Supplementary Materials and Methods

Kinase inhibition assays using recombinant substrates

CDK1/cyclin B and CDK2/cyclin E kinases were produced in Sf9 insect cells via baculoviral infection, while CDK5/p35, CDK7/cyclin H/MAT1, and CDK9/cyclin T1 were purchased from ProQinase (Freiburg, Germany) and assayed as described previously (1). The kinase reactions were assayed with 1 mg/ml histone H1 (for CDK2 and CDK5) or $(YSPTSPS)_2KK$ peptide (for CDK7 and CDK9) in the presence of 15/0.15/1.5/1.5 mM ATP (for CDK2/CDK5/CDK7CDK9), 50 μCi [γ-^{33}P]ATP and of the test compound in a final volume of 10 ml. The reaction buffer contained 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 mM Na-orthovanadate, 1.2 mM DTT, 2.5 mg/50 ml PEG20.000. The reactions were stopped by adding 5 ml of 3% H_3PO_4. Aliquots were spotted onto P-81 phosphocellulose (Whatman, GE Healthcare Biosciences, Pittsburgh, USA), washed 3 times with 0.5% H_3PO_4 and finally air-dried. Kinase inhibition was quantified using a FLA-7000 digital image analyzer (Fujifilm, Tokyo, Japan). The concentration of the test compounds required to decrease the CDK activity by 50% was determined from dose-response curves and designated as IC_{50}.

Analysis of proliferation

6 x 10^4 cells were seeded on 12-well plates and incubated with inhibitors at different concentrations for 3 days. Cell numbers were determined at various time points after trypsinization using a cell counter (CASY, Schärfe Systems, Reutlingen, Germany). Three independent experiments were performed in triplicates.

Supplementary Figure Legends

Supplementary Figure S1.The structure of BA-12 and BP-14. A, BA-12 (2-[[[2-[(4-aminocyclohexyl)amino]-9-cyclopentyl-purin-6-yl]amino]methyl]-4-chloro-phenol). B, BP-14 (N2-(4-aminocyclohexyl)-9-cyclopentyl-N6-[[6-(2-furyl)-3-pyridyl]methyl]purine-2,6-diamine).

Supplementary Figure S2. Cell viability and CDK inhibition of Hep3B and 3sp hepatoma cells. A and B, dose-dependent effects of BA-12 (A) and BP-14 (B) on the viability of human Hep3B and 3sp hepatoma cells. Cell viability was determined by MTT assay. Error bars depict SD from at least three individual experiments.

Supplementary Figure S3. Proliferation of HCC cells after exposure to BA-12 or BP-14. A and B, Proliferation kinetics of HepG2, PLC and Hep3B cells after treatment with different concentrations of BA-12 (A) or BP-14 (B). Error bars depict SD from at least three individual experiments.

Supplementary Table S1: IC_{50} values of BA-12 and BP-14 in hepatoma cell lines

Supplementary Table S2: Selectivity of BA-12 and BP-14 on protein kinases using recombinant CDK substrates

References

1. Zatloukal M, Jorda R, Gucky T, Reznickova E, Voller J, Pospisil T, et al. Synthesis and in vitro biological evaluation of 2,6,9-trisubstituted purines targeting multiple cyclin-dependent kinases. European journal of medicinal chemistry. 2012:Epub 2012 Jul 10.