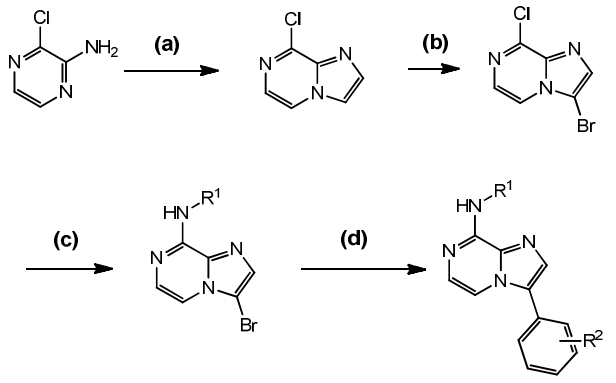
Supporting Information

Chemistry – synthetic methods and analytical data

General Details

Commercial chemicals and solvents were of reagent grade and used without further purification. Reactions sensitive to moisture or air were performed under nitrogen using anhydrous solvents and reagents. The progress of reactions was determined by either analytical thin layer chromatography (TLC), performed with TLC Silica gel 60 F254, or liquid chromatography−mass spectrometry (LC-MS). Purification was by either mass directed reverse phase HPLC or silica gel column chromatography which was performed with the indicated solvent and using silica gel 60. Chemical yields are not optimized. The purity of all compounds screened in biological assays was determined by HPLC/MS to be >95% (UV Diode Array 210-400nm). NMR experiments were recorded on a 400 MHz machine and are referenced to residual solvent signals: CDCl3 (δ 7.26) or DMSO-d6 (δ 2.54). Chemical shifts are reported in δ units (parts per million) and are assigned as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), multiplet (m), broad signal (br), or very broad signal (vbr). Coupling constants (J) are reported in hertz (Hz).

Scheme S1. General synthetic route



Step a: 8-Chloro-imidazo[1,2-a]pyrazine

Bromoacetaldehyde diethyl acetal (200 ml, 1.3 mol) was added to 48ml of 48% HBr. The reaction mixture was refluxed for 1.5 hours then poured onto a suspension of $NaHCO_3$ (100g) in propan-2-ol (1.6 L). The resulting solid was filtered off and 2-amino-3-chloropyrazine (51.8g, 0.4mol) was added to the solution, which was then refluxed for 2 hours. The reaction mixture was cooled and allowed to stand overnight to yield a white solid, which was collected by filtration and washed with propan-2-ol and Et_2O. The solid was added to a saturated solution of $NaHCO_3$ (500ml) and DCM (1L). The aqueous layer was separated from the organic solvent and re-extracted with DCM (2 x 250 mL). The organic layers were combined and dried over $MgSO_4$, filtered and evaporated to dryness, to afford 51.9g of 8-Chloro-imidazo[1,2-a]pyrazine as a pale brown solid that was used without further purification. Yield 96%. ^{1}H NMR (DMSO-d_6): δ ppm 8.65 (1 H, d, J = 4.47 Hz), 8.11 (1 H, s), 7.75 (1 H, d, J = 4.47 Hz). MS (ESI) *m/z* = 154 $[M+H]^+$.

Step b: 3-Bromo-8-chloro-imidazo[1,2-a]pyrazine

To a solution of 1.53g (0.01mol) of 8-chloro-imidazo[1,2-a]pyrazine in 30ml of DCM was added 1.78g (0.01mol) of N-bromosuccinimide and the reaction stirred at room temperature for 2 hours. After this time the reaction mixture was washed with a saturated aqueous solution of Na_2CO_3 (2 x 20 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give 3-bromo-8-chloro-imidazo[1,2-a]pyrazine, which was used without further purification. Yield 96%. ^{1}H NMR (DMSO-d_6) δ ppm 8.51 (2 H, d, J = 4.60 Hz), 8.06 (2 H, s), 7.89 (1 H, s). MS (ESI) *m/z* = 233 $[M+H]^+$.

Step c: General procedure

To a solution of 3-bromo-8-chloro-imidazo[1,2-a]pyrazine (2.0g, 8.6mmol) in 20ml of ^{i}BuOH was added the appropriate amine hydrochloride (9.5mmol) and DIPEA (3.7ml, 21.5mol). The reaction was stirred and heated to 108^{o}C for 16 hours. After this time the reaction was allowed to cool, resulting in a thick precipitate. The precipitate was filtered and washed with diethyl ether to give the desired product as an off white solid that was used without further purification.

4-[(3-Bromo-imidazo[1,2-a]pyrazin-8-ylamino)-methyl]-benzenesulfonamide

Prepared as describe in the general procedure for **Step c**, starting with 3-bromo-8-chloro-imidazo[1,2-a]pyrazine and 4-aminomethyl-benzenesulfonamide hydrochloride.

Yield 38 %. ^{1}H NMR (DMSO-d_6) δ ppm 8.43-8.36 (1 H, m), 7.77 (2 H, d, J = 8.20 Hz), 7.74 (1 H, s), 7.61 (1 H, d, J = 4.67 Hz), 7.53 (2 H, d, J = 8.12 Hz), 7.41 (1 H, d, J = 4.67 Hz), 7.30 (2 H, s), 4.76 (2 H, d, J = 6.28 Hz). MS (ESI) *m/z* = 383 $[M+H]^+$.

3-bromo-N-[(4-methylsulfonylphenyl)methyl]imidazo[1,2-a]pyrazin-8-amine

Prepared as describe in the general procedure for **Step c**, starting with 3-bromo-8-chloro-imidazo[1,2-a]pyrazine and (4-methylsulfonylphenyl)methanamine hydrochloride. Yield 52%. ^{1}H NMR (DMSO-d_6) δ ppm 8.15 (1 H, t, J = 6.31 Hz), 7.75 (1 H, s), 7.61 (1 H, d, J = 7.79 Hz), 7.52 (1 H, d, J = 7.73 Hz), 7.41 (1 H, t, J = 7.72 Hz), 7.34 (1 H, d, J = 4.63 Hz), 7.19 (1 H, d, J = 4.64 Hz), 4.58 (2 H, d, J = 6.33 Hz), 3.01 (3 H, s). MS (ESI) *m/z* = 382 $[M+H]^+$.

4-(1-(3-(4-hydroxyphenyl)imidazo[1,2-*a*]pyrazin-8-ylamino)ethyl)benzenesulfonamide

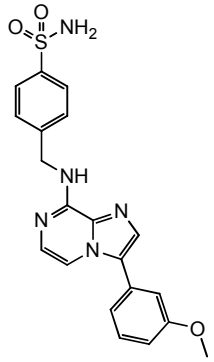
3-Bromo-8-chloro-imidazo[1,2-a]pyrazine (238mg, 0.86mmol), 4-(1-aminoethyl)benzenesulfonamide hydrochloride (300mg, 1.29 mmol) and DiPEA (0.374ml, 2.15 mmol) were added to a microwave tube. DMSO (4 ml) was added and the vessel was sealed and heated in the microwave at 150°C for 30 minutes. After this time the solution was worked up by diluting with DCM, washing with $NaHCO_3$, water and brine. The organic layer was dried using $MgSO_4$, filtered and concentrated under reduced pressure to give the title compound as an off white solid. Yield 47%. ^{1}H NMR (DMSO-d_6) δ ppm 8.20 (1 H, d, J = 7.99 Hz), 7.77 (2 H, d, J = 8.21 Hz), 7.74 (1 H, s), 7.63 (2 H, d, J = 8.20 Hz), 7.58 (1 H, d,

J = 4.66 Hz), 7.38 (1 H, d, J = 4.67 Hz), 7.28 (2 H, s), 5.48-5.42 (1 H, m), 1.60 (3 H, d, J = 7.06 Hz). MS (ESI) *m/z* = 397 $[M+H]^+$.

Step d: General Procedure

The bromide from **Step c** (0.26mmol), appropriate boronic acid (0.39mmol) and aqueous Cs_2CO_3 (0.176ml, 0.065mmol, 3.7M solution) were weighed into a tube. DMF (3 ml) was added and the mixture was deoxygenated by bubbling N_2 through the solution for 30 minutes. [1,1′-bis(diphenyl phosphino) ferrocene]dichloropalladium(II) (21 mg, 0.026 mmol) was added, the tube was sealed and then heated at 90°C for 16 hours. The reaction was diluted with EtOAc and washed with saturated aqueous NH_4Cl, water and brine. The organic layer was dried with $MgSO_4$, filtered and concentrated under reduced pressure before being purified by reverse phase preparative HPLC.

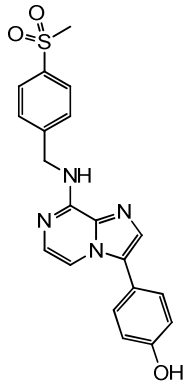
Compound 1. 4-[[[3-(3-methoxyphenyl)imidazo[1,2-a]pyrazin-8-yl]amino]methyl]benzene sulfonamide



Prepared as describe in the general procedure for **Step d**, starting with 4-[(3-Bromo-imidazo[1,2-a]pyrazin-8-ylamino)-methyl]-benzenesulfonamide and (3-methoxyphenyl)boronic acid. Yield 32%.

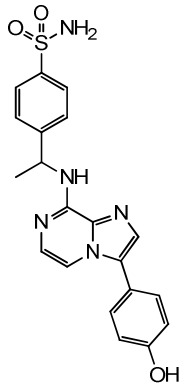
^{1}H NMR (DMSO-d_6) δ ppm 8.31 (1 H, t, J = 6.25 Hz), 7.79 (4 H, d, J = 8.52 Hz), 7.58-7.46 (3 H, m), 7.35-7.19 (5 H, m), 7.07 (1 H, d, J = 8.37 Hz), 4.79 (2 H, d, J = 6.20 Hz), 3.87 (3 H, s). MS (ESI) *m/z* = 410 $[M+H]^+$.

Compound 2. 4-[8-[(4-methylsulfonylphenyl)methylamino]imidazo[1,2-a]pyrazin-3-yl]phenol



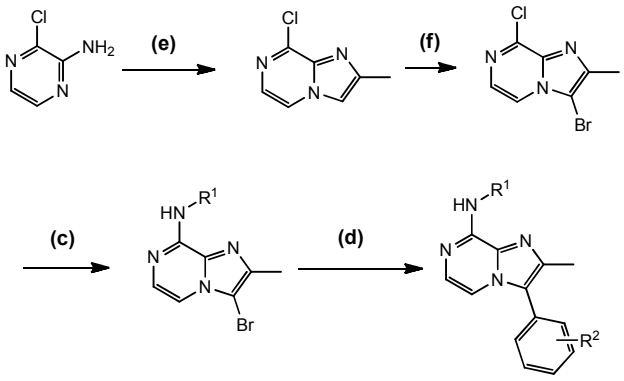
Prepared as describe in the general procedure for **Step d**, starting with 3-bromo-N-[(4-methylsulfonyl phenyl)methyl]imidazo[1,2-a]pyrazin-8-amine and (4-hydroxyphenyl)boronic acid. Yield 20%. ^{1}H NMR (DMSO-d_6) δ ppm 9.84 (1 H, s), 8.30 (1 H, t, J = 6.32 Hz), 7.89 (2 H, d, J = 8.22 Hz), 7.68 (1 H, d, J = 4.79 Hz), 7.65-7.62 (3 H, m), 7.49-7.45 (2 H, m), 7.28 (1 H, d, J = 4.77 Hz), 6.99-6.95 (2 H, m), 4.81 (2 H, d, J = 6.30 Hz), 3.20 (3 H, s). MS (ESI) *m/z* = 395 $[M+H]^+$.

Compound 6. 4-(1-(3-(4-hydroxyphenyl)imidazo[1,2-*a*]pyrazin-8-ylamino)ethyl)benzene sulfonamide



Prepared as describe in the general procedure for **Step d**, starting with 4-(1-(3-(4-hydroxyphenyl)imidazo[1,2-*a*]pyrazin-8-ylamino)ethyl)benzenesulfonamide and (4-hydroxyphenyl) boronic acid. Yield 52%. ^{1}H NMR (DMSO-d_6) δ ppm 8.24 (1 H, s), 8.00 (1 H, d, J = 8.03 Hz), 7.78 (2 H, d, J = 8.02 Hz), 7.65 (4 H, d, J = 9.50 Hz), 7.45 (2 H, d, J = 8.13 Hz), 7.30-7.20 (3 H, m), 6.96 (2 H, d, J = 8.13 Hz), 5.49-5.43 (1 H, m), 1.62 (3 H, d, J = 7.02 Hz). MS (ESI) *m/z* = 410 $[M+H]^+$.

Scheme S2. General synthetic route



Step e: 8-Chloro-2-methyl-imidazo[1,2-a]pyrazine

A solution of 2-amino-3-chloropyrazine (10g, 77.5 mmol) and chloroacetone (30mL, 387mmol) in methanol (30ml) was refluxed for 48 hours. The reaction mix was worked up by diluting with DCM, then washing with $NaHCO_3$. The organic layer was dried with $MgSO_4$, filtered and concentrated under reduced pressure. The crude material was loaded onto silica and purified by chromatography using 0-100% EtOAc in iso-Hexane to give 8-Chloro-2-methyl-imidazo[1,2-a]pyrazine as an off white solid. Yield 36%. ^{1}H NMR (DMSO-d$_6$) δ ppm 8.60 (1 H, d, J = 4.47 Hz), 8.06 (1 H, s), 7.70 (1 H, d, J = 4.47 Hz), 2.46 (3 H, s). MS (ESI) *m/z* = 169 $[M+H]^+$.

Step f: 3-Bromo-8-chloro-2-methyl-imidazo[1,2-a]pyrazine

N-bromosuccinimide (12.71g, 0.07mol) was added to a solution of 8-chloro-2-methyl-imidazo[1,2-a]pyrazine (10g, 0.06mol) in 100ml of DCM and the reaction was stirred at room temperature for 2.5 hours. After this time the solution was washed with saturated aqueous solution of Na_2CO_3 (2 x 20 ml), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give 3-bromo-8-chloro-2-methyl-imidazo[1,2-a]pyrazine). Yield 92%. ^{1}H NMR (DMSO-d$_6$) δ ppm 8.43 (1 H, d, J = 4.55 Hz), 7.86 (1 H, d, J = 4.56 Hz), 2.47 (3 H, s). MS (ESI) *m/z* = 247 $[M+H]^+$.

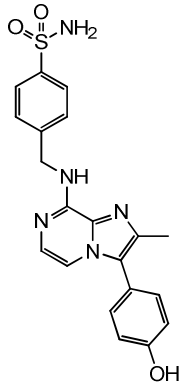
Step c: 4-[(3-Bromo-2-methyl-imidazo[1,2-a]pyrazin-8-ylamino)-methyl]- benzenesulphonamide

Prepared as described in the general procedure for **step c** starting with 4-aminomethyl-benzenesulfonamide hydrochloride and 3-bromo-8-chloro-2-methyl-imidazo[1,2-a]pyrazine. Yield 30%. ^{1}H NMR (DMSO-d$_6$) δ ppm 8.36-8.29 (1 H, m), 7.77 (2 H, d, J = 8.07 Hz), 7.58-7.47 (3 H, m), 7.37 (1 H, d, J = 4.69 Hz), 7.30 (2 H, s), 4.74 (2 H, d, J = 6.29 Hz), 2.39 (3 H, s). MS (ESI) *m/z* = 397 $[M+H]^+$.

Step c: 3-bromo-2-methyl-N-[(3-methylsulfonylphenyl)methyl]imidazo[1,2-a]pyrazin-8-amine

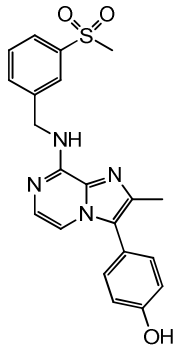
Prepared as described in the general procedure for **Step c** starting with (3-methylsulfonylphenyl) methanamine and 3-bromo-8-chloro-2-methyl-imidazo[1,2-a]pyrazine. Yield 50%. ^{1}H NMR (DMSO-d_6) δ ppm 8.15 (1 H, t, J = 6.31 Hz), 7.75 (1 H, s), 7.61 (1 H, d, J = 7.79 Hz), 7.52 (1 H, d, J = 7.73 Hz), 7.41 (1 H, t, J = 7.72 Hz), 7.34 (1 H, d, J = 4.63 Hz), 7.19 (1 H, d, J = 4.64 Hz), 4.58 (2 H, d, J = 6.33 Hz), 3.01 (3 H, s), 2.19 (3 H, s). MS (ESI) *m/z* = 396 $[M+H]^+$.

Compound 3. 4-[[[3-(4-hydroxyphenyl)-2-methyl-imidazo[1,2-a]pyrazin-8-yl]amino]methyl] benzenesulfonamide



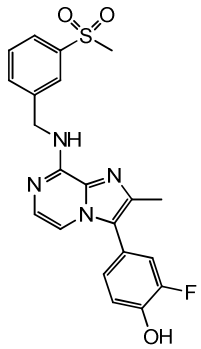
Prepared as described in the general procedure for **Step d** starting with 4-[(3-bromo-2-methyl-imidazo[1,2-a]pyrazin-8-ylamino)-methyl]- benzenesulfonamide and (4-hydroxyphenyl)boronic acid. Yield 50%. ^{1}H NMR (DMSO-d_6) δ ppm 9.83 (1 H, s), 8.21-8.16 (1 H, m), 7.77 (2 H, d, J = 8.18 Hz), 7.53 (2 H, d, J = 8.13 Hz), 7.41 (1 H, d, J = 4.73 Hz), 7.38-7.32 (2 H, m), 7.30 (2 H, s), 7.20 (1 H, d, J = 4.73 Hz), 7.00-6.96 (2 H, m), 4.75 (2 H, d, J = 6.26 Hz), 2.39 (3 H, s). MS (ESI) *m/z* = 410 $[M+H]^+$.

Compound 4. 4-[2-methyl-8-[(3-methylsulfonylphenyl)methylamino]imidazo[1,2-a]pyrazin-3-yl] phenol



Prepared as described in the general procedure for **Step d** starting with 3-bromo-2-methyl-N-[(3-methylsulfonylphenyl)methyl]imidazo[1,2-a]pyrazin-8-amine and (4-hydroxyphenyl)boronic acid. Yield 30%. ^{1}H NMR (DMSO-d_6) δ ppm 8.38 (1 H, s), 8.28 (1 H, t, J = 6.36 Hz), 7.99-7.93 (1 H, m), 7.81 (1 H, d, J = 7.79 Hz), 7.73 (1 H, d, J = 7.77 Hz), 7.67-7.58 (1 H, m), 7.42 (1 H, t, J = 4.74 Hz), 7.35 (2 H, d, J = 8.33 Hz), 7.21 (1 H, dd, J = 8.89, 4.73 Hz), 6.98 (2 H, d, J = 8.32 Hz), 4.78 (2 H, t, J = 6.26 Hz), 3.22 (3 H, s), 2.39 (3 H, s). MS (ESI) *m/z* = 409 $[M+H]^+$.

Compound 5. 2-fluoro-4-[2-methyl-8-[(3-methylsulfonylphenyl)methylamino]imidazo[1,2-a]pyrazin-3-yl]phenol



Prepared as described in the general procedure for **Step d** starting with 3-bromo-2-methyl-N-[(3-methylsulfonylphenyl)methyl]imidazo[1,2-a]pyrazin-8-amine and (3-fluoro-4-hydroxy-phenyl)boronic acid. Yield 46%. ^{1}H NMR (DMSO-d_6) δ ppm 8.26 (1 H, t, J = 6.31 Hz), 7.97 (1 H, s), 7.82 (1 H, d, J = 7.77 Hz), 7.74 (1 H, d, J = 7.71 Hz), 7.62 (1 H, t, J = 7.71 Hz), 7.47 (1 H, d, J = 4.71 Hz), 7.38-7.32 (1 H, m), 7.24 (1 H, d, J = 4.72 Hz), 7.21-7.12 (2 H, m), 4.79 (2 H, d, J = 6.28 Hz), 2.55 (3 H, s), 2.40 (3 H, s). MS (ESI) *m/z* = 427 $[M+H]^+$.

Antiviral assays – materials and methods

Assay preparation:

Poliovirus (PV) and coxsackievirus (CV):

Vero cells, subcultured in cell growth medium [MEM Rega3 (Cat. N°19993013; Invitrogen) supplemented with 10% FCS (Integro), 5ml 200mM L-glutamine (25030024) and 5ml 7.5% sodium bicarbonate (25080060)] at a ratio of 1:4 and grown for 7 days in 150cm² tissue culture flasks (Techno Plastic Products), were harvested and a cell suspension was prepared with a cell density of 25 000 cells/50µl in assay medium (MEM Rega3, 2% FCS, 5ml L-glutamine and 5ml sodium bicarbonate) of which 50µl was seeded per well at the end of the assay setup.

Human rhinovirus (hRV):

Hela Rh cells, subcultured in cell growth medium [MEM (Cat. N°21090; Invitrogen) supplemented with 10% FCS (Integro), 5ml 200mM L-glutamine (25030024) and 10ml 1M HEPES (15630)] at a ratio of 1:10 and grown for 3 - 4 days in 150cm² tissue culture flasks (Techno Plastic Products), were harvested and a cell suspension was prepared with a cell density of 15 0000 cells/50µl in assay medium (MEM, 2% FCS, 5ml L-glutamine and 10ml HEPES) of which 50µl was seeded per well at the end of the assay setup.

Enterovirus (EV):

RD cells, subcultured in cell growth medium [MEM Rega3 (Cat. N°19993013; Invitrogen) supplemented with 10% FCS (Integro), 5ml 200mM L-glutamine (25030024) and 5ml 7.5% sodium bicarbonate (25080060)] at a ratio of 1:4 and grown for 7 days in 150cm² tissue culture flasks (Techno Plastic Products), were harvested and a cell suspension was prepared with a cell density of 25 000 cells/50µl in assay medium (MEM Rega3, 2% FCS, 5ml L-glutamine and 5ml sodium bicarbonate) of which 50µl was seeded per well at the end of the assay setup.

Antiviral and cytotoxicity determinations

Compounds were prepared as DMSO stock solution with a final compound concentration of 10mM.

The compound profiling setup was performed employing a Freedom EVO200 liquid handling platform (Tecan). The evaluation of the cytostatic/cytotoxic as well as the antiviral effect of each compound was performed in parallel within one run.

Three 8-step 1-to-5 dilution series were prepared. The highest concentrations of each of the three dilution series were prepared as a 3-step 1-to-3 dilution series. Integration of all generated data points yields a duplicate 24-step dose-response curve. Furthermore, because each random (duplicate) data point (green arrow) intermits in between two (duplicate) data points of two other dilution series (red arrows), the result obtained for each data point is cross-checked within the experiment. The highest concentration of DMSO in the assay (at 100µM final concentration) was determined to be 2%.

Compound dilutions were prepared in assay medium added to empty wells (picornaviruses: 96-well microtiter plates, Falcon, BD) or in the medium present on top of pre-seeded cells (BVDV and HCV,

seeded one day before setup of the experiment, see above). Subsequently, no virus (HCV), 100µl of a 2x virus dilution (BVDV), or 50µl of a 4x virus dilution in assay medium (picornaviruses; assay medium supplemented with 15ml MgCl 1M (Sigma, M1028) in case of hRV) was added followed by addition of 50µl of cell suspension (picornaviruses). The assay plates were returned to the incubator for 2-3 (picornavirus, 35°C for hRV) days, a time at which maximal cytopathic effect for picornaviruses is observed.

For the evaluation of cytostatic/cytotoxic effects and for the evaluation of the antiviral effect in case of PV, CV, hRV, the assay medium was aspirated, replaced with 75µl of a 5% MTS (Promega) solution in phenol red-free medium and incubated for 1.5 hours (37°C, 5% CO2, 95-99% relative humidity). Absorbance was measured at a wavelength of 498nm (Safire², Tecan) and optical densities (OD values) were converted to percentage of untreated controls.

Analysis of the raw data, quality control of each individual dose-response curve and calculation, if possible, of the EC50, EC90 and CC50 values was performed employing ViroDM, a custom-made data processing software package. The EC50 and EC90 (values derived from the dose-response curve) represent the concentrations at which respectively 50% and 90% inhibition of viral replication would be observed. The CC50 (value derived from the dose-response curve) represent the concentration at which the metabolic activity of the cells would be reduced to 50 % of the metabolic activity of untreated cells.

Microsoft Excel software was used to assemble the 3 dose-response curves obtained for each compound into one graph. To this end the median ± standard deviation (SD) was calculated for each concentration. One curve (coloured green) is obtained for the residual metabolic activity and one curve (coloured red) for the anti-replicon effect.

The EC50, EC90 and CC50 ± SD were, whenever possible, calculated respectively as the median of all the EC50, EC90 or CC50 values derived from the 3 individual dose-response curves. The selectivity index (SI), indicative of the therapeutic window of the compound, was calculated as CC50/EC50. No further statistical analysis was performed.

PI4K *in vitro* activity assay.

The PI4K *in vitro* activity assay was performed as described previously (Van der Schaar HM *et. al*. Cell Res. 2012, 1–17). Briefly, recombinant PI4KIIIβ (SignalChem) or PI4KIIIα (Millipore) and their substrate phosphatidylinositol (PI) : phosphatidylserine (PS) were diluted in buffer containing Triton X-100. The reaction was started by addition of a mixture of ATP and 0.25 µCi [^{33}P]- γ-ATP. After 75-90 minutes incubation at 30°C, the reaction was terminated by addition of phosphoric acid. The incorporated radioactivity was measured by TopCount NXT™ Microplate Scintillation (PerkinElmer). Data were converted to percent inhibition of controls.