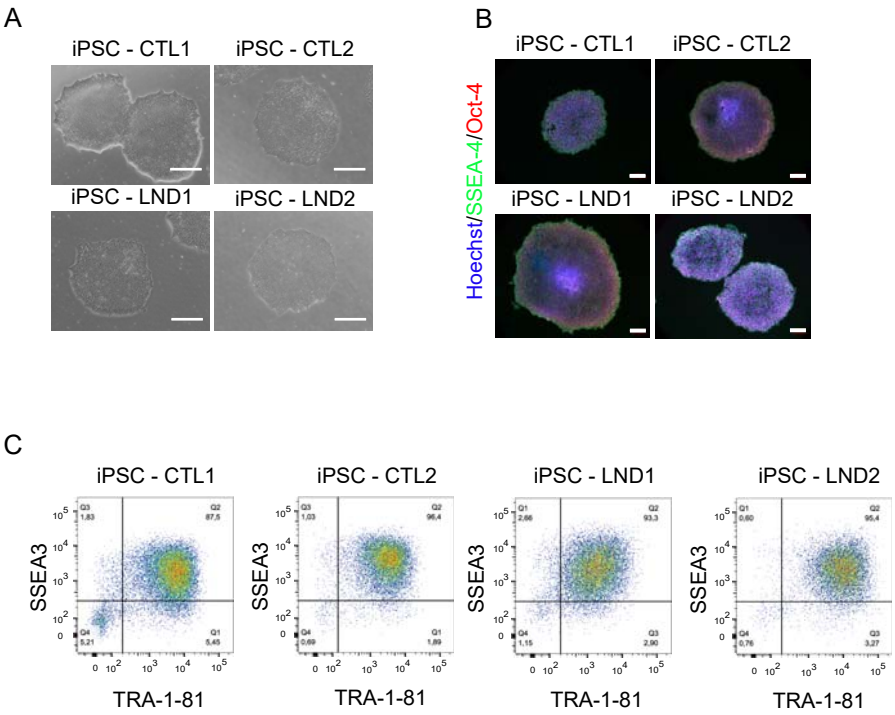


**Figure S1**: **CTL and LND donor-derived iPSC**

A - Bright field microscopy of colonies with typical iPSC morphology. Control HGPRT competent cells: CTL1 and CTL2. Lesch-Nyhan disease HGPRT- deficient cells: LND1 and LND2. Scale bar = 200 µm. B- Representative images of self-renewing HGPRT competent (CTL1 and CTL2) and deficient (LND1 and LND2) iPSC lines expressing the 2 pluripotency markers SSEA-4 (green) and Oct-4 (red) in colonies. Scale bar = 200 µm. C - Flow cytometry analysis of SSEA3 and TRA-1-81 pluripotency markers.

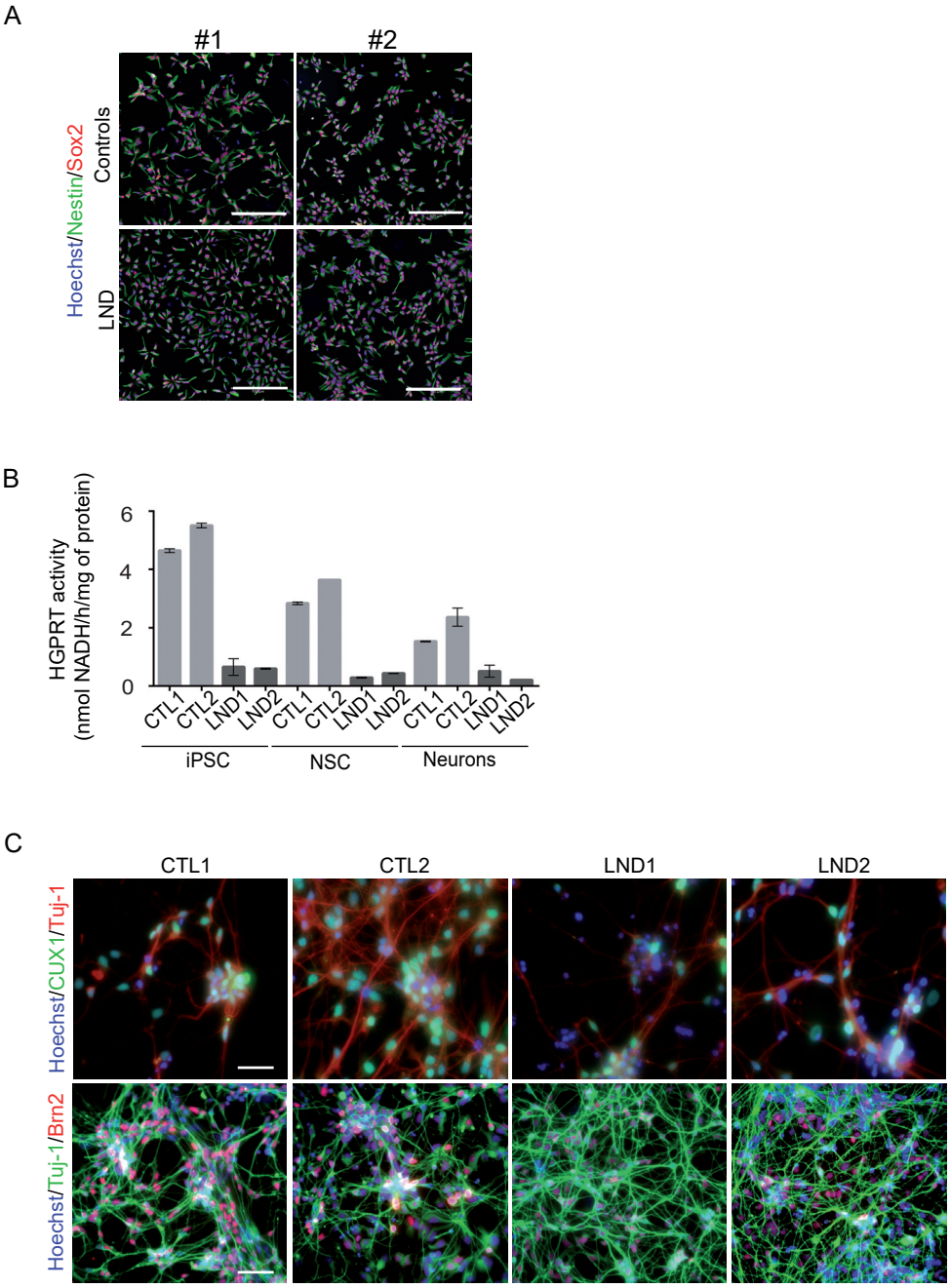


**Figure S2: Phenotypical characterization of neurons differentiated from CTL and LND stem cells**

A - Representative images of neural stem cells (NSC) expressing Nestin (green) and Sox2 (red). Scale bar = 200 µm. B - HGPRT enzymatic activity in CTL and LND-derived iPSC, NSC and neurons. The results are expressed as nmol of NADH produced per hour and normalized to total protein content. C – Immunostaining of the cortical superficial layer markers CUX1 and Brn2 in Tuj-1 positive neurons after 18 days of NSC differentiation. Scale bar: CUX1 = 25 µm, Brn2 = 50 µm.

Figure S3 - Ruillier et al- Revision

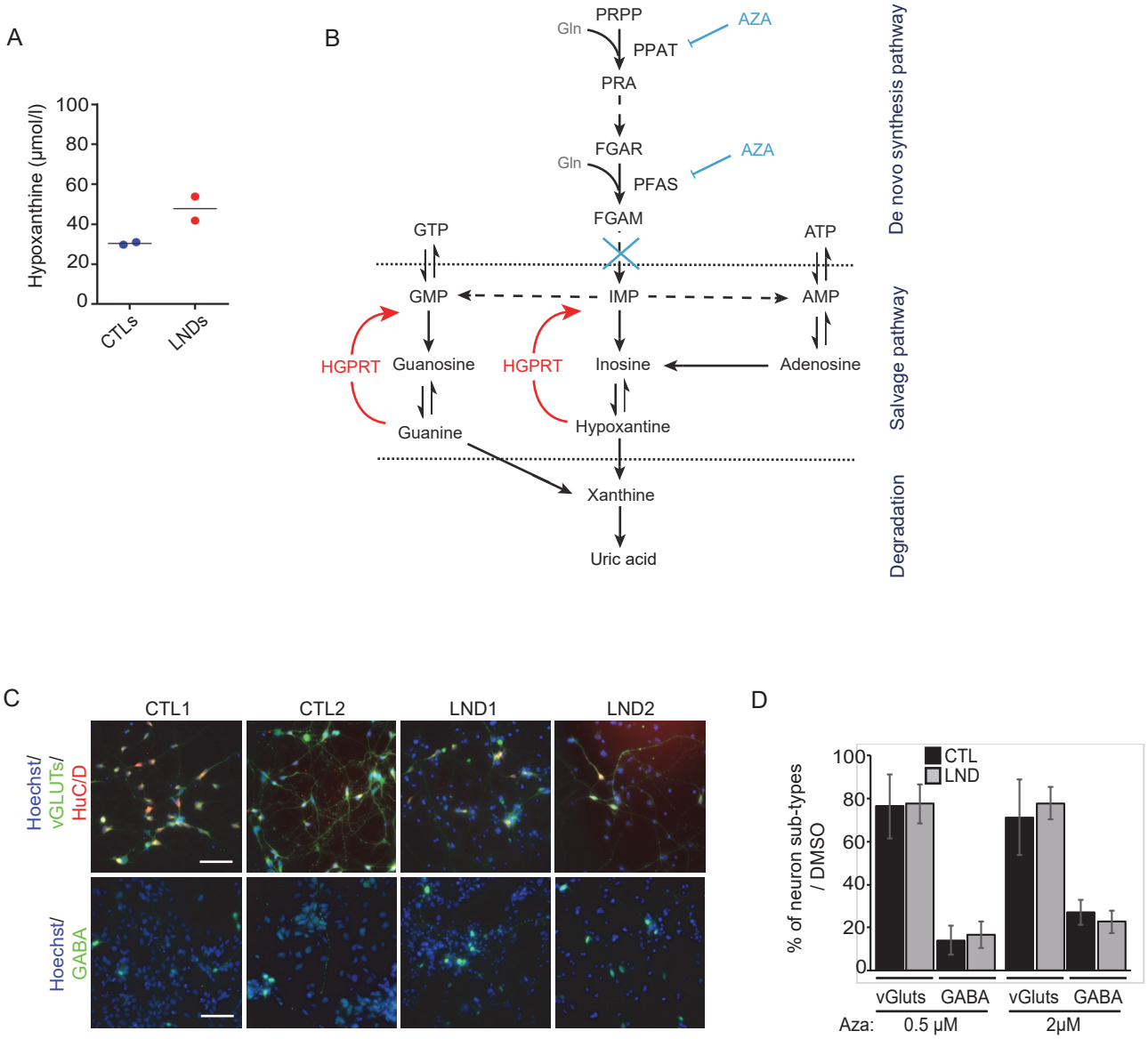


**Figure S3: Inhibition of de novo synthesis using asazerine**

A - Quantification of hypoxanthine synthesis in CTL and LND cells. B-. Inhibition of purine de novo synthesis by Azaserine. C- Immunostaining of glutamatergic and GABAergic neuronal sub-types in CTL and LND cells treated with DMSO 0.1% or azaserine 2 µM from day 5 of differentiation. Scale bar = 25µm. D- Quantification of the different neuronal sub-types after DMSO or azaserine treatment. Results are expressed as mean +/- SD of 2 cell lines in 3 biological replicate. C-D: The analysis of neuronal subtypes were performed at day 18 of differentiation.

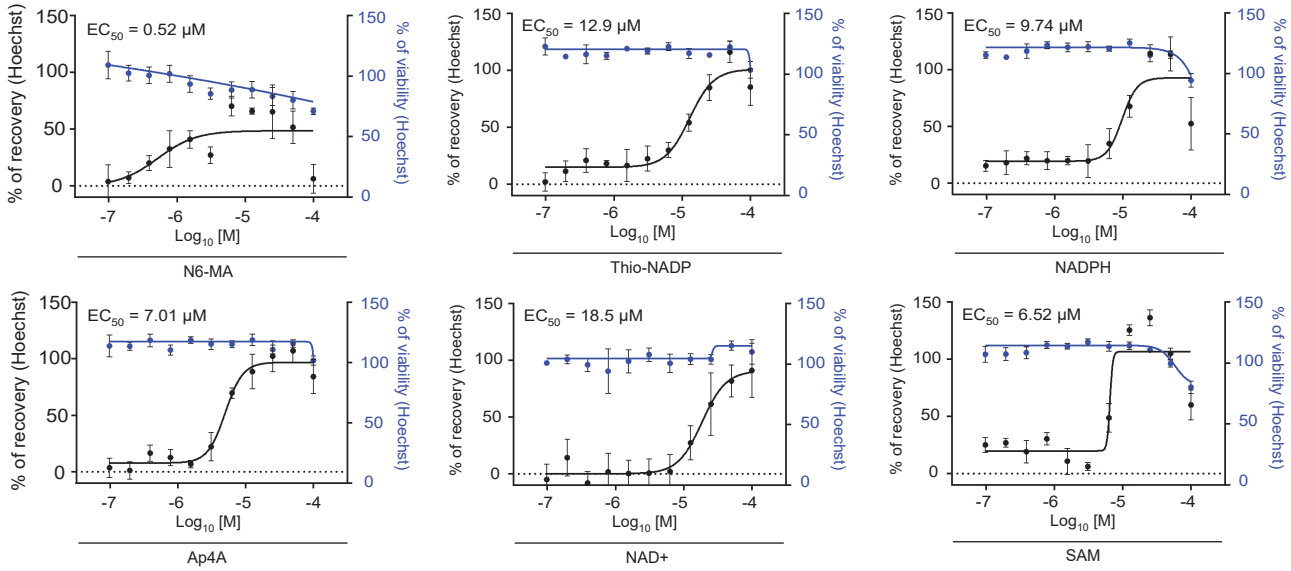


**Figure S4**: **Dose-response analysis of the 6 hit compounds efficiency using Hoechst staining as a read-out.**

The black curve corresponds to the percentage of recovery after treatment of LND1 NSC with 1.0 µM azaserine. The blue line shows the percentage of viability after treatment with the drug alone without azaserine, representing the toxicity of the compound itself. Results are expressed as mean ± SD of 4 technical replicates (2 biological replicates).

Figure S5 - Ruillier et al- Revision

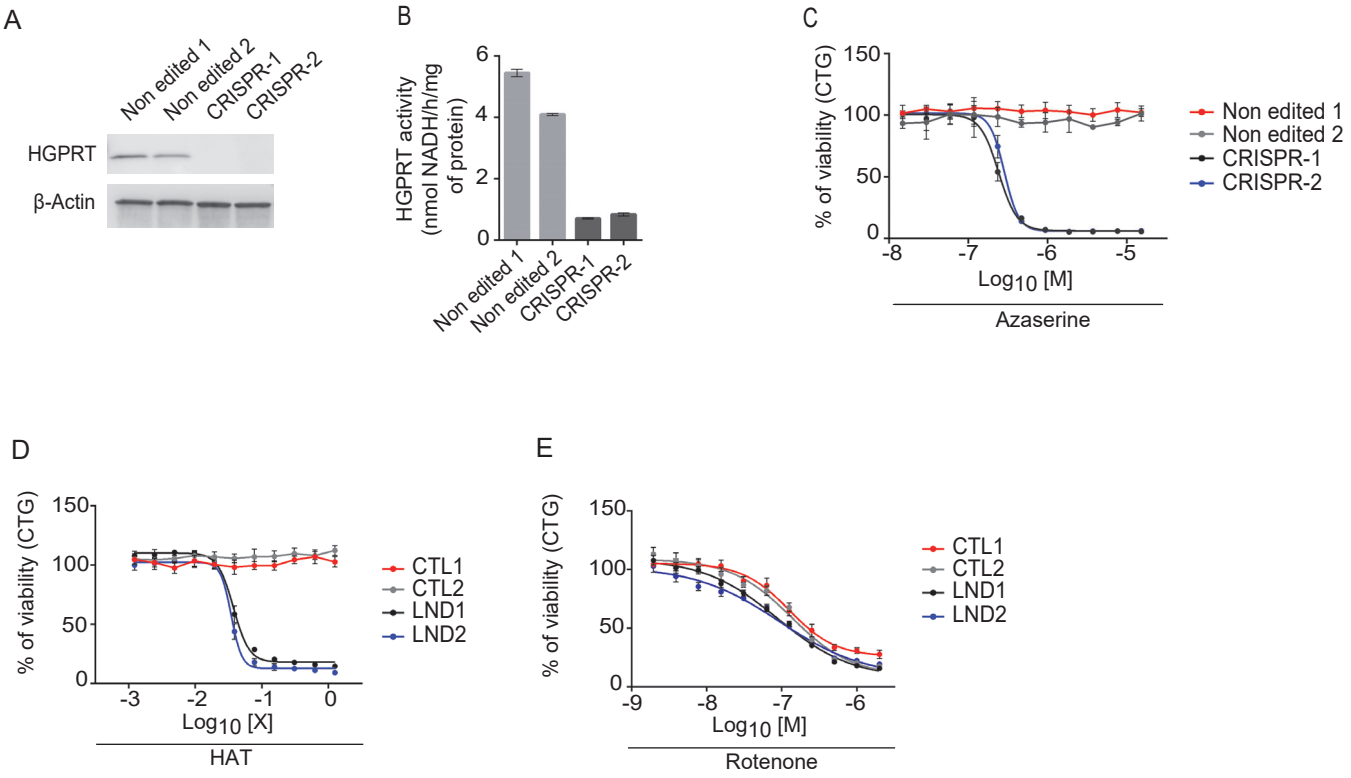


**Figure S5**: **Primary hits compounds specificity.**

A- Western-blot analysis of HGPRT protein expression in CRISPR/Cas-9 edited NSC-derived from the human embryonic stem cells line SA001 (CTL3). Two non-edited clones and 2 clones invalidated for HGPRT (CRISPR-1 and CRISPR-2) were compared. β-actin was used as a loading control. B - Enzymatic HGPRT activity measurement in the same clones. C – Azaserine selective toxic effect upon HGPRT-edited CRISPR NSC. Results are expressed as mean ± SD of 4 technical replicates. CTG: Cell-TiterGlo. D- CTG estimation of cell viability after treatment of CTL and LND cell lines with increasing concentrations of HAT medium (Hypoxanthine, Aminopterin and Thymidine containing medium). The X axis represents HAT medium concentration express as log10 (X), where the X is the dilution factor of the commercial HAT complete medium. Results are expressed as mean ± SD of 4 technical replicates and 2 biological replicates. E- CTG estimation of cell viability after treatment of CTL and LND cell lines with increasing concentrations of rotenone on CTL and LND NSC lines. X axis represents compound concentration expressed as log10 (Rotenone concentration in molar M). Results are expressed as mean ± SD of 4 technical replicates and 2 biological replicates.

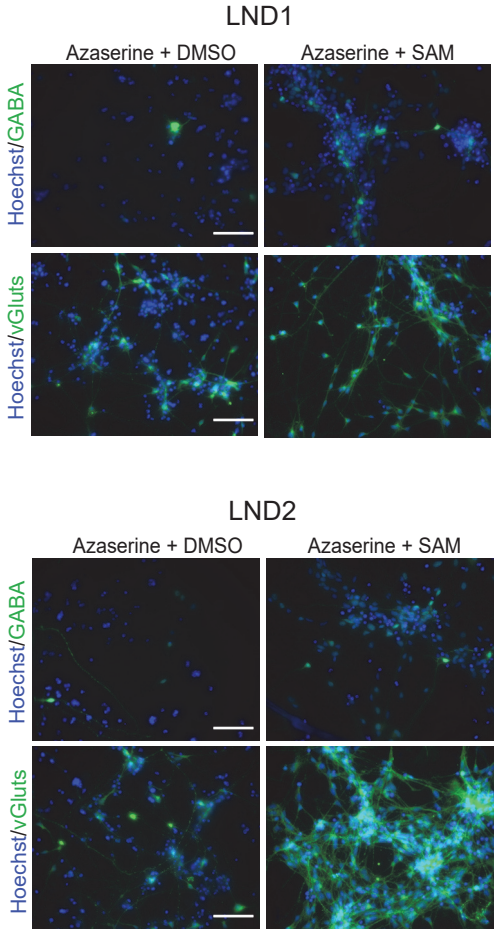


**Figure S6**: **Analysis of neuronal subtypes rescued by SAM after azaserine treatment of LND cells.**

GABA: GABAergic neurons, vGluts: Glutamatergic neurons, SAM : S-adenosylmethionine.

Scale bar = 50 µm.

**Table S1:** List of CTL and LND hiPSC or hESC lines

| Study code | Cell type | Donnor code | Cell of origin | Provider | Karyotype | HGPRT mutation | Clinical description |
|---|---|---|---|---|---|---|---|
| CTL1 | hiPSC | GM01869 | fibroblasts | Coriell, USA | 46, XY | no | unaffected |
| CTL2 | hiPSC | GM04603 | fibroblasts | Coriell, USA | 46, XY | no | unaffected |
| CTL3 | hESC | SA001 | Blastocyst | Cellartis, Sweden | 46, XY | no | unaffected |
| | | | | | | | |
| LND1 | hiPSC | GM02227 | fibroblasts | Coriell, USA | 46, XY | inv/del, ex6-9 Edwards et al, 1989 | LND |
| LND2 | hiPSC | GM23784 | fibroblasts | Coriell, USA | 46, XY | IVS7 + 5 G>A Gibbs et al, 1990 | LND |

**Table S2**: List of primary antibodies, providers and dilutions.

| Antibody | Host | Reference | Provider | Dilution |
|---|---|---|---|---|
| HPRT1 | Rabbit | 15059-1-AP | Proteintech, Rosemont, IL, USA | 1:500 |
| PRTFDC1 | Rabbit | 11986-1-AP | Proteintech, Rosemont, IL, USA | 1:250 |
| | | | | |
| SSEA-4 | Mouse | 4755S | Cell Signaling, Danvers, MA, USA | 1 :500 |
| Oct-4 | Rabbit | 2840S | Cell Signaling, Danvers, MA, USA | 1 :500 |
| Nestin | Mouse | MAB5326 | Merck, Darmstadt, Germany | 1 :500 |
| Sox2 | Rabbit | AB5603 | Merck, Darmstadt, Germany | 1:500 |
| Ki-67 | Mouse | MAB4190 | Merck, Darmstadt, Germany | 1:500 |
| HuC/D | Mouse | A-21271 | Thermo Scientific, Waltham, MA, USA | 1:250 |
| Tubulin β-3 (Tuj-1) | Rabbit | 802001 | Biolegend, San Diego, CA, USA | 1:1000 |
| | | | | |
| TRA-1-81 AF647 | Mouse | 330706 | Biolegend, San Diego, CA, USA | 2 µg/ml |
| SSEA-3 PE | Rat | 330312 | Biolegend, San Diego, CA, USA | 16 µg/ml |

**Table S3**: Table summarizing the 32 primary hits from LOPAC, Prestwick and SelleckChem libraries.

| Library | Conc. | CAS | Compound name | % Recovery | Z-Score Run | Z-Score Plate |
|---|---|---|---|---|---|---|
| | | | | | | |
| LOPAC | 10 µM | 102783-36-8 | P1,P4-Di(adenosine-5') tetraphosphate triammonium | 181.99 | 39.97 | 16.68 |
| LOPAC | 10 µM | 19254-05-8 | Thio-NADP sodium | 65.68 | 14.78 | 12.87 |
| LOPAC | 10 µM | 1867-73-8 | N6-Methyladenosine | 64.55 | 14.53 | 10.39 |
| LOPAC | 10 µM | 2646-71-1 | NADPH tetrasodium | 59.48 | 13.43 | 9.6 |
| Prestwick | 5 µM | 53-84-9 | Nadide | 17.11 | 4.26 | 6.06 |
| Prestwick | 5 µM | 66-81-9 | Cycloheximide | 13.55 | 3.49 | 4.93 |
| LOPAC | 10 µM | 85666-17-7 | Furegrelate sodium | 12.45 | 3.25 | 1.29 |
| Prestwick | 5 µM | 5536-17-4 | Vidarabine | 12.30 | 3.22 | 4.53 |
| Prestwick | 5 µM | 1867-73-8 | N6-Methyladenosine | 11.29 | 3.00 | 4.98 |
| LOPAC | 10 µM | 130506-22-8 | 6-Nitroso-1,2-benzopyrone | 9.86 | 2.69 | 1.85 |
| Prestwick | 5 µM | 21679-14-1 | Fludarabine | 9.81 | 2.68 | 5.62 |
| LOPAC | 10 µM | 134523-03-8 | Atorvastatin calcium salt trihydrate | 9.71 | 2.66 | 3.42 |
| LOPAC | 10 µM | 38819-10-2 | Ara-G hydrate | 7.46 | 2.17 | 1.91 |
| LOPAC | 10 µM | 501-36-0 | Resveratrol | 7.48 | 2.17 | 1.91 |
| Prestwick | 5 µM | 73573-88-3 | Mevastatin | 6.78 | 2.02 | 3.29 |
| | | | | | | |
| PW Phyto | 10 µM | 6027-98-1 | Harmaline hydrochloride dihydrate | 100.55 | 8.88 | 8.88 |
| | | | | | | |
| SelleckChem | 5 µM | 827022-32-2 | PD-0332991 (Palbociclib) HCl | 20.00 | 6.57 | 6.08 |
| SelleckChem | 5 µM | 827022-33-3 | PD0332991 (Palbociclib) Isethionate | 18.66 | 6.17 | 7.21 |
| SelleckChem | 5 µM | 1144068-46-1 | WYE-125132 | 14.73 | 5.01 | 4.83 |
| SelleckChem | 5 µM | 1009298-09-2 | AZD8055 | 13.72 | 4.70 | 5.48 |
| SelleckChem | 5 µM | 741713-40-6 | R547 | 10.40 | 3.72 | 3.57 |
| SelleckChem | 5 µM | 1207360-89-1 | GDC-0349 | 10.09 | 3.63 | 3.41 |
| SelleckChem | 5 µM | 343787-29-1 | CP-673451 | 9.9 | 3.57 | 3.36 |
| SelleckChem | 5 µM | 1009298-59-2 | AZD2014 | 8.56 | 3.17 | 3.67 |
| SelleckChem | 5 µM | 147526-32-7 | Pitavastatin calcium (Livalo) | 8.35 | 3.11 | 3.59 |
| SelleckChem | 5 µM | 1056634-68-4 | Momelotinib (CYT387) | 5.81 | 2.36 | 2.25 |
| SelleckChem | 5 µM | 670220-88-9 | Crenolanib (CP-868596) | 5.56 | 2.29 | 2.17 |
| SelleckChem | 5 µM | 936890-98-1 | OSI-027 | 5.52 | 2.27 | 2.17 |
| SelleckChem | 5 µM | 1224844-38-5 | INK 128 (MLN0128) | 5.46 | 2.25 | 2.58 |
| SelleckChem | 5 µM | 1223001-51-1 | Torin 2 | 5.46 | 2.25 | 2.58 |
| SelleckChem | 5 µM | 928037-13-2 | Golvatinib (E7050) | 5.20 | 2.18 | 2.49 |

| | | | | | | |
|---|---|---|---|---|---|---|
| SelleckChem | 5 μM | 50357-45-4 | Pentamidine HCl | 5.04 | 2.13 | 2.43 |