

A novel potent CDK4/6 inhibitor BPI-16350 in preclinical cancer models

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Abstract

Inhibition of the cyclin-dependent kinase (CDK) 4/6-retinoblastoma (Rb) pathway is an effective therapeutic strategy against cancer. Here, we report a novel CDK4/6 inhibitor, BPI-16350, identified from comprehensive screening of a large panel of kinases. A series of investigations to evaluate anti-tumor activity show that BPI-16350 exhibits potent antiproliferative activities by inducing G1 arrest in a wide range of human Rb-positive tumor cells. Multiple carcinoma xenografts reveal that BPI-16350 has equivalent or improved anti-tumor efficacy compared with palbociclib or abemaciclib, and achieves obvious tumor regression without body weight loss in experimental animals, where pharmacokinetic study in SD rats indicates a longer half-life of BPI-16350 with once daily. Furthermore, BPI-16350 achieves excellent brain penetration with a nearly sixfold brain-to-plasma ratio in mice, suggesting the potential of treating tumor brain metastasis. Taken together, our findings indicate that BPI-16350 is a novel CDK4/6 inhibitor with favorable pharmaceutical properties as an anti-cancer agent.

Introduction

Breast cancer has now surpassed lung cancer as the leading cause of global cancer incidence in 2020 and is the fifth leading cause of cancer mortality worldwide [1]. Hormone receptor-positive (HR+) breast cancer constitute approximately 60–70% of all breast cancers [2]. Approximately 40–50% of patients with estrogen receptor-positive breast cancer treated with adjuvant endocrine therapy that is considered the mainstay therapy for HR + breast cancer, will develop disease recurrence [3], and most patients with advanced disease develop disease progression within 1–2 years of endocrine therapy [4, 5]. Despite remarkable improvements in treatment of HR + breast cancers, a large proportion of patients eventually acquire resistance to endocrine therapy. Since treatments for patients with HR+/human epidermal growth factor receptor 2-negative (HER2-) breast cancers are still limited, novel therapies such as cyclin-dependent kinase (CDK) 4/6 inhibitors are urgently needed.

The CDK4/6-cyclin D-retinoblastoma (Rb) pathway plays essential roles in the transition from G1 to S phase of the cell cycle. The cyclin D-CDK4/6 complex phosphorylates Rb protein to cause its dissociation from E2F transcription factors, thus releasing transcriptional repression of genes important for DNA synthesis and S-phase progression [6, 7]. Alterations in several cell cycle-regulating proteins have been described in breast cancer, including various cyclins, CDKs, and Rb [8, 9]. Furthermore, cyclin D1, the binding partner of CDK4/6, is often overexpressed in patients with HR+/HER2 - breast cancer, leading to continuous activation of the cyclin D1-CDK4/6 complex. Dysregulation of the cyclin D1-CDK4/6 axis has been implicated in acceleration of breast cancer from G1 to S phase, promoting cancer progression [10]. Additionally, although CDK4 and cyclin D1 are unnecessary for normal mammary gland development, they are required for development of breast malignancies, suggesting that CDK4 inhibition may suppress breast cancer cells while sparing healthy tissues [11]. Notably, the effectiveness of CDK4/6 inhibitors can be increased by combination with drugs that prevent downstream estrogen-dependent stimulation of cancer cells. Inhibition of the estrogen pathway by endocrine therapy results in downregulated cyclin D1 expression and reduced complexation of CDK4 and CDK6 [12, 13]. Therefore, selective CDK4/6 inhibitors

palbociclib, ribociclib, and abemaciclib are frequently given in combination with endocrine therapy (aromatase inhibitors or fulvestrant) for the treatment of HR+/HER2 – breast cancers.

The existing CDK4/6 inhibitor-based therapies have shown great promise in improving clinical outcomes, the side effect also stronger, which may vary in their selectivity toward other cyclin–CDK complexes. For example, diarrhea results from avemaciclib is more serious. One of the possible reasons is that it has strong inhibition on CDK9[14, 15]. Therefore, the development of better selective CDK4/6 inhibitors is expected to reduce side effects and improve safety. Based on the above reasons, we developed a selective CDK4 / 6 BPI-16350 to reduce the inhibition of CDK9 and have good drug properties

Materials And Methods

Cell lines and culture

MCF-7, ZR-75-1, T-47D, NCI-H292, MV-4-11, MDA-MB-231, MDA-MB-361, MDA-MB-468, JeKo-1, COLO 205, and BT-474 cell lines were purchased from ATCC (Manassas, VA, USA) and cultured as suggested by ATCC.

Cell viability assay

Cell viability was assayed with CellTiter-Glo® (Promega, Madison, WI, USA). Cells were seeded in 96-well microplates at a density of 3×10^3 cells/well for COLO 205 cells and 4×10^3 cells/well for the other cell lines in 100 μ L of corresponding medium. The following day, cells were treated with compounds at final concentrations of 4.6, 13.7, 41.1, 123, 370, 1111, 3333, 10000, and 30000 nM for 96 h (120 h for COLO 205). Following incubation, 60 μ L of medium was removed from each well and replaced with an equal volume of CellTiter-Glo solution. Plates were incubated for 30 min at room temperature before measuring chemiluminescence values with a microplate reader (Envision, PerkinElmer, Waltham, MA, USA). Paclitaxel (0.15, 0.45, 1.37, 4.11, 12.34, 37.03, 111.1, 333.3, and 1000 nM) was used as a positive control and DMSO were used as the vehicle control. Each experiment was performed in triplicate.

BrdU-incorporation assay

Cells were seeded in a 96-well culture plates at a density 4×10^3 cells/well for MDA-MB-361 cells or 3×10^3 cells/well for MCF-7 and T47D cells for 24 h. Cells were incubated for 96 h with indicating concentrations of compounds (4.6, 13.7, 41.1, 123, 370, 1111, 3333, 10000, and 30000 nM). Following incubation, 2 μ L of BrdU working solution was added to all wells at a 100 \times dilution overnight. The following day, culture medium was removed from wells and replaced with fixative solution for 30 min, and 0.5 μ g/mL of Anti-BrdU-Eu was added to cells. After 1-h incubation, the plates were gently washed four times and 200 μ L of DELFIA inducer was added for 30 min. Finally, europium fluorescence was measured with an Envision plate reader. The results were calculated as follows: inhibition rate = (max

fluorescence - compound fluorescence)/(max fluorescence - min fluorescence) × 100%. The concentration that caused 50% growth inhibition (IC₅₀) was calculated and used in additional assays.

Cell cycle analysis

Cells were treated with the compound for 24 h, harvested, and then stained with propidium iodide (BD Biosciences, Franklin Lakes, NJ, USA). DNA contents were determined using a FACSCalibur™ flow cytometry system (BD Biosciences). Data were analyzed using ModFit 3.0 software (BD Biosciences).

Western blotting

Following treatment with varying concentrations of drugs, cells were lysed and total cellular proteins were separated and transferred to a membrane that was subsequently incubated with antibodies. Proteins were visualized using a western blot imaging system (Clinx Science Instruments, Shanghai, China).

Tumor xenograft studies

All animal studies were performed in accordance with institutional guidelines of the American Association for Laboratory Animal Care and all protocols were approved by the Company Animal Care and Use Committee. JeKo-1, COLO 205, and MV-4-11 cells (MV-4-11 acquired from Ankai Yibo Biotechnology, Beijing, China) were grown, harvested, and resuspended in a 1:1 mixture of serum-free media and Matrigel (BD Biosciences). Subsequently, 5 × 10⁶ cells were subcutaneously injected into the rear flank of 7-week-old NOD/SCID female mice. Tumor xenografts were measured twice a week and tumor volumes were calculated according to the formula: $T_{vol} = L \times W^2 \times 0.5$, in which T_{vol} is tumor volume, L is tumor length, and W is tumor width. LY2835219 and BPI-16350 were orally administered by gavage at the indicated dose and schedule. Tumor volume and body weight were measured twice weekly. To collect tumors for biomarker analysis, animals were euthanized and the tumors were separated, flash frozen in liquid nitrogen, and stored at - 80°C until analyzed. Tumor volume data were analyzed with a two-way repeated measures analysis of variance by time and treatment using the MIXED procedure in SAS software (version 9.2; SAS Institute, Cary, NC, USA).

Statistical analysis

Statistical analyses were performed using GraphPad Prism (version 7; GraphPad Software, San Diego, CA, USA). IC₅₀ values were calculated by nonlinear regression analysis of dose-response curves. Confidence interval (CI) values were calculated using CalcuSyn software (<http://www.biosoft.com/w/calculusyn.htm>). Mean ± standard deviation (*in vitro*) and mean ± standard error of the mean (*in vivo*) represent the results of repeated experiments. Student's t test was used to test differences between groups. P-values < 0.05 were considered statistically significant.

Results

BPI-16350 is a potent and selective CDK4/6 inhibitor

The isoform selectivity profile of BPI-16350 was determined together with LY2835219 (abemaciclib) and LEE011 (ribociclib). BPI-16350 exhibited highly selective inhibition of CDK4/cyclinD1 and CDK6/cyclinD1 with IC₅₀ values of 2.21 nmol/L and 0.4 nmol/L, respectively. BPI-16350 exerted a comparable effect on CDK4/CDK6 as LY2835219 and was slightly better than ribociclib (Table 1). All three inhibitors proved to be far less active against other CDKs, such as CDK1/cyclinB, CDK2/cyclinE, CDK5/p25, and CDK7/cyclinH complexes. Notably, BPI-16350 exhibited much weaker effect on CDK9/cyclinT1, which may avoid serious gastrointestinal reactions in patients like abemaciclib caused by CDK9 inhibition[14]. To further characterize the kinase selectivity of BPI-16350, its affinities for a panel of 468 kinases were analyzed using the KINOMEscan® selectivity screening platform (DiscoverRX, Fremont, CA, USA). As shown in Fig. 1A, BPI-16350 at 100 nM or 1000 nM shows similar kinome profiling and the observed binding profile was significantly concentrated on the CGMC family of kinases, which contains CDKs and mitogen-activated protein kinases.

Table 1
BPI-16350 biochemical profiling on CDKs

IC50 nM	BPI-16350	LY2835219/Abemaciclib	LEE011/Ribociclib
CDK4/cyclin D1	2.21	1.01	5.47
CDK6/cyclin D1	0.407	0.186	1.69
CDK4/cyclin D3	4.19	3.96	5.89
CDK6/cyclin D3	10.2	22.08	70.4
CDK1/cyclin B	1600	477	30000
CDK2/cyclin E	1190	347	30000
CDK3/cyclin E	2200	4542	95100
CDK5/p25	344	227	36500
CDK7/cyclin H	715	1930	30000
CDK9/cyclinT1	91.9	6.66	501

BPI-16350 inhibited cell proliferation and viability by arresting cells at G1 phase

A BrdU incorporation assay was used to validate the anti-proliferative effect in T47D, MCF-7, and MDA-MB-361 cell lines. The results showed that BPI-16350 inhibited proliferation of these cells comparably to abemaciclib as shown in Table 2. Furthermore, combining BPI-16350 and fulvestrant yielded synergistic anti-proliferation effects on T-47D cells, an Rb + breast cancer cell line (Supplementary Fig. 1). CellTiter-Glo assay was carried out to further testify viability on additional four cell lines, COLO 205, YeKo-1, MK-4-11, and MDA-MB-468. The similar results were observed in Table 2. While BPI-16350 ($IC_{50} = 4583 \text{ nmol/L}$) showed limited effects on Rb-negative MDA-MB-468 cell. As reported, CDK4/6 inhibitors arrest the cell cycle at the G1 phase via Rb-phosphorylation inhibition[16, 17]. Thus, we next sought to define whether BPI-16350 exerted anti-proliferative effects in Rb + cells through G1-phase block. After treating an extensive panel of Rb + cancer cell lines and MDA-MB-468 (Rb-negative) with BPI-16350 or abemaciclib for 24h, the cell-cycle was detected by flowcytometry. The two compounds had similar G1 phase arrest ratios in Rb + cells (Table 2). Indeed, the results showed that G1 arrest by CDK4/6 inhibitors occurred in a dose-dependent manner, the proportion of cells in G0/G1 phase increased from about 50–95% following treatment with 3 nM to 20 μM BPI-16350, however no such effect was seen in Rb-negative MDA-MB-468 cells at concentrations up to 20 μM (Fig. 1B).

Table 2
BPI-16350 efficiently inhibits cell proliferation and viability

Assay	Cell line	Genotype	IC50 (nM)	
			BPI-16350	Abemaciclib
Cell Proliferation	T-47D	Rb+	37.2	/
	MDA-MB-361	Rb+	56.8	43.2
	MCF-7	Rb+	84.8	73.3
Cell Viability	JeKo-1	Rb+	36.4	21.0
	MV-4-11	Rb+	87.0	39.1
	COLO 205	Rb+	371.8	169.0
	MDA-MB-468	Rb-	4583	1754
G1 arrest	MDA-MB-361	Rb+	5.4	4.8
	BT-474	Rb+	6.9	12.2
	T-47D	Rb+	14.2	12.5
	ZR-75-1	Rb+	20.9	31.4
	MDA-MB-231	Rb+	28.2	31.0
	JeKo-1	Rb+	28.5	29.3
	MV-4-11	Rb+	59.7	44.4
	COLO 205	Rb+	93.0	85.9
	NCI-H1299	Rb+	113.7	119.1
	MDA-MB-468	Rb-	> 20000	> 20000

BPI-16350 inhibited tumor growth in human xenograft models

Animal experiments were performed to further evaluate the anti-tumor efficacy of BPI-16350 against a panel of tumor xenografts *in vivo*. The results showed that BPI-16350 have stronger suppression on tumor growth. And tumor volume and weight are shown in Fig. 1C. For COLO 205 xenografts, BPI-16350 inhibited tumor growth by 71.7%, 89.5%, and 98.5% at dosages of 37.5, 75, and 150 mg/kg, respectively (Supplementary Table 1). Tumor xenografts of JeKo-1 and MV-4-11 were also investigated to further confirm anti-tumor effects. The results were in accordance with those observed in the COLO 205 model, with the TGI ranging from 73.8–104.5% for JeKo-1 and 33.2–67.7% for MV-4-11, at 25 mg/kg to 100

mg/kg dosages of BPI-16350. And all the animals were well tolerated with no significant body weight loss except for the 150 mg/kg group. In addition, synergetic effects were also observed in MCF-7 xenograft models for combination of BPI-16350 and fulvestrant, and there were no significant toxicities for neither combination therapy nor monotherapy (Supplementary Fig. 1).

Pharmacokinetic/pharmacodynamic studies of BPI-16350

The pharmacokinetic profiles of BPI-16350 were evaluated in SD rats. Correlations between drug exposure and dose after repeated dose administration were analyzed. Single-dose and repeated-dose administration of 30 mg/kg BPI-16350 were compared in Table 4. The drug exposure (AUC_{0-inf}) increased in proportion with doses ranging from 10 to 90 mg/kg (Table 3).

Table 3
Pharmacokinetic Parameters of BPI-16350 in S-D rats

parameter	unit	PO	PO(first time)	PO(seven times)	PO
		10 mg·kg ⁻¹ ₁	30 mg·kg ⁻¹	30 mg·kg ⁻¹	90 mg·kg ⁻¹
T _{1/2}	h	8.3 ± 2.8	9.7 ± 2.4	37 ± 20	27 ± 11
AUC _{0-inf}	ng·h·mL ⁻¹	5500 ± 1243	34700 ± 18106	144000 ± 83885	98600 ± 22266
MRT _{0-inf}	h	10 ± 1	23 ± 4	56 ± 35	52 ± 10
MRT _{0-t}	h	9.7 ± 1.3	23 ± 4	33 ± 9	39 ± 1
AUC _{0-inf_dose}	ng·h·kg·(mL·mg) ⁻¹	550 ± 124	1160 ± 604	NA	980 ± 195
F _{n=6}	%	32.7 ± 7.4	68.8 ± 35.9	NA	58.5 ± 11.6
T _{max}	h	4.0 ± 0	10 ± 7	9.3 ± 11.2	32 ± 0
C _{max}	ng·mL ⁻¹	517 ± 115	1030 ± 482	2420 ± 1631	1530 ± 326
AUC _{0-inf} area under the curve from 0 h after administration to infinite time point, C _{max} maximum concentration, MRT mean residence time, T _{1/2} terminal half-life, T _{max} time to C _{max} , F distribution per unit of body weight					

Table 4
Brain penetration data in COLO 205 xenograft study with repeated dosing

Does (mg/kg)	Time (h)	Brain Concentration (ng/mL)	Plasma Concentration (ng/mL)	Brain/Plasma ratio
50 BID	8	4899	1652	3.04
	24	3359	1207	2.88
100 QD	8	12266	2088	5.93
	24	10086	1698	5.97

To investigate the correlation between drug exposure and target inhibition level, Rb-phosphorylation was further evaluated in COLO 205 tumor xenografts. Both drugs were quickly absorbed into the plasma by 1 h and remained relatively high after 6 h. The max concentration in tumor occurred after approximately 6 h and remained high after 12 h. Interestingly, concentrations of BPI-16350 were much higher in tumor (9150 ± 700 ng/mL) than plasma (571 ± 88 ng/mL) at 6 h (supplementary Table 2). Similarly, rates of phospho-Rb inhibition increased quickly ($42.5\% \pm 26.8\%$ after 1 h) and peaked ($66.5\% \pm 8\%$) at 6 h (Fig. 1D). These findings also revealed that phospho-Rb correlates positively with BPI-16350 concentration.

BPI-16350 achieves BBB penetration

Brain metastasis is a great obstacle to prolonging patient survival. Accordingly, the ability of new drugs to penetrate the BBB needs consideration. Therefore, we evaluated BPI-16350 concentrations in plasma and brain tissues respectively. The results show that BPI-16350 penetrated the BBB to achieve higher concentrations in brain comparable to time-matched plasma concentrations in COLO 205 xenograft mice. We also found that once-daily oral administration at 100 mg/kg was much better than twice-daily at 50 mg/kg, which may due to the compound prefers to accumulate in brain rather than in plasma at higher a dose (Table 4). Thus, BPI-16350 has good BBB penetration which makes it potential for treating brain metastases.

Discussion

Great achievements have been announced for CDK4/6 inhibitors in many clinical studies and these encouraging results have prompted clinicians to change treatment paradigms for metastatic HR+/HER2 – breast cancers. At present, three orally bioavailable CDK4/6 inhibitors, palbociclib (Ibrance, PD0332991; Pfizer, New York, NY, USA), ribociclib (Kisqali, LEE011; Novartis, Basel, Switzerland), and abemaciclib (Verzenio, LY2835219; Eli Lilly and Company, Indianapolis, IN, USA), have been approved by the United States Food and Drug Administration for treatment of patients with HR+/HER2 – metastatic breast cancer [18, 19]. These CDK4/6 inhibitors can be administered as monotherapy in heavily pretreated patients, as combination therapy with aromatase inhibitors for initial therapy, or as combination therapy with

fulvestrant after disease progression following first-line endocrine therapy. However, because these drugs are expensive or unavailable to patients in China, development of new CDK4/6 inhibitors with improved efficacy and reduced toxicity is urgently needed in China.

In this study, BPI-16350, a novel CDK4/6 inhibitor developed by the Chinese pharmaceutical company Betta Pharmaceutical (Zhejiang, China), was shown to exhibit potent antitumor activity both *in vivo* and *in vitro*. BPI-16350, which exhibited strong kinase selectivity comparable to abemaciclib, inhibited the proliferation and viability of Rb-positive cancer cell lines by inducing cell cycle arrest in G1 phase. In xenograft models, BPI-16350 administration could suppress tumor growth in a dose-dependent manner. Importantly, BPI-16350 exhibited favorable pharmacokinetic and pharmacodynamic properties without causing noticeable toxicity in animal models. Our results also demonstrate that combination therapy with fulvestrant yielded greater inhibition of proliferation than individual treatment in cancer cell lines similar with previous studies [20, 21]. The potent anti-tumor activity and enhanced characteristics revealed by our experiments provide a strong rationale for ongoing clinical trials of BPI-16350. Indeed, we observed clinical benefit and better tolerance during period of dose escalation in a phase I clinical trial of one PR.

In previous studies, abemaciclib was reported to be structurally different from palbociclib and ribociclib, and demonstrated stronger (nanomolar) selectivity for CDK4 and CDK6 [22]. BPI-16350 has a similar structure (data not shown) and better selectivity towards CDK4/cyclin D1 and CDK6/cyclin D1 compared with abemaciclib. Notably, BPI-16350 had a weaker effect on other CDKs compared with abemaciclib, especially CDK9, for which abemaciclib is a potent inhibitor [23, 24]. Inhibition of CDK9 can modify the glycogen synthase kinase 3-mediated effects of abemaciclib, leading to observations of specific intestinal toxicity [24–26]. Thus, it is predicted that BPI-16350 may have fewer intestinal reactions, such as diarrhea and nausea. Indeed, in an ongoing phase I study, BPI-16350 elicited controllable diarrhea less than or equivalent to grade I CTCAE5.0 in a majority of subjects, which may be explained by its lack of inhibition for CDK9. We also observed substantially lower incidence and severity of myelotoxicity following continuous dose scheduling of BPI-16350, which is not achieved with 1-week drug holiday of palbociclib or ribociclib [27, 28]. Moreover, oral administration of BPI-16350 is more convenient (once a day) than abemaciclib (twice a day) to reach an effective biological dose. Consistent with the preclinical results, detected concentrations of BPI-16350 were lower in plasma, tumor, and brain following twice-daily compared with once-daily administration at the same total dosage.

A distinct feature of abemaciclib is its ability to cross the BBB; fortunately, because of its similar molecular structure, BPI-16350 exhibits BBB permeability in rodents. Breast cancer brain metastasis, one of the most common forms of breast cancer metastasis, has a poor prognosis [29, 30]. In a comparative study of breast cancers with brain metastasis, median overall patient survival was 7.1 months for HR+/HER2-, 18.9 months for HR+/HER2+, 13.1 months for HER2-, and 4.4 months for triple-negative forms [31]. The poor prognosis after brain metastasis may result from the BBB removing drug substances such as chemotherapeutic agents, targeted agents, and toxins from the brain [32, 33]. The BBB is constructed of specialized blood vessel structures comprising endothelial cells, astrocytes, pericytes, and neurovascular units [34]. Endothelial cells express several transporters (e.g., P-glycoprotein

and multidrug-resistance proteins) that act as efflux pumps [35, 36]. Our exploratory results indicate that BPI-16350 has high permeability in Caco-2 cells and, although less obvious, appears a less likely substrate for efflux compared with P-glycoprotein and breast cancer resistance protein; moreover, BPI-16350 had a certain inhibitory effect on these proteins. Importantly, BPI-16350 was observed to have a six-fold brain-to-plasma ratio following 100 mg/kg administration in mice, indicating high brain-penetration potential. Consistent with preclinical results, a phase I study of BPI-16350 in patients with breast cancer brain metastasis revealed that all extracranial lesions were regrade and intracranial multiple lesions were continually stable without out of group. However, more evidence is needed to establish whether BPI-16350 influences and permeates the BBB.

In conclusion, BPI-16350 is a potent and orally available CDK4/6 inhibitor with broad-spectrum anti-tumor effects against Rb-positive cancer cell lines. Moreover, BPI-16350 exhibited feasibility, antitumor efficacy, higher safety, and a high degree of BBB permeability indicating great potential for the treatment of brain metastases and tumors of the central nervous system.

Declarations

Ethics approval and consent to participate

The study protocol was approved by Institutional Animal Care and Use Committee (IACUC) of the institutions where the experiments were conducted.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

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Competing interests

The authors disclose no competing interests.

Authors' Contributions

Conception and design: Shui-ping Gao, Ru-Jiao Liu, Yi-qian Wang, Hong Chen, Hui-juan Zhang, Tian-yi Ma, Jian Zhang, Jian Zhang

Development of methodology: Shui-ping Gao, Ru-Jiao Liu , Yi-qian Wang, Hong Chen, Hui-juan Zhang, Jian Zhang

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Writing, review, and/or revision of the manuscript: Shui-ping Gao, Ru-Jiao Liu , Hong Chen, Jian Zhang

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Shui-ping Gao, Ru-Jiao Liu , Yi-qian Wang, Hong Chen, Hui-jua Zhang, Jian Zhang

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Compliance with Ethical Standards

Disclosure of potential conflicts of interest

The authors disclose no potential conflicts of interest.

Research involving Human Participants and/or Animals

The use and welfare of laboratory animals complied with Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

Informed consent

Not applicable.

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Figures

Figure 1

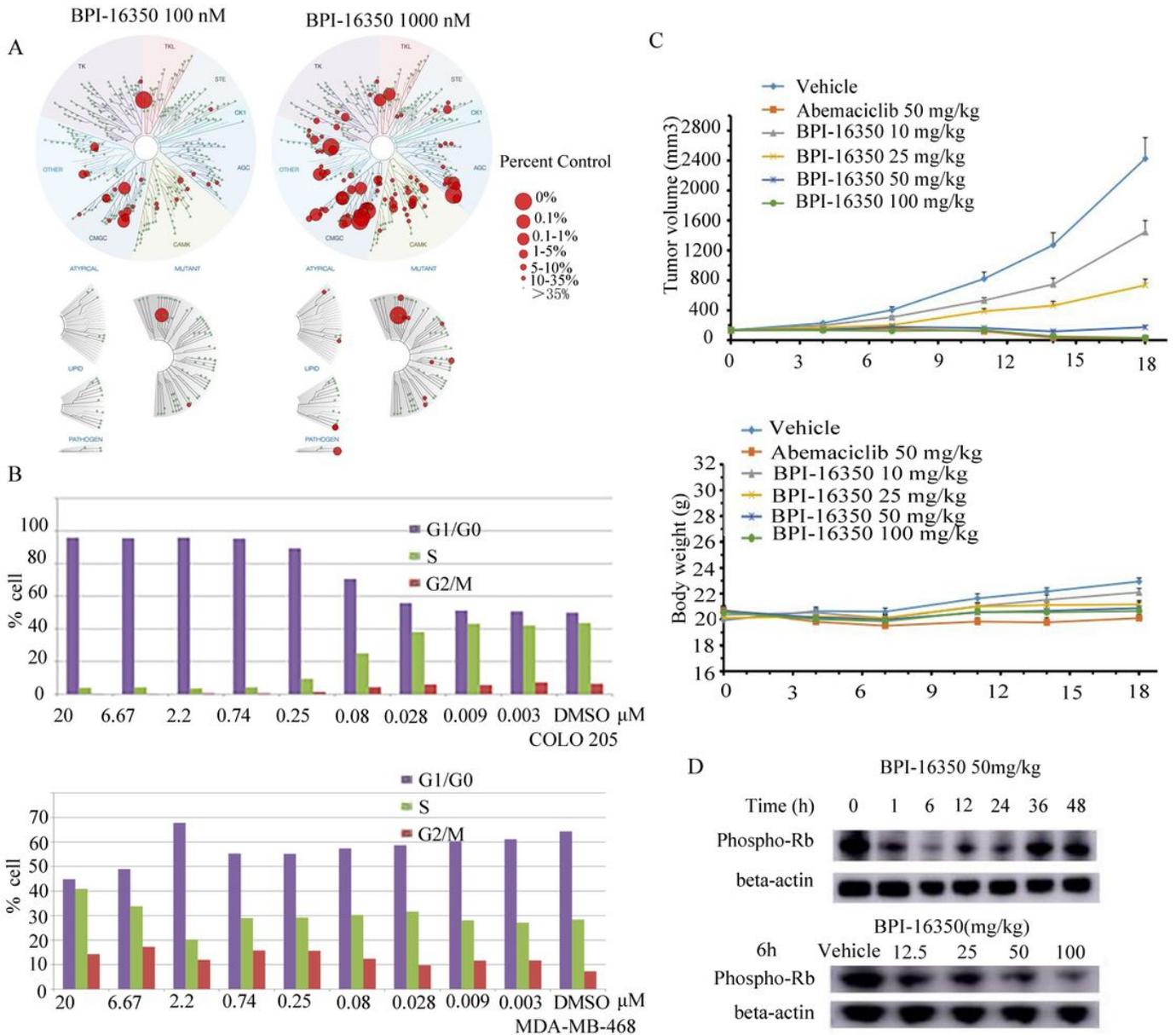


Figure 1

Anovel potent CDK4/6 inhibitor BPI-16350. A. KINOMEScan dendrogram for biochemical kinase selectivity profile against 468 kinases. Single point binding at 100 nM (left) and 1 μ M (right) BPI-16350. B. Cells treated with increasing concentrations of compound BPI-16350 for 24h and cell cycle activity profiled by flow cytometry from 20 μ M to 0.003 μ M. C. Antitumor activity of BPI-16350 in human Jeko-1 xenograft

model. Tumor volumes (up) were measured and body weights(low) were determined at the indicated times. Tumor volumes are presented as means \pm SEM. D. Therapeutically active doses of BPI-16350 in vivo caused the downregulation of retinoblastoma protein (Rb) Ser780 in CoLo 205 xenograft tumor tissues. Phospho-Rb, phosphorylated Rb. PK/PD correlation of single dose BPI-16350 (50 mg/kg) in COLO 205 xenograft model (upper group).PK/PD correlation of single dose BPI-16350 in COLO 205 xenograft model at 6h (lower group)

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