Design of First-in-Class Dual EZH2/HDAC Inhibitor: Biochemical Activity and Biological Evaluation in Cancer Cells

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Supporting Information

Contents

Experimental Section:

EXPERIMENTAL SECTION

Chemistry. Melting points were determined on a Buchi 530 melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz on a Bruker AC 400 spectrometer; chemical shifts are reported in δ (ppm) units relative to the internal reference tetramethylsilane (Me4Si). EIMS spectra were recorded with a Fisons Trio 1000 spectrometer; only molecular ions (M+) and base peaks are given. All compounds were routinely checked by TLC, ^{1}H NMR, and Mass spectra. TLC were performed on aluminumbacked silica gel plates (Merck DC, Alufolien Kieselgel 60 F254) with spots visualized by UV light. All solvents were reagent grade and, when necessary, were purified and dried by standard methods. Concentration of solutions after reactions and extractions involved the use of a rotary evaporator operating at reduced pressure of ca. 20 Torr. Organic solutions were dried over anhydrous sodium sulfate. Elemental analysis has been used to determine purity of the final described compounds, that is, >95%. Analytical results are within ±0.40% of the theoretical values (Table S1). All chemicals were purchased from Sigma Aldrich Chimica, Milan (Italy), or from Alfa Aesar, Karlsruhe (Germany), and were of the highest purity.

Procedure for the synthesis of the *tert*-butyl ester 5-methyl-1-(3-nitrophenyl)-1*H*-pyrazole-4-carboxylate (6). (3-Nitrophenyl)hydrazine hydrochloride (5.67 mmol, 1.0 eq, 1.07 g) and triethylamine (6.24 mmol, 1.1 eq, 0.87 mL) were added to a solution of *tert*-butyl (*Z*)-2-((dimethylamino)methylene)-3-oxobutanoate (5.67 mmol, 1.0 eq, 1.20 g) in dry ethanol (10 mL) and the resulting mixture was heated to 80 °C for 1 h. The reaction was quenched with water (50 mL) and the product **6** precipitated as a brownish solid that was filtered, dried in oven and triturated in petroleum ether. Yield = 87%; m.p. = 101-102 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): δ 1.56 (9H, s, -COOC(C*H*$_3$)$_3$), 2.47 (3H, s, pyrazole -C*H*$_3$), 7.68-7.77 (1H, m, benzene proton), 7.87-7.94 (1H, m, benzene proton), 8.07 (1H, s, pyrazole proton), 8.14-8.22 (1H, benzene proton), 8.44-8.50 (1H, m, benzene proton). MS (ESI): *m/z* $[M+H]^+$: 304.12.

Procedure for the synthesis of the 2,5-dimethyl-1-(3-nitrophenyl)-1*H*-pyrrole-3-carboxylic acid (7). In a sealed tube, 2,5-dimethyl-1-(3-nitrophenyl)-1*H*-pyrrole (18.6 mmol, 1.0 eq, 4.02 g) was dissolved in dry dichloroethane (20 mL) and the solution was cooled to 0 °C. Trichloroacetyl chloride (55.8 mmol, 3.0 eq, 6.23 mL) was added dropwise. The solution was stirred at 0 °C for 10 min, let warm to room temperature and finally heated to 70 °C for 3 h. After this time, the volatiles were removed *in vacuo*. The residue was dissolved in a 1:1 EtOH:THF mixture. The solution was cooled to 0 °C and a 2N aqueous solution of KOH (186 mmol, 10 eq, 10.4 g) was added dropwise. The reaction mixture was stirred at room temperature for 1 h. After this time, the organic solvents were removed *in vacuo*. The byproducts were removed by extraction from the basic aqueous layer with

ethyl acetate (3 × 20 mL). The aqueous layer was then cooled to 0 °C and acidified till pH 2 by addition of 2N HCl aqueous solution. The brownish precipitated solid **7** was filtered, rinsed with distilled water and dried in oven (60 °C). Yield = 41.3%; m. p. = 109-110 °C. ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 1.96 (3H, s, pyrrole -C*H*$_3$), 2.24 (3H, s, pyrrole -C*H*$_3$), 6.28 (1H, s, pyrrole proton), 7.84-89 (2H, m, benzene protons), 8.16-8.19 (1H, m, benzene proton), 8.36-8.39 (1H, m, benzene proton), 11.78-11.81 (1H, bs, COO*H*) ppm. MS (ESI): *m/z* [M-H]$^-$: 260.08.

Procedure for the synthesis of *tert*-butyl 2,5-dimethyl-1-(3-nitrophenyl)-1*H*-pyrrole-3-carboxylate (8). *N*,*N*-Dimethylformamide di-*tert*-butyl acetal (23.1 mmol, 3.0 eq, 5.5 mL) was added to a solution of 2,5-dimethyl-1-(3-nitrophenyl)-1*H*-pyrrole-3-carboxylic acid **7** (7.68 mmol, 1.0 eq, 2.0 g) in dry toluene (10 mL). The resulting mixture was stirred at 90 °C for 4 h. After this time, the reaction was quenched with brine (20 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine (3 × 10 mL), dried with sodium sulphate, filtered and evaporated *in vacuo*. The reaction crude was purified by column chromatography (SiO_2 eluting with chloroform/methanol 20/1) to give the pure **8**. Yield = 72%; m. p. = 98-99 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): δ 1.49 (9H, s, -COOC(C*H*$_3$)$_3$), 1.93 (3H, s, pyrrole -C*H*$_3$), 2.22 (3H, s, pyrrole -C*H*$_3$), 6.30 (1H, s, pyrrole proton), 7.46-7.49 (1H, m, benzene proton), 7.60-7.68 (1H, t, benzene proton), 8.00-8.03 (1H, t, benzene proton), 8.24-8.29 (1H, m, benzene proton) ppm. MS (ESI): *m/z* [M+H]$^+$: 317.14.

General procedure for the synthesis of *tert*-butyl 1-(3-aminophenyl)-1*H*-pyrazole/pyrrole-carboxylates (9, 10). Example: *tert*-butyl 1-(3-aminophenyl)-5-methyl-1*H*-pyrazole-4-carboxylate (9). Metallic zinc (34.2 mmol, 7.25 eq, 2.23 g) and ammonium chloride (9.43 mmol, 2.0 eq, 0.504 g) were added to a solution of *tert*-butyl 5-methyl-1-(3-nitrophenyl)-1*H*-pyrazole-4-carboxylate **6** (4.71 mmol, 1.0 eq, 1.43 g) in 1:1 1,4-dioxane:water mixture. The resulting solution was stirred at 50 °C for 2 h. The reaction was subsequently quenched with a saturated solution of sodium carbonate (20 mL) and extracted with dichloromethane (3 × 10 mL). The combined organic phases were washed with brine (3 × 10 mL), dried with sodium sulphate, filtered and evaporated under reduced pressure to obtain a crude product that was purified by column chromatography (SiO_2 eluting with *n*-hexane/ethyl acetate 2.5/1) to give the pure **9**. Yield = 87.7%; m. p. = 109-110 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): δ 1.56 (9H, s, -COOC(C*H*$_3$)$_3$), 2.46 (3H, s, pyrazole -C*H*$_3$), 4.54 (2H, bs, -N*H*$_2$), 6.62-6.65 (1H, m, benzene proton), 7.27-7.32 (1H, m, benzene proton), 7.34-7.42 (2H, m, benzene protons), 8.00 (1H, s, pyrazole proton) ppm. MS (ESI): *m/z* [M+H]$^+$: 274.15.

***Tert*-butyl 1-(3-aminophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylate (10).** Synthesized from **8** as described for **9**. Yield = 78.2%; m. p. = 123-124 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): δ 1.49 (9H, s, -COOC(C*H*$_3$)$_3$), 1.91 (3H, s, pyrrole -C*H*$_3$), 2.21 (3H, s, pyrrole -C*H*$_3$), 3.74 (2H, bs, -N*H*$_2$),

6.22 (1H, s, pyrrole proton), 6.35-6.39 (1H, m, benzene proton), 6.44-6.48 (1H, m, benzene proton), 6.64-6.68 (1H, m, benzene proton) ppm. MS (ESI): *m/z* $[M+H]^+$: 287.17.

General procedure for the synthesis of the *tert*-butyl 1-(3-(8-methoxy-8-oxooctanamido)phenyl)-1*H*-pyrazole/pyrrole-carboxylates (11, 12). Example: *tert*-butyl 1-(3-(8-methoxy-8-oxooctanamido)phenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylate (12). Triethylamine (2.6 mmol, 2.5 eq, 0.36 mL) and methyl 8-chloro-8-oxooctanoate (2.09 mmol, 2.0 eq, 0.3 mL) were added to a solution of *tert*-butyl 1-(3-aminophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylate **10** (1.05 mmol, 1.0 eq, 0.3 g) in dry dichloromethane (7 mL). The resulting mixture was stirred for 3 h at room temperature. After this time, the reaction was quenched with water (15 mL) and extracted with dichloromethane (3 × 10 mL). The combined organic layers were washed with a saturated solution of sodium carbonate (3 × 10 mL), 2N HCl aqueous solution (3 × 10 mL), and brine (3 × 10 mL), and were dried with sodium sulphate. The crude residue obtained after the evaporation of the solvent *in vacuo* was purified by column chromatography (SiO_2 eluting with ethyl acetate/*n*-hexane 1/2) to give the pure **12** as a dense yellow liquid. Yield = 91.4%. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): δ 1.36-1.41 (4H, m, -C*H*$_2$C*H*$_2$), 1.58 (9H, s, -COOC(C*H*$_3$)$_3$), 1.63-1.67 (2H, m, -C*H*$_2$), 1.75-1.79 (2H, m, -C*H*$_2$) 1.99 (3H, s, pyrrole -C*H*$_3$), 2.28 (3H, s, pyrrole -C*H*$_3$), 2.32-2.36 (2H, t, -C*H*$_2$), 2.42-2.45 (2H, t, -C*H*$_2$), 3.68 (3H, s, -COOC*H*$_3$), 6.32 (1H, s, pyrrole proton), 6.91 (1H, d, benzene proton), 7.43 (2H, m, benzene protons), 7.49 (1H, s, -CON*H*-), 7.63 (1H, d, benzene proton) ppm. MS (ESI): *m/z* $[M+H]^+$: 267.26.

***Tert*-butyl 1-(3-(8-methoxy-8-oxooctanamido)phenyl)-5-methyl-1*H*-pyrazole-4-carboxylate (11).** Synthesized from **9** as described for **12**. Yield = 87.4%. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): δ 1.31-1.39 (4H, m, -NHCOCH$_2$CH$_2$C*H*$_2$C*H*$_2$CH$_2$CH$_2$COOCH$_3$), 1.56 (9H, s, -COOC(C*H*$_3$)$_3$), 1.57-1.69 (4H, m, -NHCOCH$_2$C*H*$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$COOCH$_3$), 2.26-2.37 (4H, m, -NHCOC*H*$_2$- and -C*H*$_2$COOCH$_3$), 2.46 (3H, s, pyrazole -C*H*$_3$), 3.64 (3H, s, -COOC*H*$_3$), 7.41-7.48 (1H, m, benzene proton), 7.50-7.59 (2H, m, benzene protons), 7.81-7.85 (1H, m, benzene proton), 8.05 (1H, s, pyrazole proton), 8.64 (1H, s, -CON*H*-) ppm. MS (ESI): *m/z* $[M+H]^+$: 444.24.

General procedure for the synthesis of the 1-(3-(8-methoxy-8-oxooctanamido)phenyl)-1*H*-pyrazole/pyrrole-carboxylic acids (13, 14). Example: 1-(3-(8-methoxy-8-oxooctanamido)phenyl)-5-methyl-1*H*-pyrazole-4-carboxylic acid (13). The *tert*-butyl 1-(3-(8-methoxy-8-oxooctanamido)phenyl)-5-methyl-1*H*-pyrazole-4-carboxylate **11** (0.753 mmol, 1.0 eq, 0.334 g) was dissolved in dry dichloromethane (5 mL) and cooled to 0 °C. Trifluoroacetic acid (3.76 mmol 5.0 eq, 0.288 mL) was slowly added and the resulting solution was first stirred at 0 °C for 10 min and then let warm to room temperature. The reaction was monitored and other little addictions of trifluoroacetic acid have been made at 0 °C (15 eq were added overall). When TLC analysis showed

the complete conversion of the starting material, the reaction was quenched with water (15 mL) and extracted by dichloromethane (3 × 10 mL). The combined organic phases were washed with brine (3 × 10 mL), dried with sodium sulphate, filtered and evaporated under reduced pressure. Trituration in petroleum ether allowed to obtain the pure acid **13**. Yield = 77.0%; m. p. = 127-128 °C. ^{1}H NMR (DMSO-d_6, 400 MHz, δ; ppm): δ 1.28-1.42 (4H, m, -NHCOCH$_2$CH$_2$C*H*$_2$C*H*$_2$CH$_2$CH$_2$COOCH$_3$), 1.52-1.71 (4H, m, -NHCOCH$_2$C*H*$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$COOCH$_3$), 2.24-2.37 (4H, m, -NHCOC*H*$_2$- and -C*H*$_2$COOCH$_3$), 2.62 (3H, s, pyrazole -C*H*$_3$), 3.63 (3H, s, -COOC*H*$_3$), 7.37-7.45 (2H, m, benzene protons), 7.56-7.61 (1H, m, benzene proton), 7.87-7.91 (1H, m, benzene proton), 8.09 (1H, s, pyrazole proton), 9.70 (1H, s, -CON*H*-), 12.66 (1H, bs, -COO*H*) ppm. MS (ESI): *m/z* [M-H]$^-$: 387.18.

1-(3-(8-methoxy-8-oxooctanamido)phenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (14). Synthesized from **12** as described for **13**. Yield = 85.2%, m.p. = 138-139 °C. ^{1}H NMR (DMSO-d_6, 400 MHz, δ; ppm): δ 1.27-1.40 (4H, m, -NHCOCH$_2$CH$_2$C*H*$_2$C*H*$_2$CH$_2$CH$_2$COOCH$_3$), 1.52-1.70 (4H, m, -NHCOCH$_2$C*H*$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$COOCH$_3$), 2.16 (3H, s, pyrrole -C*H*$_3$), 2.24-2.37 (4H, m, -NHCOC*H*$_2$- and -C*H*$_2$COOCH$_3$), 2.43 (3H, s, pyrrole -C*H*$_3$), 3.63 (3H, s, -COOC*H*$_3$), 5.87 (1H, s, pyrrole proton), 7.25-7.32 (1H, m, benzene proton), 7.34-7.44 (2H, m, benzene protons), 7.68-7.73 (1H, m, benzene proton), 9.65 (1H, s, -CON*H*-), 12.42 (1H, bs, -COO*H*) ppm. MS (ESI): *m/z* [M-H]$^-$: 400.20.

General procedure for the synthesis of the methyl 8-((3-(((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)carbamoyl)-1*H*-pyrazole/pyrrole-1-yl-phenyl)amino)-8-oxooctanates (15, 16). Example: methyl 8-((3-(3-(((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)carbamoyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)phenyl)amino)-8-oxooctanoate (16). The 1-(3-(8-methoxy-8-oxooctanamido)phenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid **14** (0.580 mmol, 1.0 eq, 0.232 g) was dissolved in dry *N*,*N*-dimethylformamide (5 mL). Triethylamine (4.05 mmol, 7.0 eq, 0.57 mL) and 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU Reagent) (0.695 mmol, 1.2 eq, 0.223 g) were added under nitrogen atmosphere and the mixture was stirred for 40 min. After this time the (4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methylamine hydrochloride (0.638 mmol, 1.1 eq, 0.120 g) was added under nitrogen atmosphere. The resulting solution was stirred at room temperature overnight. The day after the reaction was quenched with brine (10 mL) and the precipitated colorless solid was filtered, dried in oven and purified by column chromatography (SiO_2 eluting with chloroform/methanol 30/1) to give the pure intermediate **16**. Yield = 71%; m.p. = 167-168 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): δ 1.31-1.39 (4H, m, -NHCOCH$_2$CH$_2$C*H*$_2$C*H*$_2$CH$_2$CH$_2$COOCH$_3$), 1.54-1.71 (4H, m, -NHCOCH$_2$C*H*$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$COOCH$_3$), 2.07 (3H, s, pyrrole -C*H*$_3$), 2.10 (3H, s, pyridone -C*H*$_3$), 2.23 (3H, s, pyridone -C*H*$_3$), 2.25-2.37 (4H, m, -NHCOC*H*$_2$- and -C*H*$_2$COOCH$_3$), 2.45 (3H, s, pyrrole

-C*H*$_3$), 3.59 (3H, s, -COOC*H*$_3$), 4.42 (1H, s, -CONHC*H*$_2$-), 5.83 (1H, s, pyrrole proton), 6.03 (1H, s, pyridone –C*H*-), 7.24-7.30 (1H, m, benzene proton), 7.36-7.46 (2H, m, benzene protons), 7.52.7.57 (1H, m, benzene proton), 8.54-8.61 (1H, t, -CON*H*CH$_2$-), 9.17 (1H, s, -N*H*CO-), 10.06 (1H, s, -N*H* pyridone) ppm. MS (ESI): *m/z* $[M+H]^+$: 535.28.

Methyl 8-((3-(4-(((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)carbamoyl)-5-methyl-1H-pyrazol-1-yl)phenyl)amino)-8-oxooctanoate (15). Synthesized from **13** as described for **16**. Yield = 75.4%, m.p. = 165-166 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): δ 1.29-1.41 (4H, m, -NHCOCH$_2$CH$_2$C*H*$_2$C*H*$_2$CH$_2$CH$_2$COOCH$_3$), 1.54-1.71 (4H, m, NHCOCH$_2$C*H*$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$COOCH$_3$), 2.11 (3H, s, pyridone -C*H*$_3$), 2.23 (3H, s, pyridone -C*H*$_3$), 2.25-2.38 (4H, m, -NHCOC*H*$_2$- and -C*H*$_2$COOCH$_3$), 2.61 (3H, s, pyrazole -C*H*$_3$), 3.64 (3H, s, -COOC*H*$_3$), 4.27 (1H, d, -CONHC*H*$_2$-), 6.03 (1H, s, pyridone –C*H*-), 7.41-7.48 (1H, m, benzene proton), 7.49-7.61 (2H, m, benzene protons), 7.82.7.88 (1H, m, benzene proton), 8.00 (1H, s, pyrazole proton), 8.60-8.67 (1H, t, -CON*H*CH$_2$-), 9.24 (1H, s, -N*H*CO-), 10.08 (1H, s, -N*H* pyridone) ppm. MS (ESI): *m/z* $[M+H]^+$: 522.26.

General procedure for the Synthesis of the 8-((3-(((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)carbamoyl)-1*H*-pyrazole/pyrrole-1-yl-phenyl)amino)-8-oxooctanoic acids (17, 18). Example: 8-((3-(4-(((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)carbamoyl)-5-methyl-1*H*-pyrazol-1-yl)phenyl)amino)-8-oxooctanoic acid (17). An aqueous solution of lithium hydroxide monohydrate (0.399 mmol, 2.0 eq, 0.017 g) was added to a solution of methyl 8-((3-(4-(((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)carbamoyl)-5-methyl-1*H*-pyrazol-1-yl)phenyl)amino)-8-oxooctanoate **15** (0.199 mmol, 1.0 eq, 0.104 g) in tetrahydrofuran (3 mL). The resulting mixture was stirred at room temperature overnight. The day after the reaction was complete. The tetrahydrofuran was evaporated *in vacuo* and the 2N HCl solution was added until pH 2 at 0 °C. The product **17** precipitated as a colorless solid that was filtered and dried in oven. Yield = 50.1%; m.p. = 142-143 °C. ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 1.27-1.42 (4H, m, NHCOCH$_2$CH$_2$C*H*$_2$C*H*$_2$CH$_2$CH$_2$COOCH$_3$), 1.49-1.70 (4H, m, NHCOCH$_2$C*H*$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$COOCH$_3$), 2.04 (3H, s, pyridone -C*H*$_3$), 2.22 (3H, s, pyridone -C*H*$_3$), 2.23-2.37 (4H, m, -NHCOC*H*$_2$- and -C*H*$_2$COOCH$_3$), 2.62 (3H, s, pyrazole -C*H*$_3$), 4.29 (2H, d,-CONHC*H*$_2$-), 6.03 (1H, s, pyridone -C*H*-), 7.36-7.46 (2H, m, benzene proton), 7.55-7.61 (1H, m, benzene proton), 7.86-7.91 (1H, m, benzene proton), 8.00 (1H, s, pyrazole proton), 8.91-8.98 (1H, m, -CON*H*CH$_2$-), 9.70 (1H, s, -N*H*CO-), 11.31 (1H, s, pyridone -N*H*-), 11.68 (1H, bs, -COO*H*) ppm. MS (ESI): *m/z* $[M-H]^-$: 520.27

8-((3-(3-(((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)carbamoyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)phenyl)amino)-8-oxooctanoic acid (18). Synthesized from **16** as described for **17**. Yield

= 47.8%, m.p. = 141-142 °C. ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 1.27-1.41 (4H, m, -NHCOCH$_2$CH$_2$C*H*$_2$C*H*$_2$CH$_2$CH$_2$COOCH$_3$), 1.49-1.70 (4H, m, -NHCOCH$_2$C*H*$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$COOCH$_3$), 2.04 (3H, d, pyridone -C*H*$_3$), 2.07 (3H, s, pyrrole -C*H*$_3$), 2.22 (3H, s, pyridone -C*H*$_3$), 2.23-2.37 (4H, m, -NHCOC*H*$_2$- and -C*H*$_2$COOCH$_3$), 2.43 (3H, s, pyrrole -C*H*$_3$), 4.27 (2H, d,- CONHC*H*$_2$-), 5.83 (1H, s, pyrrole proton), 6.03 (1H, s, pyridone -C*H*-), 7.25-7.32 (1H, m, benzene proton), 7.35-7.43 (2H, m, benzene protons), 7.69-7.74 (1H, m, benzene proton), 8.99-9.03 (1H, t, -CON*H*CH$_2$-), 9.65 (1H, s, -N*H*CO-), 11.31 (1H, s, pyridone -N*H*-), 11.69 (1H, bs, -COO*H*) ppm. MS (ESI): *m/z* [M-H]$^-$: 507.25.

General procedure for the synthesis of *N*^{1}-(3-(4-(((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)carbamoyl)-5-methyl-1H-pyrazol-1-yl)phenyl)-*N*^{8}-hydroxyoctanediamide (4) and *N*^{1}-(3-(3-(((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)carbamoyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)phenyl)-*N*^{8}-hydroxyoctanediamide (5). Example *N*^{1}-(3-(3-(((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)carbamoyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)phenyl)-*N*^{8}-hydroxyoctanediamide (5). The 8-((3-(3-(((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)carbamoyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)phenyl)amino)-8-oxooctanoic acid **18** (0.40 mmol, 1.0 eq, 0.203 g) was dissolved in dry *N*,*N*-dimethylformamide (3 mL). Triethylamine (1.60 mmol, 4.0 eq, 0.22 mL) and 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU Reagent) (0.481 mmol, 1.2 eq, 0.154 g) were added under nitrogen atmosphere and the mixture was stirred for 40 min. After this time the *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (1.20 mmol, 3.0 eq, 0.141 g) was added under nitrogen atmosphere. The resulting solution was stirred at room temperature for 1 h. After this time the reaction was considered complete by TLC analysis thus brine (10 mL) was added to the reaction mixture and the colorless solid of the protect hydroxamate precipitated. The solid was filtered, dried in oven and purified by column chromatography (SiO_2 eluting with chloroform/methanol 20/1) to give pure protected hydroxamate (1.0 eq, 0.203 g, 0.343 mmol) which was immediately dissolved in tetrahydrofuran (3 mL) and reacted with the 4M HCl solution in 1,4-dioxane (1.71 mmol, 5.0 eq, 0.43 mL) at 0 °C and then the precipitation of a colorless solid was observed. The resulting mixture was stirred at room temperature and the reaction was monitored. Other little addictions of the 4M HCl solution in 1,4-dioxane have been made at 0 °C (10 eq added overall). When TLC analysis showed the complete conversion of the starting material, diethyl ether was added and the solid of the final hydroxamate *N*^{1}-(3-(3-(((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)carbamoyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)phenyl)-*N*^{8}-hydroxyoctanediamide **5** was filtered and dried in oven. Yield = 36%; m. p. = 170-171 °C. ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 1.28-1.41 (4H, m, -NHCOCH$_2$CH$_2$C*H*$_2$C*H*$_2$CH$_2$CH$_2$COOCH$_3$), 1.47-1.70 (4H, m, -NHCOCH$_2$C*H*$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$COOCH$_3$), 1.98-2.00 (2H, m, -C*H*$_2$COOCH$_3$),

2.03 (3H, d, pyridone -C*H*$_3$), 2.07 (3H, s, pyrrole -C*H*$_3$), 2.22 (3H, d, pyridone -C*H*$_3$), 2.29-2.37 (2H, m, -NHCOC*H*$_2$-), 2.43 (3H, s, pyrrole -C*H*$_3$), 4.28 (2H, d, -CONHC*H*$_2$-), 5.83 (1H, s, pyrrole -C*H*-), 6.03 (1H, s, pyridone -C*H*-), 7.25-7.32 (1H, m, benzene proton), 7.35-7.43 (2H, m, benzene protons), 7.69-7.74 (1H, m, benzene proton), 8.99-9.03 (1H, t, -CON*H*CH$_2$-), 9.39 (1H, bs, -CONHO*H*), 9.65 (1H, s, -N*H*CO-), 10.06 (1H, bs, -CON*H*OH), 11.31 (1H, s, pyridone -N*H*-) ppm. ^{13}C NMR (*d*$_6$-DMSO): δ 12.04, 13.25, 18.51, 19.12, 24.71, 25.27, 29.10, 31.14, 32.77, 37.34, 104.73, 111.13, 114.23, 114.25, 115.55, 116.02, 126.64, 130.05, 130.34, 131.52, 138.62, 139.45, 151.55, 157.99, 163.61, 167.55, 170.14, 170.76 ppm. MS (ESI): *m/z* [M]$^+$: 536.28.

***N*^{1}-(3-(4-(((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)carbamoyl)-5-methyl-1*H*-pyrazol-1-yl)phenyl)-*N*^{8}-hydroxyoctanediamide (4).** Yield = 36%; m. p. = 168-170 °C. ^{1}H NMR (DMSO-*d*$_6$, 400 MHz, δ, ppm): δ 1.27-1.42 (4H, m, -NHCOCH$_2$CH$_2$C*H*$_2$C*H*$_2$CH$_2$CH$_2$COOCH$_3$), 1.47-1.70 (4H, m, -NHCOCH$_2$C*H*$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$COOCH$_3$), 1.98-2.01 (1H, m, -C*H*$_2$COOCH$_3$), 2.04 (3H, d, pyridone -C*H*$_3$), 2.22 (3H, d, pyridone -C*H*$_3$), 2.29-2.38 (2H, m, -NHCOC*H*$_2$-), 2.62 (3H, s, pyrazole -C*H*$_3$), 4.28 (2H, d,- CONHC*H*$_2$-), 6.03 (1H, s, pyridone -C*H*-), 7.36-7.46 (2H, m, benzene proton), 7.54-7.60 (1H, m, benzene proton), 7.86-7.91 (1H, m, benzene proton), 8.00 (1H, s, benzene proton), 8.91-8.98 (2H, m, -CON*H*CH$_2$-), 9.39 (1H, bs, -CONHO*H*), 9.70 (1H, s, -N*H*CO-), 10.06 (1H, bs, -CON*H*OH), 11.31 (1H, s, pyridone -N*H*-) ppm. ^{13}C NMR (*d*$_6$-DMSO): δ 10.97, 18.88, 22.64, 25.05, 25.54, 28.15, 28.18, 32.24, 36.98, 37.39, 109.73, 110.79, 112.95, 118.62, 120.36, 120.49, 129.49, 136.41, 137.57, 138.83, 140.89, 141.21, 150.76, 163.52, 164.56, 170.65, 174.34. MS (ESI): *m/z* [M+H]$^+$: 523.26.

Table S1. Elemental analysis of the final compounds **4** and **5**.

compd	MW	%, calculated			%, found		
		C	H	N	C	H	N
4	522.61	62.05	6.56	16.08	62.38	6.71	15.77
5	535.65	65.03	6.96	13.07	64.82	6.87	13.39

Biochemical assays

Biochemical inhibition screening against HDAC1-6, -8 and EZH2/PRC2 were performed according to our previous descriptions.[1, 2]

Cell lines and culture conditions

U937, NB4 and THP1 human acute myeloid leukemia cell lines, SH-N-SK neuroblastoma, U-87 glioblastoma cell lines were maintained in RPMI 1640 medium. RH4 fusion-positive alveolar rhabdomyosarcoma cell line was cultured in DMEM. Media were both supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine and 1% penicillin-streptomycin. Cells were cultured at 37 °C in a humidified atmosphere 95% air/5% CO_2. The experiments were performed with early (3-20) cell passages.

Cell Proliferation Assay

The inhibitory effect of different drugs was assessed in a panel of acute myeloid leukemia and solid tumor cell lines protocol by quantitation of the ATP present in metabolically active cells using CellTiter-Glo Luminescent cell viability assay (Promega Italia s.r.l., Milano, Italy) in triplicate following manufacturer's instructions. Data were analyzed by the Chou-Talalay method to determine the combination index (CI), a well-established index of the interaction between two drugs.[3] CI values of <1, =1, and >1 indicate synergistic, additive, and antagonistic effects, respectively.

Western Blot Analysis

The effect of different drugs on protein acetylation and methylation mark was assessed by western blot analysis. Cells were lysed and total extracts were fractionated by SDS-PAGE, transferred to a nitrocellulose filter, and subjected to immunoblot assay, as previously described.[4, 5] Immunodetection was done using the following antibodies Ac-H3 and Hsp70/72 (EMD Millipore Corporation, Billerica, MA, USA), H3K27me3 (Cell Signaling, Beverly, MA, USA), Ac-Tubulin (K40), Vinculin, β-actin (Sigma Aldrich, Merck Life Science S.r.l., Milano, Italy) and MYOG (F5D) (Developmental Studies Hybridoma Bank at the University of Iowa, (DSHB) Iowa City, IA, USA). Horseradish peroxidase–conjugated secondary antibodies binding were visualized by enhanced chemiluminescence according to manufacturer's specification and recorded on autoradiography film (Amersham GE HEALTHCARE BioScience Corporate Piscataway, NJ, USA).

The primary antibodies used for Snail and E-cadherin immunoblotting were: α-Snail (Cell Signaling Technology, Danvers, Massachusetts), α-E-cadherin (BD transduction laboratories, Franklin Lakes, New Jersey), and α-tubulin (SantaCruz Biotechnology, Inc., CA), used as a loading control. The immune complexes were detected with horseradish peroxidase-conjugated species-specific secondary antiserum (Bio-Rad Laboratories, Hercules, CA) then by enhanced chemiluminescence reaction (Bio-Rad Laboratories, Hercules, CA).

Flow cytometric analysis of cell cycle, apoptosis and cell differentiation

Flow cytometric analyses were performed to evaluate cell cycle distribution by propidium iodide (PI) staining at the reported time points[5] and apoptosis by the Annexin V apoptosis detection kit (BD Pharmingen, San Diego, CA, USA), according to the manufacturer's recommendations. To detect myeloid differentiation, leukemic cells were analyzed for the CD11b expression by using a FITC-CD11b antibody (BD bioscience) as previously described.[4]

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