Paolo Mellini, Biagina Marrocco, Diana Borovika, Lucia Polletta, Ilaria Carnevale, Serena Saladini, Giulia Stazi, Clemens Zwergel, Peteris Trapencieris, Elisabetta Ferretti, Marco Tafani,* Sergio Valente,* and Antonello Mai*

Pyrazole-based inhibitors of Enhancer of Zeste Homolog 2 induce apoptosis and autophagy in cancer cells

SUPPLEMENTARY MATERIAL

Content:

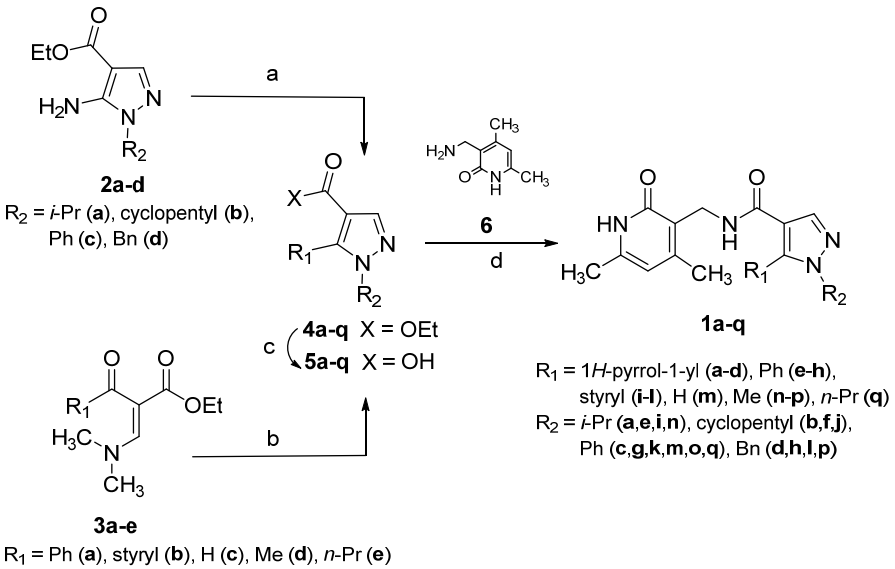
Chemistry

The ethyl esters **4a-q**, key intermediate compounds for the synthesis of the desired pyrazoles **1a-q**, were prepared either by Clauson-Kaas reaction between the known 5-amino-4-ethoxycarbonyl pyrazoles **2a-d** [1] and 2,5-dimethoxytetrahydrofuran in glacial acetic acid at 80°C (compounds **4a-d**), or from the enamines **3a-e** prepared according to the literature [2], which underwent cyclocondensation with the proper commercial hydrazines in ethanol at 80°C (compounds **4e-q**). The esters **4a-q** were hydrolyzed in basic conditions with 2 N potassium hydroxide in ethanol to give the corresponding 4-pyrazolecarboxylic acids **5a-q**, which in presence of triethylamine, *N,N,N′,N′*-tetramethyl-*O*-(benzotriazol-1-yl)uronium tetrafluoroborate (TBTU), dry *N,N*-dimethylformamide were coupled under nitrogen atmosphere with the amine **6** [3] to yield the final amides **1a-q** (see ref. 4 for **1o**,**p**) (Scheme S1).

The *N*-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-2-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)acetamide **1r** was prepared by coupling the known 2-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)acetic acid [5] with **6** following the same procedure described for **1a-q.**

Physical, chemical and spectral data for intermediate compounds **2-5** and the final compounds **1a-r** are reported below in Tables S1-S5.

Scheme S1. Synthesis of **1a-q**.



Reagents and conditions (a) 2,5-dimethoxytetrahydrofuran, glacial acetic acid, 80°C, (1-6 h); (b) proper hydrazine, ethanol, 80°C or rt; (c) 2 N potassium hydroxide, ethanol, rt, overnight; (d) TBTU, triethylamine, dry DMF, N2, rt, overnight.

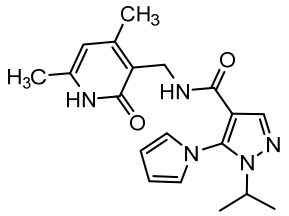
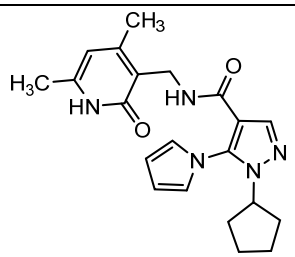
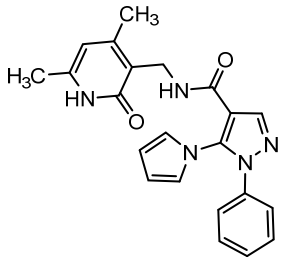
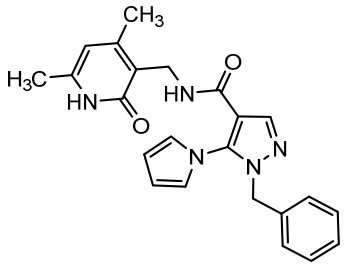
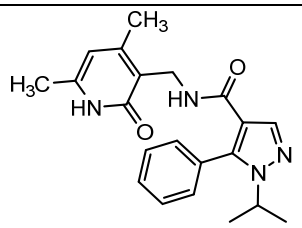
Experimental Section

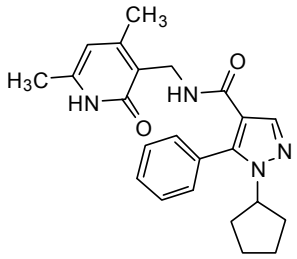
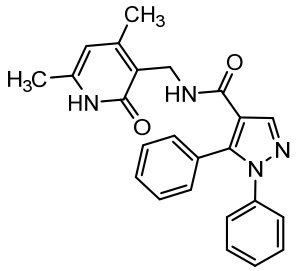
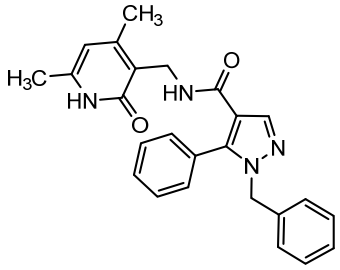
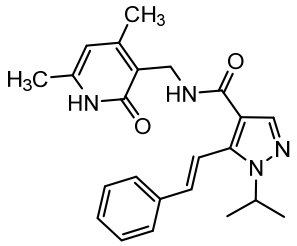
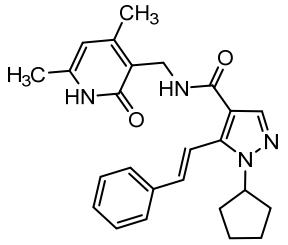
General procedure for the synthesis of 5-pyrrolyl-pyrazole ethyl esters 4a-d. 2,5-Dimethoxytetrahydrofuran (6.72 mmol) was added to a solution of the known appropriate 1-substituted ethyl 5-amino-1*H*-pyrazole-4-carboxylate **2** (4.48 mmol) in acetic acid (6 ml), and the reaction mixture was refluxed for 1 h. Then, 5 ml of toluene were added, the solution was concentrated under reduced pressure, and the residue was purified by chromatography on silica gel (eluent: ethyl acetate:*n*-hexane = 1:8) to furnish the desired pyrrole derivative (see Table S3).

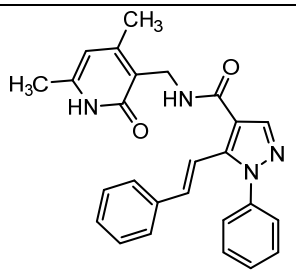
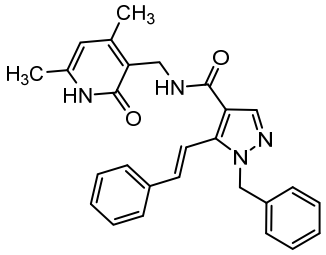
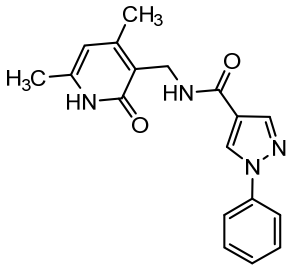
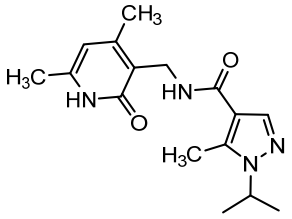
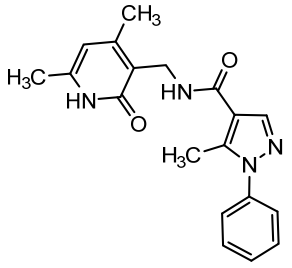
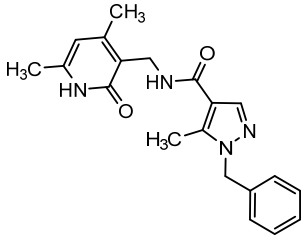
General procedure for the synthesis of pyrazole esters 4e-q. The proper hydrazine (1.01 mmol) and triethylamine (2.02 mmol) were added to a solution of the known appropriate ethyl 2-acyl-3-(dimethylamino)acrylate **3** (1.01 mmol) in ethanol (5 ml), and the reaction mixture was refluxed for 2 h. After the completion of the reaction, the solvent was evaporated by 90% and water (20 ml) was added to the residue. The aqueous phase was extracted with dichloromethane (3 × 50 ml), the organic phase washed with sodium hydrogen carbonate (2 × 15 ml) and sodium chloride (2 × 10 ml) saturated solutions, dried over sodium sulphate and concentrated under reduced pressure to obtain the ethyl 1*H*-pyrazole-4-carboxylates **4e-q** (see Table S3), differently substituted at the N1 and C5 positions, which were used in the following step without further purification.

General procedure for the synthesis of pyrazole-4-carboxylic acids 5a-q. The appropriate ethyl 1*H*-pyrazole-4-carboxylate **4** (1.82 mmol) was suspended in ethanol (6 ml), then 2 N KOH (3.64 ml) was added and the resulting mixture was left stirring at room temperature. After 16 h the reaction was complete, the ethanol was concentrated under vacuum and 2 N HCl was added to the residue till pH = 2. The obtained precipitate was filtered off, washed with distilled water (3 × 3 ml) and dried at 60°C to give the corresponding 1,5-disubstituted 1*H*-pyrazole-4-carboxylic acid **5** (see Table S4).

Table S1. Chemical and Physical Data of Compounds **1a-r**.

Cpd	Lab Code	Molecule structure	m. p. (°C)	recrystallization solvent	yield (%)
1a	MC3600		229-231	methanol	66
1b	MC3601		208-210	acetonitrile/methanol	61
1c	MC3584		232-235	methanol	68
1d	MC3589		187-189	acetonitrile	65
1e	MC3599		211-213	acetonitrile/methanol	67

1f	MC3602		213-215	acetonitrile/methanol	59
1g	MC3590		206-208	acetonitrile/methanol	57
1h	MC3598		181-183	acetonitrile	62
1i	MC3625		223-225	methanol	64
1j	MC3627		192-194	acetonitrile	63

1k	MC3648		231-233	methanol	62
1l	MC3630		195-197	acetonitrile	58
1m	MC3715		>250	methanol	67
1n	MC3690		194-196	acetonitrile	57
1o [4,6]	MC3629		225-227	methanol	60
1p [4]	MC3702		219-221	methanol	51

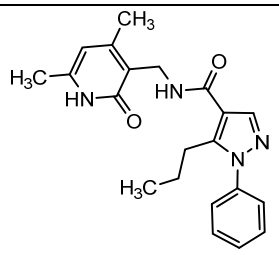
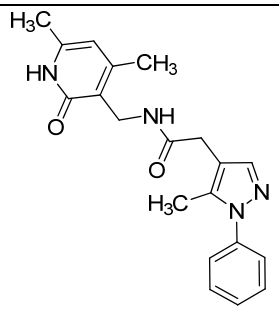
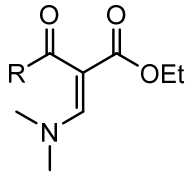
1q	MC3623		204-206	acetonitrile/methanol	60
1r	MC3710		215-217	acetonitrile/methanol	66

Table S2. Chemical and Physical Data of the Intermediates **3a-e**



3a-e

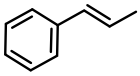
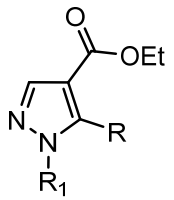
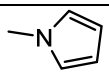
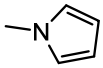
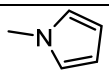
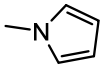
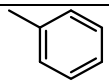
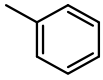
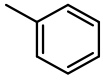
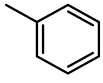
Cpd	**R**	**m. p. (°C)**	**recrystallization solvent**	**yield (%)**
3a [7]		64-66	cyclohexane	78
3b [8]		oil	-	72
3c [9]	-H	150-152	toluene	65
3d [10]	$-CH_3$	43-45	hexane	77
3e [8]	$-CH_2CH_2CH_3$	112-114	cyclohexane	69

Table S3. Chemical and Physical Data of the Intermediates **2a-d** and **4a-q.**

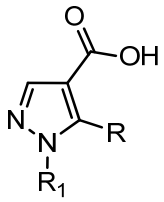


2a-d, 4a-q

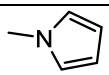
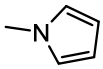
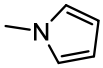
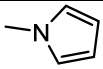
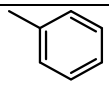
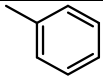
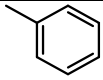
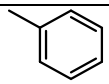
Cpd	**R**	**R_1**	**m. p. (°C)**	**recrystallization solvent**	**yield (%)**
2a [11]	-NH_2	*i*-Pr	62-63	cyclohexane	92
2b [12]	-NH_2	cyclopentyl	oil	-	95
2c [13]	-NH_2	Ph	107-109	cyclohexane	81
2d [14]	-NH_2	Bn	109-111	cyclohexane	82
4a		*i*-Pr	oil	-	67
4b		cyclopentyl	oil	-	77
4c [15]		Ph	111-113	cyclohexane	88
4d		Bn	44-45	*n*-hexane	77
4e		*i*-Pr	oil	-	75
4f		cyclopentyl	oil	-	68
4g [16]		Ph	113-115	cyclohexane	66
4h [17]		Bn	68-70	*n*-hexane	73
4i	-CH=CHPh	*i*-Pr	oil	-	70

4j	-CH=CHPh	cyclopentyl	oil	-	67
4k [18]	-CH=CHPh	Ph	oil	-	69
4l [19]	-CH=CHPh	Bn	oil	-	65
4m [20]	-H	Ph	98-100	cyclohexane	66
4n [21]	$-CH_3$	*i*-Pr	oil	-	70
4o [22]	$-CH_3$	Ph	56-58	*n*-hexane	88
4p	$-CH_3$	Bn	oil	-	82
4q [16]	$-CH_2CH_2CH_3$	Ph	112-114	cyclohexane	92

Table S4. Chemical and Physical Data of the Intermediates **5a-q.**



5a-q

Cpd	**R**	**R_1**	**m. p. (°C)**	**recrystallization solvent**	**yield (%)**
5a		*i*-Pr	138-140	toluene	87
5b		cyclopentyl	167-168	toluene/acetonitrile	93
5c [23]		Ph	184-186	acetonitrile	89
5d		Bn	170-172	toluene/acetonitrile	77
5e		*i*-Pr	164-166	toluene/acetonitrile	73
5f		cyclopentyl	196-197	acetonitrile	68
5g [22]		Ph	177-178	toluene/acetonitrile	83
5h [24]		Bn	166-168	toluene/acetonitrile	76
5i	-CH=CHPh	*i*-Pr	167-170	toluene/acetonitrile	80
5j	-CH=CHPh	cyclopentyl	191-193	acetonitrile	85
5k	-CH=CHPh	Ph	176-177	toluene/acetonitrile	83
5l	-CH=CHPh	Bn	136-140	toluene	78
5m [25]	-H	Ph	225-227	methanol	88
5n [21]	$-CH_3$	*i*-Pr	151-153	toluene	92

5o [22]	$-CH_3$	Ph	163-165	toluene/acetonitrile	86
5p [4]	$-CH_3$	Bn	167-169	toluene/acetonitrile	77
5q [22]	$-CH_2CH_2CH_3$	Ph	111-113	cyclohexane	71

Table S5. Elemental Analyses for Compounds **1a-r**.

compd	MW	calculated, %			found, %		
		C	H	N	C	H	N
1a	353.43	64.57	6.56	19.82	64.78	6.67	19.59
1b	379.46	66.47	6.64	18.46	66.23	6.51	18.72
1c	387.44	68.20	5.46	18.08	68.04	5.55	18.32
1d	401.47	68.81	5.77	17.44	68.59	5.65	17.69
1e	364.45	69.21	6.64	15.37	69.44	6.75	15.19
1f	390.49	70.75	6.71	14.35	70.98	6.84	14.11
1g	398.47	72.34	5.57	14.06	72.59	5.68	13.82
1h	412.49	72.80	5.86	13.58	73.04	5.99	13.27
1i	390.49	70.75	6.71	14.35	70.57	6.59	14.52
1j	416.53	72.09	6.78	13.45	71.88	6.70	13.64
1k	424.50	73.56	5.70	13.20	73.82	5.88	12.95
1l	438.53	73.95	5.98	12.78	74.17	6.08	12.54
1m	322.37	67.07	5.63	17.38	67.29	5.74	17.17
1n	302.38	63.55	7.33	18.53	63.89	7.45	18.28
1o	336.40	67.84	5.99	16.66	68.02	6.10	16.39
1p	350.42	68.55	6.33	15.99	68.36	6.29	16.17
1q	364.45	69.21	6.64	15.37	69.44	6.79	15.08
1r	350.42	68.55	6.33	15.99	68.42	6.39	16.13

Spectral Data for Compounds 1a-r.

1a, *N*-((4,6-Dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-1-isopropyl-5-(1*H*-pyrrol-1-yl)-1*H*-pyrazole-4-carboxamide

^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 1.32 (d, 6H, (CH_3)$_2$CH-, *J*= 6.4 Hz), 2.09 (s, 3H, 4,6 dimethylpyridone –CH_3), 2.10 (s, 3H, 4,6 dimethylpyridone –CH_3), 3.88 (m, 1H, (CH_3)$_2$C*H*-), 4.13 (d, 2H, -CH_2NH-, *J*= 3.6 Hz), 5.82 (s, 1H, -C*H*- pyridone proton), 6.29 (dd, 2H, pyrrole protons, *J*= 5.6 Hz), 6.95 (dd, 2H, pyrrole protons, *J*= 2.0 Hz), 7.14 (d, 1H, -CH_2N*H*-, *J*= 3.6 Hz), 8.00 (s, 1H, pyrazole proton), 11.46 (s, 1H, pyridone -N*H*) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, δ, ppm): δ 18.5, 19.1, 21.7 (2C), 32.8, 51.4, 100.2, 111.1, 112.9 (2C), 116.0, 121.6 (2C), 138.1, 147.3, 151.6, 158.0, 163.6, 165.1 ppm; MS (EI) calc: *m/z*: 353.1852 [M]$^+$ found: *m/z*: 353.1845 [M]$^+$

1b, 1-Cyclopentyl-*N*-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-(1*H*-pyrrol-1-yl)-1*H*-pyrazole-4-carboxamide

^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 1.53 (m, 2H, cyclopentyl protons), 1.78 (m, 2H, cyclopentyl protons), 1.87 (m, 4H, cyclopentyl protons), 2.09 (s, 3H, 4,6 dimethylpyridone –CH_3), 2.10 (s, 3H, 4,6 dimethylpyridone –CH_3), 4.07 (m, 1H, cyclopentyl (CH_2)$_2$C*H*-), 4.12 (d, 2H, -CH_2NH-, *J*= 4.8 Hz), 5.82 (s, 1H, -C*H*- pyridone proton), 6.29 (d, 2H, pyrrole protons, *J*= 5.4 Hz), 6.94 (d, 2H, pyrrole protons, *J*= 2.0 Hz), 7.15 (t, 1H, -CH_2N*H*-, *J*= 3.6 Hz), 7.98 (s, 1H, pyrazole proton), 11.46 (s, 1H, pyridone -N*H*) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, δ, ppm): δ 18.5, 19.1, 24.6 (2C), 32.8, 33.4 (2C), 62.2, 99.8, 111.1, 112.9 (2C), 116.0, 121.6 (2C), 139.1, 146.4, 151.6, 158.0, 163.6, 165.1.ppm; MS (EI) calc: *m/z*: 379.2008 [M]$^+$ found: *m/z*: 379.2011 [M]$^+$

1c, *N*-((4,6-Dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-1-phenyl-5-(1*H*-pyrrol-1-yl)-1*H*-pyrazole-4-carboxamide

^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 2.11 (s, 3H, 4,6 dimethylpyridone –CH_3), 2.50 (s, 3H, 4,6 dimethylpyridone –CH_3), 4.17 (d, 2H, -CH_2NH-, *J*= 4.8 Hz), 5.84 (s, 1H, -C*H*- pyridone proton), 6.17 (d, 2H, aromatic protons, *J*= 1.6 Hz), 6.85 (d, 2H, aromatic protons, *J*= 1.6 Hz), 7.11 (d, 2H, aromatic protons, *J*= 8.0 Hz), 7.36 (d, 2H, aromatic protons, *J*= 6.4 Hz), 7.49 (t, 1H, -CH_2N*H*-, *J*= 4.0 Hz), 8.24 (s, 1H, pyrazole proton), 11.48 (s, 1H, pyridone -N*H*) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, δ, ppm): δ 18.5, 19.1, 32.8, 101.3, 111.1, 112.9 (2C), 116.0, 121.6 (2C), 125.2 (2C), 127.2, 129.5 (2C), 138.5, 139.6, 141.7, 151.6, 158.0, 163.6, 165.1 ppm; MS (EI) calc: *m/z*: 387.1695 [M]$^+$ found: *m/z*: 387.1689 [M]$^+$

1d, 1-Benzyl-*N*-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-(1*H*-pyrrol-1-yl)-1*H*-pyrazole-4-carboxamide

^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 2.09 (s, 3H, 4,6 dimethylpyridone –CH_3), 2.10 (s, 3H, 4,6 dimethylpyridone –CH_3), 4.13 (d, 2H, -CH_2NH-, *J*= 4.8 Hz), 5.01 (s, 2H,-NCH_2Ph), 5.82 (s, 1H, -C*H*- pyridone proton), 6.26 (s, 2H, pyrrole protons), 6.87 (s, 2H, pyrrole protons, *J*= 1.6 Hz), 6.96 (d, 2H, aromatic protons, *J*= 6.8 Hz), 7.27 (m, 3H, aromatic protons), 7.36 (t, 1H, -CH_2N*H*-, *J*= 4.0 Hz), 8.02 (s, 1H, pyrazole proton), 11.46 (s, 1H, pyridone -N*H*) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, δ, ppm): δ 18.5, 19.1, 32.8, 51.1, 100.5, 111.1, 112.9 (2C), 116.0,

121.6 (2C), 127.6, 128.4 (2C), 129.0 (2C), 136.3, 139.6, 141.5, 151.6, 158.0, 163.6, 165.1 ppm; MS (EI) calc: *m/z*: 401.1852 $[M]^+$ found: *m/z*: 401.1845 $[M]^+$

1e, *N*-((4,6-Dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-1-isopropyl-5-phenyl-1*H*-pyrazole-4-carboxamide

^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 1.31 (d, 6H, $(CH_3)_2$CH-, *J*= 5.6 Hz), 2.07 (s, 3H, 4,6 dimethylpyridone –CH_3), 2.10 (s, 3H, 4,6 dimethylpyridone –CH_3), 4.15 (d, 2H, -CH_2NH-, *J*= 4.8 Hz), 4.17 (m, 1H, $(CH_3)_2CH$-), 5.82 (s, 1H, -C*H*- pyridone proton), 7.28 (t, 1H, -CH_2N*H*-, *J*= 4.0 Hz), 7.35 (m, 2H, aromatic protons), 7.48 (m, 3H, aromatic protons), 7.96 (s, 1H, pyrazole proton), 11.44 (s, 1H, pyridone -N*H*) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, δ, ppm): δ 18.5, 19.1, 21.7 (2C), 32.8, 53.1, 111.1, 116.0, 124.5, 128.5 (2C), 128.6, 128.9 (2C), 130.8, 141.5, 151.6, 153.9, 158.0, 163.6, 165.3 ppm; MS (EI) calc: *m/z*: 364.1899 $[M]^+$ found: *m/z*: 364.1901 $[M]^+$

1f, 1-Cyclopentyl-*N*-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-phenyl-1*H*-pyrazole-4-carboxamide

^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 1.51 (m, 2H, cyclopentyl protons), 1.80 (m, 2H, cyclopentyl protons), 1.89 (m, 4H, cyclopentyl protons), 2.07 (s, 3H, 4,6 dimethylpyridone –CH_3), 2.10 (s, 3H, 4,6 dimethylpyridone –CH_3), 4.10 (d, 2H, -CH_2NH-, *J*= 5.2 Hz), 4.33 (m, 1H, cyclopentyl proton -N-C*H*-), 5.82 (s, 1H, -C*H*- pyridone proton), 7.30 (t, 1H, -CH_2N*H*-, *J*= 4.8 Hz), 7.34 (m, 2H, aromatic protons), 7.48 (m, 3H, aromatic protons), 7.95 (s, 1H, pyrazole proton), 11.44 (s, 1H, pyridone -N*H*) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, δ, ppm): δ 18.5, 19.1, 24.6 (2C), 32.8, 33.4 (2C), 64.1, 111.1, 116.0, 122.8, 128.5 (2C), 128.6, 128.9 (2C), 130.8, 142.0, 151.6, 154.1, 158.0, 163.6, 165.3 ppm; MS (EI) calc: *m/z*: 390.2056 $[M]^+$ found: *m/z*: 390.2060 $[M]^+$

1g, *N*-((4,6-Dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxamide

^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 2.10 (s, 3H, 4,6 dimethylpyridone -CH_3), 2.11 (s, 3H,4,6 dimethylpyridone -CH_3), 4.17 (d, 2H, -CH_2NH-, *J*= 5.2 Hz), 5.84 (s, 1H, -C*H*- pyridone proton), 7.17 (d, 2H, aromatic protons, *J*= 6.4 Hz), 7.24 (d, 2H, aromatic protons, *J*= 6.4 Hz), 7.32 (m, 6H, aromatic protons), 7.60 (t, 1H, -CH_2N*H*-, *J*= 4.8 Hz), 8.15 (s, 1H, pyrazole proton), 11.46 (s, 1H, pyridone –N*H*) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, δ, ppm): δ 18.5, 19.1, 32.8, 111.1, 116.0, 124.1 (2C), 125.8, 127.1, 128.4 (2C), 128.6, 128.9 (2C), 129.1 (2C), 130.6, 139.6, 140.2, 146.0, 151.6, 158.0, 163.6, 165.3 ppm; MS (EI) calc: *m/z*: 398.1743 $[M]^+$ found: *m/z*: 398.1740 $[M]^+$

1h, 1-Benzyl-*N*-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-phenyl-1*H*-pyrazole-4-carboxamide

^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 2.08 (s, 3H, 4,6 dimethylpyridone -CH_3), 2.10 (s, 3H, 4,6 dimethylpyridone -CH_3), 4.11 (d, 2H, -CH_2NH-, *J*= 4.0 Hz), 5.14 (s, 2H, -NCH_2Ph), 5.82 (s, 1H, -C*H*- pyridone proton), 6.90 (d, 2H, aromatic protons, *J*= 5.6 Hz), 7.27 (m, 5H, aromatic protons), 7.43 (m, 4H, aromatic protons and -CH_2N*H*-), 8.01 (s, 1H, pyrazole proton), 11.45 (s, 1H, pyridone –N*H*) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, δ, ppm): δ 18.5, 19.1, 32.8, 52.9, 111.1, 116.0, 120.6, 127.6, 128.0 (2C), 128.4 (2C), 128.6 (3C), 128.9 (2C), 130.9, 136.0, 140.6, 142.5, 151.6, 158.0, 163.6, 165.3 ppm; MS (EI) calc: *m/z*: 412.1899 $[M]^+$ found: *m/z*: 412.1893 $[M]^+$

1i, *N*-((4,6-Dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-1-isopropyl-5-styryl-1*H*-pyrazole-4-carboxamide

^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 1.40 (d, 6H, $(CH_3)_2$CH-, *J*= 5.6 Hz), 2.10 (s, 3H, 4,6 dimethylpyridone –CH_3), 2.15 (s, 3H, 4,6 dimethylpyridone –CH_3), 4.24 (d, 2H, -CH_2NH-, *J*= 4.0 Hz), 4.82 (m, 1H, $(CH_3)_2CH$-), 5.84 (s, 1H, -C*H*- pyridone proton), 7.18 (d, 1H, vinyl proton, *J*= 16.8 Hz), 7.38 (m, 4H, aromatic protons and vinyl proton), 7.59 (m, 2H, aromatic protons), 7.83 (t, 1H, -CH_2N*H*-, *J*= 5.0 Hz),

7.87 (s, 1H, pyrazole proton), 11.46 (s, 1H, pyridone -N*H*) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, δ, ppm): δ 18.5, 19.1, 21.7 (2C), 32.8, 52.3, 111.1, 116.0, 121.8, 122.0, 126.3 (2C), 129.0 (2C), 129.8, 131.6, 136.6, 143.8, 145.3, 151.6, 158.0, 163.6, 165.4 ppm; MS (EI) calc: *m/z*: 390.2056 $[M]^+$ found: *m/z*: 390.2058 $[M]^+$

1j, 1-Cyclopentyl-*N*-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-styryl-1*H*-pyrazole-4-carboxamide

^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 1.64 (m, 2H, cyclopentyl protons), 1.83 (m, 2H, cyclopentyl protons), 1.97 (m, 2H, cyclopentyl protons), 2.06 (m, 2H, cyclopentyl protons), 2.10 (s, 3H, 4,6 dimethylpyridone –CH_3), 2.16 (s, 3H, 4,6 dimethylpyridone –CH_3), 4.24 (d, 2H, -C*H*$_2$NH-, *J*= 5.2 Hz), 4.98 (m, 1H, cyclopentyl proton -N-C*H*-), 5.84 (s, 1H, -C*H*- pyridone proton), 7.20 (d, 1H, vinyl proton, *J*= 16.8 Hz), 7.38 (m, 4H, aromatic protons and vinyl proton), 7.34 (m, 1H, aromatic proton), 7.41 (m, 3H, aromatic and vinyl protons), 7.60 (d, 2H, aromatic protons, *J*= 7.2 Hz), 7.85 (t, 1H, -CH_2N*H*- *J*= 5.1 Hz), 7.87 (s, 1H, pyrazole proton), 11.45 (s, 1H, pyridone -N*H*) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, δ, ppm): δ 18.5, 19.1, 24.6 (2C), 32.8, 33.4 (2C), 63.9, 111.1, 116.0, 120.6, 122.1, 126.3 (2C), 129.0 (2C), 129.8, 131.6, 136.6, 143.4, 144.3, 151.6, 158.0, 163.6, 165.4 ppm; MS (EI) calc: *m/z*: 416.2212 $[M]^+$ found: *m/z*: 416.2215 $[M]^+$

1k, *N*-((4,6-Dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-1-phenyl-5-styryl-1*H*-pyrazole-4-carboxamide

^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 2.12 (s, 3H, 4,6 dimethylpyridone –CH_3), 2.19 (s, 3H, 4,6 dimethylpyridone –CH_3), 4.31 (d, 2H, -C*H*$_2$NH-, *J*= 4.0 Hz), 5.87 (s, 1H, -C*H*- pyridone proton), 6.85 (d, 1H, vinyl proton, *J*= 16.8 Hz), 7.32 (m, 6H, aromatic protons and vinyl proton), 7.53 (m, 5H, aromatic protons), 8.09 (t, 1H, -CH_2N*H*-, *J*= 5.1 Hz), 8.12(s, 1H, pyrazole proton), 11.47 (s, 1H, pyridone -N*H*) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, δ, ppm): δ 18.5, 19.1, 32.8, 111.1, 116.0, 117.9, 122.2, 123.0 (2C), 126.3 (2C), 127.2, 129.0 (2C), 129.1 (2C), 129.8, 132.6, 136.6, 136.7, 139.5, 141.8, 151.6, 158.0, 163.6, 165.4 ppm; MS (EI) calc: *m/z*: 424.1899 $[M]^+$ found: *m/z*: 424.1903 $[M]^+$

1l, 1-Benzyl-*N*-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-styryl-1*H*-pyrazole-4-carboxamide

^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 2.11 (s, 3H, 4,6 dimethylpyridone –CH_3), 2.18 (s, 3H, 4,6 dimethylpyridone –CH_3), 4.27 (d, 2H, -C*H*$_2$NH-, *J*= 4.4 Hz), 5.57 (s, 2H, -NC*H*$_2$-Ph), 5.86 (s, 1H, -C*H*- pyridone proton), 7.14 (d, 2H, aromatic protons), 7.24-7.40 (m, 7H, aromatic protons and vinyl protons), 7.47 (d, 3H, aromatic protons, *J*= 7.6 Hz), 7.95 (s, 1H, pyrazole proton), 7.97 (t, 1H, -CH_2N*H*-, *J*= 5.8 Hz), 11.46 (s, 1H, pyridone -N*H*) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, δ, ppm): δ 18.5, 19.1, 32.8, 52.8, 111.1, 116.0, 118.8, 121.7, 126.3 (2C), 127.6, 128.0 (2C), 128.4 (2C), 129.0 (2C), 129.8, 131.1, 136.0, 136.6, 140.7, 142.8, 151.6, 158.0, 163.6, 165.4 ppm; MS (EI) calc: *m/z*: 438.2056 $[M]^+$ found: *m/z*: 438.2051 $[M]^+$

1m, *N*-((4,6-Dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-1-phenyl-1H-pyrazole-4-carboxamide

^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 2.13 (s, 3H, 4,6 dimethylpyridone –CH_3), 2.19 (s, 3H,4,6 dimethylpyridone –CH_3), 4.28 (d, 2H, -C*H*$_2$NH-, *J*= 4.8 Hz), 5.88 (s, 1H, -C*H*- pyridone proton), 7.35 (t, 1H, aromatic proton), 7.52 (m, 2H, aromatic protons), 7.82 (d, 2H, aromatic protons, *J*= 7.6 Hz), 8.05 (t, 1H, -CH_2N*H*-, *J*= 4.8 Hz), 8.15 (s, 1H, pyrazole proton), 8.98 (s, 1H, pyrazole proton), 11.50 (s, 1H, pyridone –N*H*) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, δ, ppm): δ 18.5, 19.1, 32.8, 111.1, 116.0, 119.2 (2C), 119.4, 127.5, 129.4 (2H), 132.3, 136.8, 139.4, 151.6, 158.0, 162.3, 163.61.ppm; MS (EI) calc: *m/z*: 322.1430 $[M]^+$ found: *m/z*: 322.1433 $[M]^+$

1n, *N*-((4,6-Dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-1-isopropyl-5-methyl-1*H*-pyrazole-4-carboxamide

^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 1.33 (d, 6H, (CH_3)$_2$CH-, *J*= 4.4 Hz), 2.11 (s, 3H, 4,6 dimethylpyridone –CH$_3$), 2.15 (s, 3H, 4,6 dimethylpyridone –CH$_3$), 2.50 (s, 3H, 5 methylpyrazole –CH$_3$), 4.22 (d, 2H, -CH_2NH-, *J*= 4.8 Hz), 4.51 (m, 1H, (CH$_3$)$_2$C*H*-), 5.85 (s, 1H, -C*H*- pyridone proton), 7.76 (t, 1H, -CH$_2$N*H*-, *J*= 7.6 Hz), 7.87 (s, 1H, pyrazole proton), 11.47 (s, 1H, pyridone –N*H*) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, δ, ppm): δ 11.9, 18.5, 19.1, 21.7 (2C), 32.8, 51.0, 111.1, 116.0, 125.9, 147.2, 148.8, 151.6, 158.0, 163.6, 165.9.ppm; MS (EI) calc: *m/z*: 362.1743 [M]$^+$ found: *m/z*: 362.1744 [M]$^+$

1o, *N*-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carboxamide

^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 2.13 (s, 3H, 4,6-dimethylpyridone -CH$_3$), 2.19 (s, 3H, 4,6-dimethylpyridone -CH$_3$), 2.51 (s, 3H, 5-methylpyrazole -CH$_3$), 4.27 (d, 2H, -CH_2NH, *J*= 4.8 Hz), 5.87 (s, 1H, -CH pyridone proton), 7.53 (m, 5H, aromatic protons), 7.96 (t, 1H, -CH$_2$N*H*, *J*= 4.8 Hz), 8.13 (s, 1H, pyrazole proton), 11.48 (br s, 1H, pyridone NH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, δ, ppm): δ 11.1, 18.5, 19.1, 32.8, 111.1, 116.0, 123.3 (2C), 126.9, 127.2, 129.1 (2C), 139.2, 142.6, 146.6, 151.5, 158.0, 163.6, 165.9 ppm; MS (EI) calc: m/z: 336.1586 [M]$^+$ found: m/z: 336.1590 [M]$^+$.

1p, 1-Benzyl-*N*-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-methyl-1*H*-pyrazole-4-carboxamide

^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 2.11 (s, 3H, 4,6 dimethylpyridone –CH$_3$), 2.16 (s, 3H, 4,6 dimethylpyridone –CH$_3$), 2.44 (s, 3H, 5 methylpyrazole –CH$_3$), 4.22 (d, 2H, -CH_2NH-, *J*= 5.2 Hz), 5.31 (s, 2H, -NCH_2-Ph), 5.85 (s, 1H, -C*H*- pyridone proton), 7.09 (d, 2H, aromatic protons, *J*= 6.8 Hz), 7.31 (m, 3H, aromatic protons), 7.84 (t, 1H, -CH$_2$N*H*-, *J*= 4.8 Hz), 7.94 (s, 1H, pyrazole proton), 11.47 (s, 1H, pyridone –N*H*) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, δ, ppm): δ 11.8, 18.5, 19.1, 32.8, 52.6, 111.1, 116.0, 123.6, 127.6, 128.4 (2C), 128.7 (2C), 136.1, 147.4, 150.1, 151.6, 158.0, 163.6, 165.9 ppm; MS (EI) calc: *m/z*: 350.1743 [M]$^+$ found: *m/z*: 350.1739 [M]$^+$

1q, *N*-((4,6-Dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-1-phenyl-5-propyl-1*H*-pyrazole-4-carboxamide

^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 0.72 (t, 3H, CH_3CH$_2$CH$_2$-, *J*= 7.2 Hz), 1.40 (m, 2H, CH$_3$CH_2CH$_2$-), 2.13 (s, 3H, 4,6 dimethylpyridone –CH$_3$), 2.17 (s, 3H,4,6 dimethylpyridone –CH$_3$), 2.92 (t, 2H, CH$_3$CH$_2$CH_2-), 4.28 (d, 2H, -CH_2NH-, *J*= 7.6 Hz), 5.87 (s, 1H, -C*H*- pyridone proton), 7.50 (m, 5H, aromatic protons), 7.95 (t, 1H, -CH$_2$N*H*-, *J*= 4.0 Hz), 8.11 (s, 1H, pyrazole proton), 11.50 (s, 1H, pyridone –N*H*) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, δ, ppm): δ 13.6, 18.5, 19.1, 21.8, 25.0, 32.8, 111.1, 116.0, 119.9, 124.2 (2C), 127.2, 128.9 (2C), 139.8, 145.7, 146.6, 151.6, 158.0, 163.6, 165.6 ppm; MS (EI) calc: *m/z*: 364.1899 [M]$^+$ found: *m/z*: 364.1904 [M]$^+$

1r, *N*-((4,6-Dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-2-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)acetamide

^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): δ 2.11 (s, 3H, 4,6 dimethylpyridone –CH$_3$), 2.13 (s, 3H, 4,6 dimethylpyridone –CH$_3$), 2.27 (s, 3H, pyrazole –CH$_3$), 3.26 (s, 2H, -CH_2CONH-), 4.09 (d, 2H, -CH_2NH-, *J*= 5.2 Hz), 5.85 (s, 1H, -C*H*- pyridone proton), 7.39 (t, 1H, aromatic proton, *J*= 7.6 Hz), 7.42-7.54 (m, 5H, aromatic and pyrazole protons), 7.91 (t, 1H, -CH$_2$N*H*-, *J*= 4.4 Hz), 11.47 (s, 1H, pyridone -N*H*) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, δ, ppm): δ 10.3, 18.5, 19.1, 30.3, 33.0, 109.1, 111.1, 116.0, 123.3 (2C), 127.2, 129.1 (2C), 136.6, 138.0, 139.3, 151.6, 158.0, 163.6, 170.8 ppm; MS (EI) calc: *m/z*: 356.1743 [M]$^+$ found: *m/z*: 356.1747 [M]$^+$

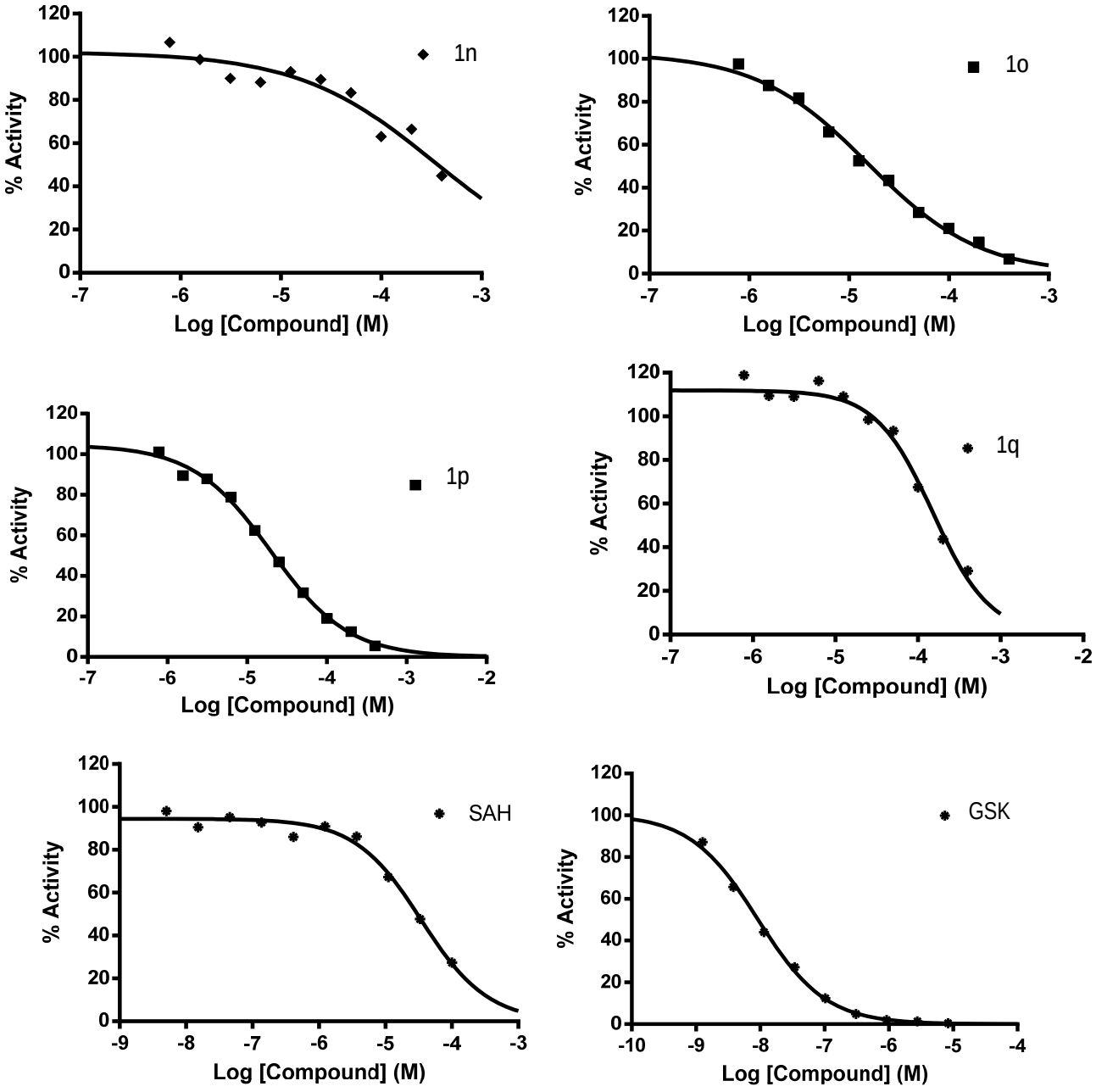


Figure S1. Determination of IC_{50} values against EZH2/PRC2 for **1n**, **1o**, **1p**, and **1q**. SAH and GSK126 have been used as reference drugs.

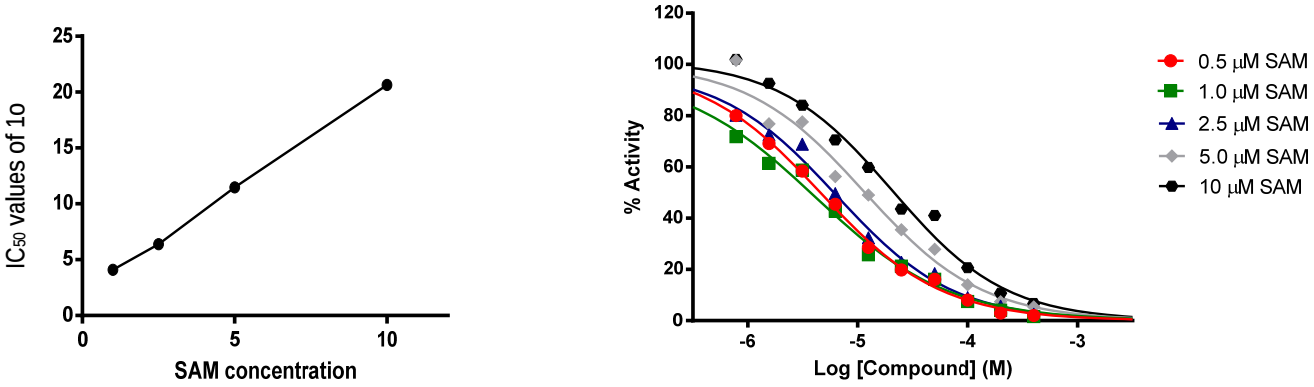


Figure S2. SAM competition experiments. Left, dependence of **1o** IC_{50} values with increasing SAM concentration. Right, inhibitory effects of **1o** vs EZH2/PRC2 at increasing SAM concentrations.

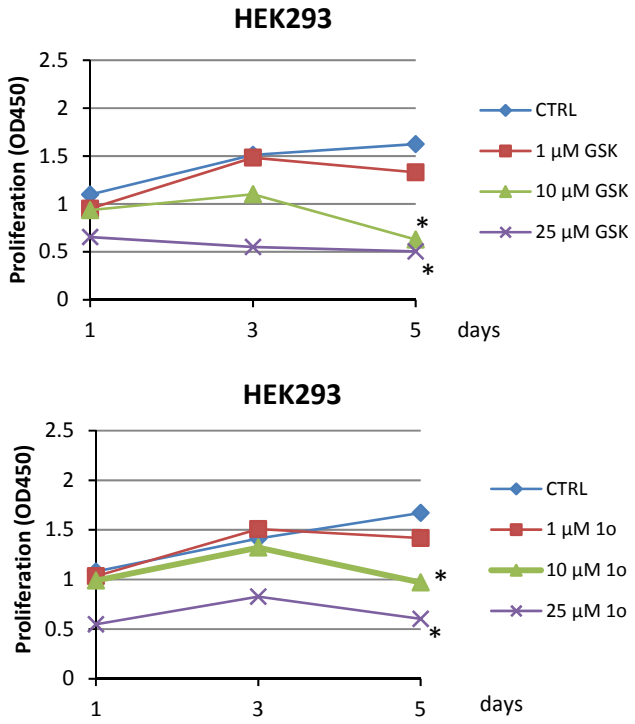


Figure S3. Cytotoxicity of **1o** and GSK126 in non-cancer cells. Antiproliferative effects of GSK126 (up) and of **1o** (down) in human embryonic kidney HEK293 cells treated with 1, 10 and 25 µM of the compound for 5 days (MTS Cell Proliferation Assay).

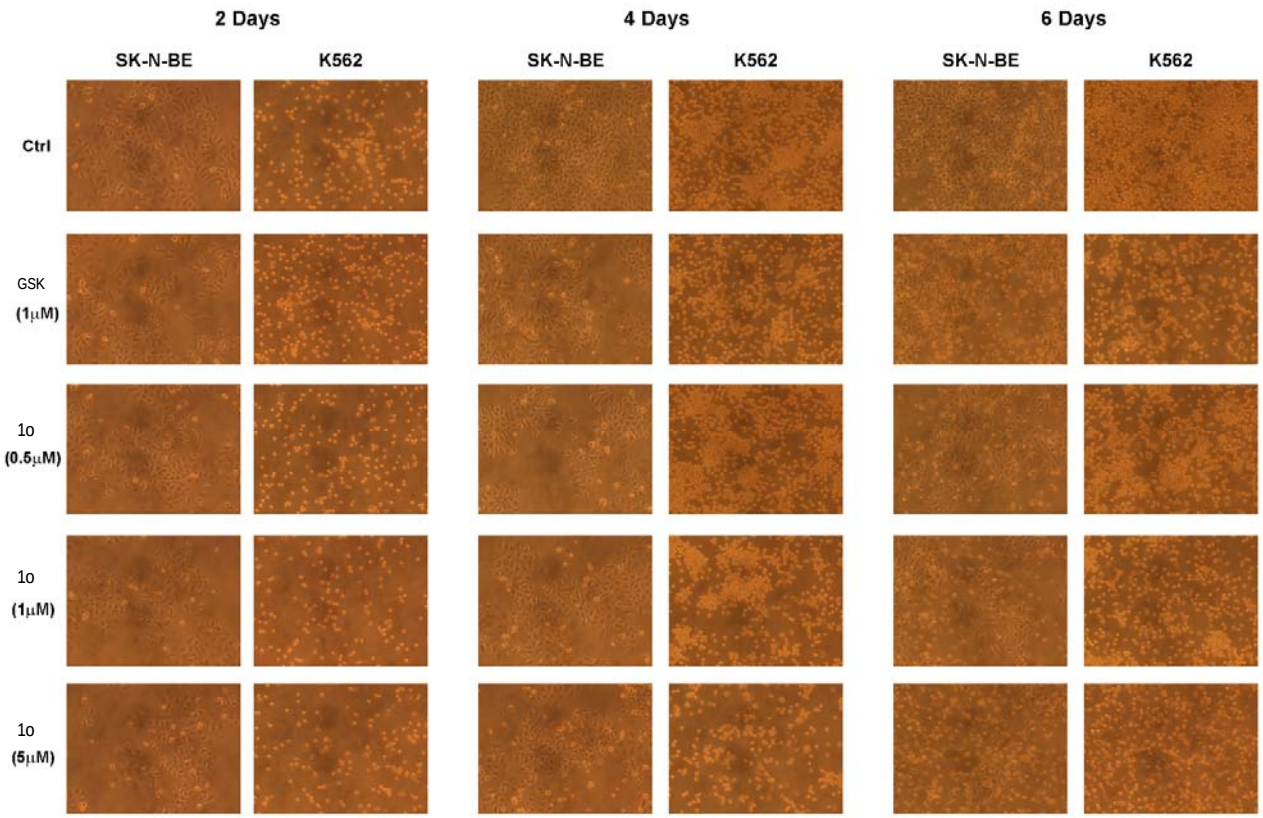


Figure S4. SK-N-BE and K562 cells were either left untreated or treated with 0.5, 1 and 5 μM **1o** for 2, 4 and 6 days. GSK126 (1 μM) was used as a reference drug. Cell morphology was evaluated by optical microscopy and picture taken.

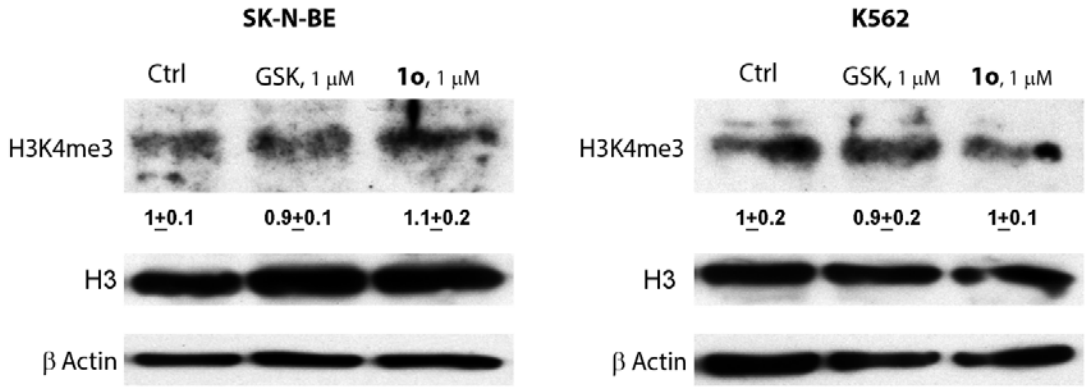


Figure S5. Western blot analysis showing the levels of H3K4me3 and histone H3 after treatment with GSK126 and **1o** (both at 1 μM) in SK-N-BE and K562 cells. The relative densitometric analyses for H3K4me3 (arbitrary units) are reported. β-Actin was used for normalization.

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