**Overcoming multidrug-resistance *in vitro* and *in vivo* using the novel P-glycoprotein inhibitor 1416**

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**Synopsis**

MDR (multidrug-resistance) represents a major obstacle to successful cancer chemotherapy and is usually accomplished by overexpression of P-gp (P-glycoprotein). Much effort has been devoted to developing P-gp inhibitors to modulate MDR. However, none of the inhibitors on the market have been successful. 1416 [1-(2,6-dimethylphenoxy)-2-(3,4-dimethoxyphenylethylamino)propane hydrochloride (phenoprolamine hydrochloride)] is a new VER (verapamil) analogue with a higher IC50 for blocking calcium channel currents than VER. In the present paper, we examined the inhibition effect of 1416 on P-gp both *in vitro* and *in vivo*. 1416 significantly enhanced cytotoxicity of VBL (vinblastine) in P-gp-overexpressed human multidrug-resistant K562/ADM (adriamycin) and KBV cells, but had no such effect on the parent K562 and KB cells. The MDR-modulating function of 1416 was further confirmed by increasing intracellular Rh123 (rhodamine123) content in MDR cells. Human K562/ADM xenograft-nude mice model verified that 1416 potentiates the antitumour activity of VBL in *vivo*. RT-PCR (reverse transcriptase-PCR) and FACS analysis demonstrated that the expression of MDR1/P-gp was not affected by 1416 treatment. All these observations suggest that 1416 could be a promising agent for overcoming MDR in cancer chemotherapy.

**Key words:** calcium antagonism, multidrug-resistance, P-glycoprotein, phenoprolamine hydrochloride, verapamil.


**INTRODUCTION**

MDR (multidrug resistance) is one of the major impediments for effective chemotherapy in cancer patients as cancer cells may become cross-resistant to a broad spectrum of chemotherapeutic agents after a single drug treatment [1,2]. Despite many complicated mechanisms of MDR such as blocking cell apoptosis, alteration of the drug target protein, activation of DNA repair, decreased drug uptake and increased drug efflux [3], cancer cells can escape from the toxic effect of drugs mainly due to the highly expressed membrane transporters that pump drugs out of cells [2]. The most prevalent and first discovered transporter is P-gp (P-glycoprotein), which belongs to the ABC (ATP-binding cassette) family [4]. Thus inhibiting or blocking the molecular ‘pumps’ is one of the strategies to reverse MDR in cancer treatment.

P-gp is a 170 kDa transmembrane glycoprotein encoded by the human MDRI gene that is located on chromosome 7q21 [5]. It has two homologous halves each with a TMD (transmembrane domain) and a NBD (nucleotide-binding domain) located towards the cytoplasmic face of the membrane [6]. A wide variety of compounds with a tendency towards amphipathic, lipid-soluble and cationic at physiological pH were transported out of cells by P-gp in an ATP-dependent manner [7,8]. Most anticancer drugs including Vinca alkaloids, anthracyclines, epipodophyllotoxins, colchicine and taxanes have the features mentioned above and thus can easily be expelled out of cancer cells since P-gp tends to highly expressed on cell surface of neoplastic tissues [9]. Against P-gp target, three generations of inhibitors have been developed: first-generation inhibitors including VER (verapamil) [10], cyclosporine A [11] etc., were limited by unacceptable toxicity [12]; second generation agents such as dexverapamil [13], PSC833...
VER, we hypothesize 1416 may also have MDR reversal ability. VER is the well-known and first discovered MDR-modifying agent, whose presence is required to reverse MDR, which generates considerable cardiovascular toxicity due to its calcium antagonism effect [21]. A new VER structure analogue, 1416 [1-(2,6-dimethylphenoxy)-2-(3,4-dimethoxyphenylethylamino)propane hydrochloride] (Figure 1) was found to have α1-adrenoceptor antagonism as well as calcium channel blocking activity. Therefore it was originally used to treat against hypertension and arrhythmia [22–24]. Considering the structural and functional similarity between 1416 and VER, we hypothesize 1416 may also have MDR reversal ability. In the present study, we evaluated the ability of 1416 in reversing P-gp-mediated drug resistance in cultured cell lines and in xenograft nude mouse model.

MATERIALS AND METHODS

Chemicals

1416 was synthesized based on the method described by Xia et al. [25]. FITC-labelled monoclonal anti-human P-gp antibody 17F9 was acquired from BD Biosciences Pharmingen. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) and Rh123 (Rhodamine123) were purchased from Sigma. Cell culture reagents were purchased from Invitrogen. ADM (adriamycin), VBL (vincristine) and VER were provided by their pharmaceutical manufacturers. All other chemicals and reagents were of analytical grade and obtained commercially.

Cells and cell culture

Human chronic myelogenous leukaemia cell K562 and its ADM-resistant counterpart K562/ADM were obtained from China Pharmaceutical University (Nanjing, China). The MDR human oral carcinoma line KBV cell and its sensitive parental line KB were purchased from Nanjing KeyGen Biotech. All cells were maintained in the RPMI 1640 medium supplemented with 10% foetal bovine serum and 1% penicillin/streptomycin at 37 °C in a humidified atmosphere containing 5% CO2. Cells were checked routinely and found to be free of contamination by mycoplasma or fungi. All cell lines used were more than 3 months thawed from frozen stocks. For the maintenance of the MDR phenotype, 1000 ng/ml ADM and 200 ng/ml Vincristine were added to the medium of K562/ADM and KBV cell lines, respectively; the drugs were removed 2 weeks before the experiment.

Detection of P-gp expression on the cell surface

To analyse the surface P-gp expression level, K562 and K562/ADM cells were collected and counted. KB and KBV cells were trypsinized with 0.25% trypsin and counted. Cells (106) were rinsed and dissolved with ice-cold PBS containing 1% BSA and incubated with 20 μl of the FITC-labelled monoclonal anti-(human P-gp) antibody 17F9 at 4 °C for 30 min in dark. After incubation, cells were washed and resuspended in chilled PBS. The binding affinity of the FITC-labelled P-gp antibody was analysed using FACS Calibur flow cytometer. Fluorescence was measured by counting 10000 events with an excitation wavelength of 488 nm and an emission wavelength of 535 nm (FL1). Gates were set to forward and sideward scatter to exclude damaged cells and cell debris. Data were analysed by the flowjo software (Treestar).

Cellular drug sensitivity assays

VBL, an anticancer drug as well as a classic P-gp substrate [26], was employed in cellular drug sensitivity assay. Increasing concentrations of VBL dissolved in 50 μl RPMI 1640 were added to cell suspensions (105/ml, 200 μl) in 96-well culture plates and incubated for 48 h. RPMI 1640 (50 μl) without VBL was added to the cells as a negative control and RPMI 1640 (250 μl) without cells was incubated as a blank control. MTT (20 μl of 5 mg/ml) was added and incubated for an additional 4 h. The medium was then removed and DMSO was added to dissolve the formazan crystals. Absorbance was measured at A570 on a monochromator-based multifunction microplate (Tecan). The process was repeated in the presence of 1416 or VER at 10 μM final
concentration. IR (inhibitory rate) of cell growth (IR%) = (A_{negative control}−A_{drug treated})/(A_{negative control}−A_{blank})×100\%. IC_{50} values (50\% inhibitory concentration) were calculated using GraphPad Prism 5.01 and presented as means ± S.D. (n = 3). The RF (resistance fold) is calculated from the ratio of the IC_{50} values for drug-resistant cells to its parental cells. The efficacy between 1416 and VER was compared by Gaddum/Schild EC50 shift (GraphPad Prism5.01).

Estimation of intracellular Rh123 content

The fluorescent dye Rh123 has been extensively used as an index of P-gp-mediated transport in MDR cells [27]. To observe the influence of modulators on Rh123 accumulation in K562 and K562/ADM cells, 5×10^5/ml cells were loaded on to coverglass bottom dishes (MatTek) and cultured overnight in the incubator. The medium was removed and cells were washed with PBS, and a new medium containing 5 μM Rh123 was added in the absence and presence of 10 μM 1416 or VER and incubated for 30 min, the cells were then washed twice with ice-cold PBS before examining with the Zeiss 5-LIVE fast CLSM (confocal laser scanning microscopy; Carl Zeiss). Data were collected and analysed by the IMARIS software (Bitplane).

Flow cytometry was used to determine dynamics of Rh123 transport. In Rh123 accumulation assay, K562 and K562/ADM cells (10^5) were incubated with 5 μM Rh123 in the presence and in the absence of modulators for 0, 5, 10, 20, 30, 40, 50 and 60 min. At each time point, cells were collected, washed, resuspended in ice-cold PBS and kept on ice before measuring with flow cytometry. In substrate efflux studies, cells were first incubated with 5 μM Rh123 for 30 min with no modulators and then washed twice with ice-cold PBS. Then cells were resuspended in the Rh123-free RPMI 1640 medium with and without modulators for 0, 5, 10, 20, 30, 40, 50 and 60 min. Cells were collected at each time point and washed, and resuspended in ice-cold PBS then analysed for fluorescence intensity by flow cytometry.

Xenografts in nude mice

Female 5-week-old BALB/c nude mice were bought from Beijing Experimental Animal Center (Beijing, China). Experiments and protocols were approved by the Animal Care and Use Committee of Nanjing University and performed following Animal Care and Use guidelines set by the NIH (National Institute of Health, USA).

K562 cells and K562/ADM cells (8×10^6) at exponential phase were suspended in PBS and subcutaneously transplanted in the armpit of the BALB/c nude mice under sterile conditions. After the tumour diameter reached about 5 mm, the mice bearing K562 cells were randomly assigned to four groups, each containing six mice, and received various regimens every other day for 14 days.

Drugs were given to the mice at an optimal dose, based on preliminary experiments as well as previous literature [10]: (i) 0.1 ml of NS (normal saline) was injected subcutaneously as the negative control; (ii) VBL (4 mg/kg of body weight) alone; (iii) VBL (4 mg/kg of body weight) + VER (40 mg/kg of body weight); and (iv) VBL (4 mg/kg of body weight) + 1416 (40 mg/kg of body weight). The mice bearing K562/ADM cells were randomly assigned to four groups containing nine mice each and received the same regimens as K562 cell-bearing mice. Tumour volume (V) was calculated as: V (mm^3) = 1/2 × L × W^2, where L is the length and W is the width of the tumour mass (in mm). The tumour sizes were standardized in different groups by using the RTV (relative tumour volumes) calculated as: RTV = V_n/V_o, where V_n is the tumour volume at day n of treatment and V_o represents the initial tumour volume at the onset of treatment. Tumour measurements and body weight of the mice were recorded every other day. On day 15, the mice were killed. The antitumour activity was assessed in terms of the IR, which was defined as: IR (%) = (1 – mean RTV of the treated group/mean RTV of the control group)×100%

MDR1 expression at the transcriptional and translational levels

Semi-quantitative RT-PCR (reverse transcriptase-PCR) was employed to detect MDR1 mRNA expression. Total RNAs were extracted from K562 and K562/ADM cells pre-incubated with 10 μM 1416 or VER for 48 h using Trizol reagent (GIBCO). The 2 μg RNA was incubated with 2 μl of Oligo(dT) at 70°C for 5 min in ice-cold and subjected to first strand DNA synthesis using 200 units of MMLV (Moloney-murine-leukaemia) RT (Promega) in 25 μl of RT-Mix containing dNTPs. The reaction mixture was maintained at 42°C for 1 h using a Minicycler PTC-150 (MJ Research). MDR1 specific sequences were amplified by using the sense strand primer 5′-CCCATGCAAATGACAGG-3′ and the antisense strand primer 5′-GGTCAAACTCTGGTCTGCTGA-3′ which yield a 157 bp product. Housekeeping primer β-actin sequences were: 5′-TGA CGG GTG CAC CCA CAC TGTG GCC CAT CTA-3′ (sense) and 5′-CTA GAA GCA TTTGCG GTG GAC GAT GGA GGG-3′ (antisense). PCR products were separated on 1.5 % agarose gel containing 75 ng/ml of ethidium bromide. After electrophoresis, gels were examined on an ultraviolet transilluminator and photographed. The relative density of the signals from each lane of PCR products was determined by digitized analysis using the Labworks4.0 software (UVP).

Flow cytometry is widely used to determine P-gp expression at the protein level [28,29], and herein performed in K562 cells and K562/ADM cells using 17F9 monoclonal antibody as detailed above. K562/ADM cells were first incubated with 10 μM 1416 or VER for 48 h then stained with FITC-labelled antibody 17F9 for 30 min before measuring. P-gp-positive rate was calculated by the same measuring scale using the flowjo software (Treestar).

Statistical analysis

All experiments were performed in triplicate in at least three independent trials. The results are expressed as the means ± S.D. of the three independent experiments. A Student’s t test was used for each pair comparison, and results were considered significantly different when *P < 0.05 and extremely significantly different when **P < 0.01.
RESULTS AND DISCUSSION

In the present study, we have demonstrated for the first time that 1416 can reverse P-gp-mediated MDR both in vitro and in vivo. The expression of P-gp in two paired cell lines, K562 and its MDR counterpart K562/ADM, KB and its MDR counterpart KBV, were checked by flow cytometry. As shown in Figure 2(A), K562/ADM and KBV cells have greater P-gp expression levels than their parental cell lines. The ability of 1416 to enhance the cytotoxicity of chemotherapeutic drugs was examined by MTT assays. The cytotoxicity of VBL with and without modulators in two paired cells is summarized in Table 1. According to the IC50 values of VBL in different cell lines, K562/ADM cells was 80.88-fold more resistant to VBL than K562 cells and KBV cells was 114.92-fold more resistant to VBL than KB cells. Addition of 10 μM 1416 decreased the RF of VBL in two drug-resistant cells, RF decreased from 80.88 to 16.31 for K562/ADM cells and from 114.92 to 4.58 for KBV cells, respectively. The dosage of modulators used here was subtoxic.
Neither 1416 nor VER changes the IC50 values in parental cells, whereas coadministration of 1416 or VER did not enhance the effect of VBL in multidrug-resistant cells. No apparent difference was observed in MDR reversal ability of 1416 compared with VER as analysed by Schild regression (see Supplementary Figure S2 at http://www.bioscreip.org/bsr/032/bsr0320559add.htm), which suggests 1416 is as effective as VER in potentiation of VBL cytotoxicity in MDR cells. Neither 1416 nor VER changes the IC50 values in parental cells, suggesting these modulators may not have any effect on permeability of non-P-gp expressing cells.

The accumulation of the naturally fluorescing substrate of P-gp, Rh123, was directly observed under CLSM (Figure 2B). When exposed to 5 μM Rh123, P-gp overexpressed K562/ADM cells accumulated much less Rh123 than K562 cells. Addition of modulators led to an obvious increase of Rh123 content in K562/ADM cells without affecting Rh123 accumulation in K562 cells. The inhibitory effect of 1416 on P-gp pump was further confirmed by monitoring accumulation and efflux dynamics of Rh123 within 1 h by flow cytometry. As shown in Figure 2(C), the accumulation of Rh123 in K562/ADM cells was much slower than that in K562 cells and increased almost to the same level as observed in K562 cells when VER or 1416 was added. In substrate efflux assay, Rh123 was pre-incubated with cells and then expelled from K562/ADM cells much faster than from K562 cells. VER or 1416 treatment obviously decreased speed of release of Rh123 from K562/ADM cells. The fluorescent features in parental K562 cell line were not modified by VER and 1416. Such results suggested that 1416 can restore Rh123 accumulation and delay its efflux, which further indicated that P-gp efflux pump might be abrogated by 1416. Neither in VBL cytotoxicity assay nor in Rh123 fluorescence observation did 1416 have significant effect on non-P-gp-expressing parental cells, implying the potentiation of cell toxicity and alteration of fluorescent features by 1416 have a close relationship with the P-gp pump, that is to say the reverse of MDR is probably through P-gp inhibition.

The in vivo MDR reversal effect of 1416 was evaluated through human xenograft-nude mouse model. Tumour growth presented by RTV was measured and calculated as described in Materials and methods section. VBL, given at 4 mg/kg of body weight, significantly inhibited the growth of K562 tumours by 58.5 %, whereas coadministration of 1416 or VER did not enhance the antitumour activity of VBL (Figure 3A). However, VBL alone had little effect on the drug-resistant K562/ADM tumours with an IC50 of 10.4 % (P = 0.38 compared with NS control). Additionally, 1416 or VER potentiated the antitumour activity of VBL in K562/ADM xenografts and the enhancement was statistically significant in comparison with the NS-treated mice (P < 0.001) (Figure 3B). No statistical difference of tumour growth was observed between VBL plus 1416 group and VBL plus VER group (P = 0.80), implying that 1416 can enhance the antitumour activity of VBL in multidrug-resistant tumours as effectively as VER does. To monitor the toxicity of the treatment, the body weights of the mice were weighed. Neither in K562-bearing mice nor in K562/ADM-bearing mice did we see any significant body weight loss during drug administration, suggesting that this regime was safe and effective in terms of inhibition of MDR tumour growth (Figures 3C and 3D).

To assess whether 1416 could modulate MDR1 gene expression, semi-quantitative RT-PCR analysis was carried out and the results are shown in Figures 4(A) and 4(B). The expression of housekeeping gene β-actin was of a similar magnitude in K562 and K562/ADM cells either non-treated or treated by modulators. The expression of MDR1 mRNA was not detectable in K562 cells but was very high in K562/ADM cells. After modulators treatment, a slight decrease in MDR1 expression was observed in K562/ADM cells exposed to VER and no obvious change was recognized in cells exposed to 1416. When calculating the ratio of the densitometric value of MDR1 expression to β-actin expression, there was a 24.3 % (P < 0.01) reduction of MDR1 expression in VER treated K562/ADM cells while no detectable change of MDR1 expression was observed in 1416 treated K562/ADM cells (Figure 4B). The semi-quantitative RT-PCR method we used here is highly sensitive and less expensive; however, it may be influenced by several factors such as PCR amplification efficiency, electrophoresis procedures, resolution of gel images, which require considerable investments of time, resources and expertise and may cause inaccurate results [30]. Nowadays, real-time PCR is more popular, although it is simpler and faster but more expensive. The protein level of P-gp expression, which was detected by flow cytometry, showed a similar trend as MDR1 mRNA expression (Figure 4C). Using the same calculating scale, K562 cells showed a positive P-gp rate of 0.29 %, while non-treated K562/ADM cells, VER-treated K562/ADM cells and 1416-treated K562/ADM cells have positive P-gp rate of 99.6, 70.6 and 99.7 %, respectively, suggesting that 1416 does not affect P-gp expression but VER slightly inhibits P-gp expression. VER may affect the antibody:epitope interaction, but 1416 does not.

### Table 1. Effects of modulators on the VBL cytotoxicity in two pairs of parental and multidrug-resistant cell lines

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cell line . . .</th>
<th>IC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K562</td>
<td>K562/ADM</td>
</tr>
<tr>
<td>VBL</td>
<td>1.70 ± 0.23</td>
<td>137.50 ± 2.33 (80.88)</td>
</tr>
<tr>
<td>+ VER (10 μM)</td>
<td>1.63 ± 0.20</td>
<td>26.27 ± 1.31 (16.12)</td>
</tr>
<tr>
<td>+ 1416 (10 μM)</td>
<td>1.59 ± 0.25</td>
<td>25.93 ± 1.24 (16.31)</td>
</tr>
</tbody>
</table>

Cell were incubated with increasing drug concentrations with or without 10 μM modulators over 48 h, and viability was analysed by the MTT assay. The IC50 value was taken as the anticancer drug concentration that inhibits cell growth by 50 % relative to untreated control cells. Values are the means ± S.D. of at least three independent experiments. Numbers in parentheses indicate relative RF, which are the ratios of IC50 in the resistant cell to the one in the parent cell.
The P-gp substrate Rh123 efflux was reversed within 1 h after 1416 treatment (Figure 2C), whereas the half-lives of the MDR1 mRNA and P-gp were reported to be about 4–10 and 17 h, respectively [31,32] which suggested that 1416 can reverse MDR though not affecting MDR1/P-gp expression. Semi-quantitative RT-PCR and flow cytometry confirmed our hypothesis as no obvious change in MDR1/P-gp expression, was observed after 1416 treatment. Therefore 1416 would most probably have direct interaction with P-gp to inhibit its drug efflux function but not its expression.

1416 and VER have similar chemical features that are shared by other P-gp modulators, such as hydrophobicity and amphiphilicity [33], planar aromatic rings with N-aminalkyl side chain [34], positively charged under physiological conditions [35]. Such characteristics make them easily partitioned into the lipid bilayer and get an access to P-gp portals formed by TMs (transmembrane helices) 4–6 and TMs 10–12, which have been supposed to be involved in substrate binding [6,36]. The common structure of 1416 and VER (Figure 1C) has the electronegative atoms (O, N) and the π-electron system (phenyl group), which might be essential for binding to P-gp and inhibiting function of P-gp. These potential hydrogen-bonding acceptors play a critical role in contacting with the high density of hydrogen-bonding donors in P-gp transmembrane sequences: TM4, TM5, TM6, TM11 and TM12 [33]. VER has been discovered to have direct binding on P-gp and reverse MDR by competing for drug-binding sites with some anticancer drugs [37]. Taking structural and functional similarities between 1416 and VER into account, we suggest 1416 may have similar interaction with P-gp as VER does.

VER was discovered first but was limited in application as a P-gp inhibitor, as its calcium antagonism activity led to cardiac toxicity. Although 1416 and VER have a lot in common including the inhibition efficiency of P-gp, still some differences exists: 1416 exhibited a weaker inhibition effect on calcium channel than VER [22]; therefore it is possible for 1416 to circumvent unwanted cardiotoxicity; 1416 also blocks other ion channels such as Na⁺, K⁺ currents [22,38], suggesting 1416 has more complicated effect and needs further investigation. Safety of 1416 floating sustained-release tablets in healthy Chinese subjects was reported by Zhao et al. [39], which held promise for 1416 to go to clinical trial as a new MDR reversal agent. Furthermore, the structural activity of 1416 may gave us inspiration in exploring more potent drug candidates.

In conclusion, we verified the reversal effect of MDR phenotype by 1416 through inhibition of the drug efflux function of P-gp both in vitro and in vivo. This suggests that 1416 may be a potential candidate for reversing MDR in cancer chemotherapy.

Figure 3 MDR reversal ability of modulators in vivo
Effect of modulators on the antitumour activity of VBL in (A) K562 bearing and (B) K562/ADM bearing nude mice. Volume of tumour in each mouse was measured every other day for 14 days and RTV (relative tumour volume) were calculated as described in the Materials and methods section. Body weight of (C) K562 bearing and (D) K562/ADM bearing nude mice was measured every other day during the drug treatment. NS (control); VBL (4 mg/kg of body weight); VBL (4 mg/kg of body weight) + VER (40 mg/kg of body weight); VBL (4 mg/kg of body weight) + 1416 (40 mg/kg of body weight); (means ± S.D., n=6–9).
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Figure 4 RT-PCR and FACS analysis of MDR1/P-gp expression

(A) PCR products were separated on 1.5% agarose gel. From left to right, the lanes are K562, 562/ADM, K562/ADM treated with 10 μM VER and K562/ADM treated with 10 μM 1416. (B) The relative level of MDR1 expression was determined semi-quantitatively by calculating the ratio of the densitometric value of MDR1 gene expression to β-actin expression. (C) Flow cytometry assay of P-gp expression in K562 cells and K562/ADM cells either non-treated or treated by modulators.

Here we have only conducted the reversal effect study of 1416; additional studies are needed to identify the in vivo pharmacokinetics of 1416, which are in progress.

AUTHOR CONTRIBUTION

Yiqiao Hu initiated the project and gave directions and support during the whole process. Jinhui Wu revised the paper; Yan Xu and Feng Zhi investigated the feasibility and designed the whole project; they also conduct major experiments with help from Guangming Xu. Xiaolei Tang and Sheng Lu helped with synthesis and structure–activity analysis of the compounds.

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SUPPLEMENTARY ONLINE DATA

Overcoming multidrug-resistance in vitro and in vivo using the novel P-glycoprotein inhibitor 1416

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Figure S1 Effect of modulators on cell growth was carried out using an MTT assay
(A) KB Cells and (B) KBV cells were incubated with increasing concentrations of modulators over 48 h, inhibition rate (IR%) of cell growth is defined as in the Materials and methods section of the main paper. The subtoxic dosage of modulators was similar in another pair of cells: K562 and K562/ADM. Values are the means±S.D. of three independent experiments.

Figure S2 Non-linear Schild regressions were performed using least square fit with no constraints on the parameters
Cultures were cotreated with various concentrations of VBL and 10 μM VER or 1416. Cell inhibition rate was as in the Materials and methods section of the main paper. Values are the means±S.D. of three independent experiments.

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