 (d, 1H, *J*=15.9 Hz, **C=C**) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ 188.7, 153.1, 142.4, 133.4, 126.9, 126.2, 121.0, 120.0, 119.6, 106.0, 60.9, 56.3, 17.8 ppm; MS (ESI): *m/z* 394 $(M+H)^+$ HRMS (ESI): calcd for $C_{23}H_{21}ClN_3O_4S$ $(M+H)^+$ 470.0941; found: 470.0935; IR (KBr) (ν_{max}/cm^-): 2932, 2836, 2358, 1660, 1590, 1494, 1455, 1351, 1249, 1174, 1123, 1030, 1013, 838.

BIOLOGY

CYTOTOXIC ACTIVITY ASSAY USING MTT ASSAY

This assay is a quantitative colorimetric method for determination of cell survival and proliferation. The assessed parameter is the metabolic activity of viable cells. Metabolically

active cells reduce pale yellow tetrazolium salt (MTT) to a dark blue water-insoluble formazan which can be directly quantified after solubilisation with DMSO. The absorbance of the formazan directly correlates with the number of viable cells. The cells were plated in 96-well plates at a density of 2.0 x 10^4 in 200 µl of medium per well of 96 well plate. Cultures were incubated with different concentrations of test material and incubated for 48h. The medium was replaced with fresh medium containing 100µg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) for 2-3 h. The supernatant was aspirated and MTT-formazon crystals dissolved in 100 µl DMSO; OD measured at λ540 nm (reference wavelength, λ620 nm) on ELISA reader Cell viability % was calculated by comparing the absorbance of treated versus untreated cells.

CELL-CYCLE ANALYSIS

Prostate cancer cells (DU-145) were incubated for 24 h in the presence or absence of test compounds **11a**, **11b** and doxorubicin (3 µM). Cells were harvested with trypsin EDTA and fixed in ice-cold 70% ethanol at 4 ^{0}Cfor 30 min; ethanol was removed by centrifugation, and cells were stained with 1 mL of DNA staining solution (2 mg of RNase A and 0.2 mg of propidium iodide (PI)) for 30 min.The DNA content of 20000 events were measured by flow cytometry (BD FACS C anto II). Histograms were analyzed using FCS express 4 plus.[37]

HOECHST-33258 STAINING

DU-145 cells were incubated for a period of 24 h in the presence or absence of test compounds **11a**, **11b** and doxorubicin (3 µM). At the end of treatment, the medium was aspirated, cells were washed with medium without FBS, and Hoechst-33258 stain (Invitrogen cat. no. H3570) was added to the cells for 20 min at 378C under humidified atmosphere. Cells were washed again with medium. The cells were covered with medium and observed under a fluorescence microscope equipped with DAPI filter.[38]

CASPASE-3, -8 LIKE PROTEOLYTIC ACTIVITIES

After the treatments with in the presence or absence of compounds , cells were washed twice in cold DPBS and lysed in buffer containing 10 mmol/L of Tris-HCl, 10 mmol/L of NaH_2PO_4/Na_2HPO_4 (pH 7.5), 130 mmol/L of NaCl, 1% Triton, and 10 mmol/L of sodium pyrophosphate. Cell lysates were incubated with caspase-3 and -8 fluorogenic substrate *N*-acetyl-DEVD-7-amido-4-trifluoromethylcoumarin and Ac-IETD-AFC respectively at 37°C for 1 h. 7-Amido-4-trifluoromethylcoumarin liberated from the substrates was measured using a fluorescence plate reader (Tecan) with λ_{ex}=400 nm and λ_{em}=505 nm. The fluorescence intensity was normalized to the protein levels measured with the Bradford protein assay.[39]

WESTERN BLOT ANALYSIS

After the treatment of DU-145 cells with compounds **11a**, **11b** and doxorubicin at 3 µM, cells were washed cells were washed with ice cold phosphate-buffered saline (PBS) and homogenised in 100 µL of radioimmunoprecipitation assay (RIPA) buffer (20 mm Tris·HCl, pH 7.4, 2.5 mm EDTA, 1% Triton X-100, 1% sodium deoxycholate, 1% SDS, 100 mm NaCl, 100 mm NaF) containing 1 mm sodium orthovanadate and a mixture of protease inhibitors.The homogenate was centrifuged at 750 g for 10 min at 48C to pellet out the nuclei. The remaining supernatant was centrifuged for 30 min at 12000 g. Proteins were resolved on SDS-PAGE and blotted onto nitrocellulose membranes. Membranes were probed with Cdk4, p21, p27, CyclinD1, CyclinE and GAPDH procured from Cell Signaling Technology. The detection was carried out with HRP-labelled rabbit anti-rabbit/mouse IgG (Amersham) using an enhanced chemiluminescence detection system (ECL Advanced Kit, GE Health care).