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PRECLINICAL STUDIES

The novel PI3K inhibitor S1 synergizes with sorafenib in non-small cell lung cancer cells involving the Akt-S6 signaling

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Summary
Non-small cell lung cancer (NSCLC) has been the major cause of cancer-related deaths worldwide. Targeted therapy has been available as an additive strategy for NSCLC patients, but the inevitable resistance to mono-targeted agents has largely hampered its usage in the clinic. We have previously designed and synthesized a novel small molecule compound S1, 2-methoxy-3-phenylsulfonamino-5-(quinazolin-6-yl) benzamides and demonstrated its inhibition of PI3K and mTOR as well as the anti-tumor potential. In the present study, we have identified that S1 alone or combined with the multi-kinase inhibitor sorafenib can inhibit the in vitro cell proliferation of NSCLC cells (A549, NCI-H157 and 95D cells) and tumor growth in the A549 xenograft model. S1 alone produced inhibitory effects on the colony formation, cell migration and invasion and angiogenesis, with more pronounced inhibition when used with sorafenib. We further revealed that S1 mainly inhibited the Akt/S6 phosphorylation while sorafenib mostly decreased the phosphorylation of ERK. Together, the novel PI3K/mTOR inhibitor S1 per se exhibits strong anti-tumor effects in NSCLC cells and A549 xenograft, effects possibly via its inhibition of cell proliferation, invasion and migration and angiogenesis. The combination of S1 and sorafenib exerts potentiated anti-tumor effects, in which the underlying mechanisms may involve their differential modulation of the phosphorylation of Akt and S6 in the PI3K/Akt/mTOR cascades and ERK phosphorylation in the Raf/MEK/ERK pathways. The combination of S1 and sorafenib could be used as an additive approach in treating NSCLC in the clinic.

Keywords Lung cancer · Sorafenib · S1 · Angiogenesis · Phosphorylation

Introduction

Non-small cell lung cancer (NSCLC), the predominant form of lung cancer has been the major cause of cancer-related deaths worldwide, with the 5-year survival rate less than 15% [1, 2]. Although platinum-based chemotherapy decreases the lung-cancer mortality and has become the standard treatment for NSCLC, its usage in the clinic is largely restricted due to the inevitable disease progression and dosage-related toxicities and side effects [3, 4]. The development of molecular biology has elucidated several critical intracellular signaling pathways activated in NSCLC cells, including the phosphoinositide 3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway and Ras/Raf/MEK/ERK pathway [5–7], leading to molecular targeted therapies proposed as an additive strategy in treating NSCLC. Indeed, some targeted agents such as the tyrosinase kinase inhibitor (TKI) erlotinib have been shown to be superior to placebo and considered to be a reasonable alternative treatment option to single-agent chemotherapy, particularly for nonsquamous NSCLC [8]. Moreover, co-targeted inhibition of more than one molecule in the PI3K pathway or with inhibition of other cell signaling pathways represents a critical strategy to hamper the development of resistance to targeted inhibitors through bypass tracks [9]. In this regard, simultaneously targeting both PI3Kα and mTOR has been the research highlight of drug

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design and development for anti-tumor agents. Indeed, the first PI3Kα/mTOR inhibitor BEZ235 has been developed by Novartis and exhibits anti-tumor activity in NSCLC [10, 11]. GSK2126458 has been identified by GlaxoSmithKline as a potent, orally bioavailable dual inhibitor of PI3Kα and mTOR [12]. We have previously designed and synthesized a novel small molecule compound S1 (Fig. 1), 2-methoxy-3-phenylsulfonamino-5-(quinazolin-6-yl)benzamides and demonstrated its inhibition of PI3K and mTOR as well as the anti-tumor potential [13]. However, whether and how S1 exerts its possible anti-tumor effects in NSCLC has yet to be determined.

As aforementioned, co-targeting strategies by the combination of PI3K/mTOR inhibitors and inhibitors of MEK/ERK may provide a beneficial avenue to prevent resistance to targeted agents [9]. In this context, the oral multikinase inhibitor sorafenib, which targets Raf kinase in the Raf/MEK/ERK pathway as well as receptor tyrosinase kinases involved in angiogenesis, has been shown to inhibit tumor growth in various xenograft models driven by the activation of the Raf/MEK/ERK pathway [9, 14, 15]. In particular, encouraging clinical results have been obtained by using sorafenib monotherapy in patients with KRAS-mutant NSCLC [16, 17]. Nonetheless, the inevitable development of acquired resistance hinders the overall survival benefit of sorafenib [18, 19]. Accordingly, it is reasonable for the use of sorafenib in combination with the PI3K/mTOR inhibitor, such as S1, in the treatment of NSCLC.

In the present study, we have identified that S1 alone or combined with sorafenib can inhibit the in vitro cell proliferation of NSCLC cells and tumor growth in the xenograft model, possibly through intervening the colony formation, cell migration and invasion, and angiogenesis. Furthermore, the combination of S1 and sorafenib exerts potentiated anti-tumor effects, possibly by their differential modulation of the phosphorylation of Akt and S6 in the PI3K/Akt/mTOR cascades and ERK phosphorylation in the Raf/MEK/ERK pathways.

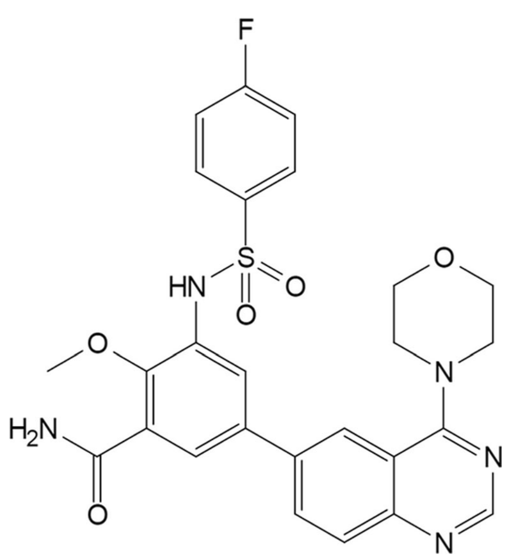


Fig. 1 Molecular structure of S1

Materials and methods

Cell lines and culture

Three NSCLC cell lines, A549, NCI-H157 and 95D were used in this study (the American Type Culture Collection). Cells were maintained in DMEM medium supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin G and 100 μg/ml streptomycin and cultured in a humidified incubator at 37 °C with 5% carbon dioxide in air. Human umbilical vein endothelial cells (HUVECs) were isolated from the human umbilical cord veins as described previously [20], and maintained in an endothelial cell medium (Sciencecell, SanDiego, USA) containing endothelial Cell Growth Supplement/heparin growth supplements (Promo Cell, Heidelberg, Germany).

Reagents

S1 was synthesized and kindly provided by Prof. Sanqi Zhang (Xi'an Jiaotong University, Xi'an, China). BEZ235 and sorafenib were purchased from Selleck Chemicals (Houston, USA). For in vitro studies, all the three drugs were dissolved in dimethyl sulfoxide (DMSO) to obtain a stock solution of 10 mM and stored at −20 °C. For in vivo studies, S1, BEZ235 and sorafenib were dissolved in NMP/PEG300 (10:90; *v*/v).

Animals

All experimental protocols were approved by the Institutional Animal Care and Use Committee and performed in accordance with the NIH guidelines for the care and use of laboratory animals (NIH publications No. 8023, revised 1978). Male BALB/c (Nu/Nu) mice (18–20 g, 6–8 weeks) were provided by Shanghai Slac Laboratory Animal Co. Ltd., Shanghai, China. Animals were housed in a temperature- and light-controlled room (12 h light-12 h dark cycle, lights on at 7:00 a.m.), with food and water ad libitum.

In vitro antiproliferative assay

Cells were seeded into a 96-well plate at a density of 4–5 × 10^3 cells per well and allowed to attach to the bottom of the plate overnight. We then treated cells with different concentrations of S1 and sorafenib for 72 h. Thereafter, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Roche Diagnostic Corporation, Indianapolis, IN, USA) was added to appropriate wells with the final concentration of 0.5 mg/ml. Following the incubation of cells with MTT for 4 h, DMSO was used to dissolve the formazan. The optical density was determined at 570 nm with a microplate reader (Varioskan Flash, Thermo Electron Corporation, Vantaa, Finland). The combinatorial effects of S1 and sorafenib were analyzed in accordance with the combination index (CI) [21] using the CompuSyn software (Combo-Syn, Inc., Paramus, USA), in which CI > 1, =1 or < 1 denotes antagonistic, additive or synergistic, respectively.

Colony formation assay

Cells were seeded in 24-well plates at a density of 200 cells per well and allowed to attach to the bottom of the well overnight. Cells were then treated with indicated drugs at the given concentration. The culture medium was replaced every 3 days. After a 12-day culturing, the cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. Colonies>10 cells were counted under a light microscope (×100 magnification; Olympus, Tokyo, Japan).

Tube formation assay

The 96-well plates were coated with Matrigel (BD Biosciences, Franklin Lakes, USA) according to the manufacturer's instructions and then returned to the incubator to polymerize for 30–40 min. The HUVECs were seeded in the aforementioned 96-well plates at a density of 2×10^5 cells/mL and treated with various given drugs for 8 h. Tube formation was photographed with an inverted microscope at the indicated time points and analyzed using an ImageJ software.

For cell invasion assay, wound healing assay and western blotting, please find the details in the supplemental data.

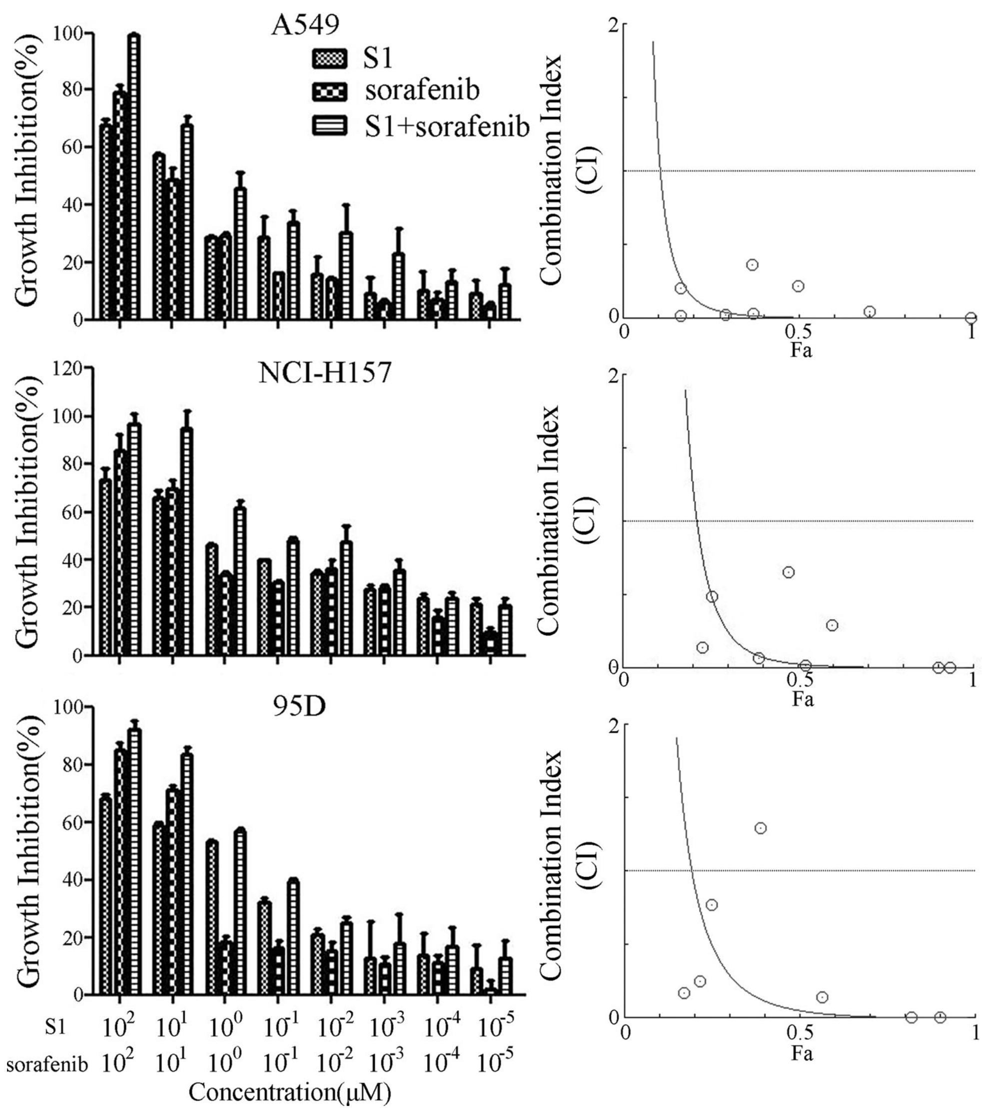


Fig. 2 S1 combined with sorafenib synergistically inhibits cell proliferation in NSCLC cell lines. The NSCLC cells including A549, NCI-H157 and 95D were seeded in 96-well plates and treated with S1/sorafenib the next day. The cellular growth was detected with colorimetric MTT assay at 72 h post-treatment. Each treatment was performed in triplicates. Data are expressed as mean ± standard deviation from three independent experiments. The combination index (CI) was calculated using the CalcuSyn software. Note a synergistic effect by S1 + sorafenib on cell proliferation (CI < 1)

In vivo anti-tumor activity and animal survival of S1 in combination with sorafenib

The A549 cell suspensions were prepared (3×10^6 cells) and injected subcutaneously into the right flank of athymic nude mice. Tumor-bearing mice were randomly divided into six groups. Animals were then treated with S1 (30 and 50 mg/kg), BEZ235 (50 mg/kg) and sorafenib alone (30 mg/kg) or in combination with S1 (30 mg/kg) once daily for 14 days. Doses were selected based on previous studies [13, 21, 22] and our preliminary data. Drugs were dissolved in NMP/PEG300 (10:90, *v*/v) and delivered by oral gavage. The tumor growth was monitored twice a week using an electronic caliper, with the volume calculated as 0.5 x length x width^2 (mm^3). Kaplan-Meier overall survival were investigated and analyzed with a Prism software (GraphPad Software, San Diego, CA). For western blot, tumor mass was removed at day 14 post-treatment and snap-frozen in liquid nitrogen.

Statistical analysis

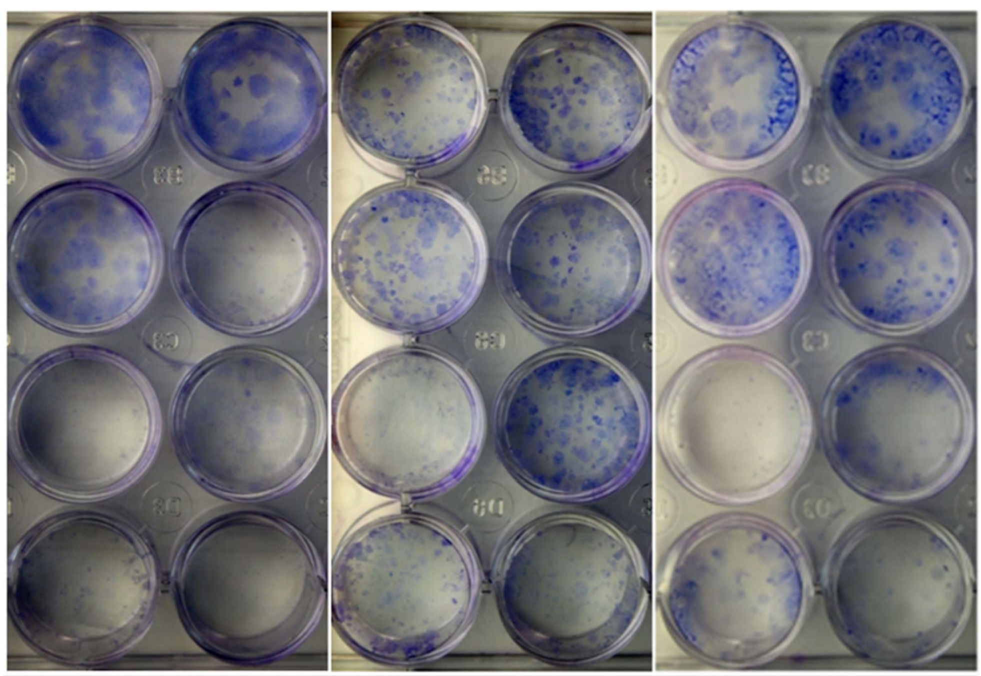
All data are expressed as mean ± standard deviation (SD). Unless otherwise stated, statistical analyses were performed using analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test. A value of $p < 0.05$ was considered statistically significant.

Results

Effects of S1 alone or in combination with sorafenib on the proliferation and colony formation of NSCLCs in vitro

The three NSCLC cell lines (A549, NCI-H157 and 95D) were used in the experiment due to their differential expression of several key signaling molecules in the PI3K/Akt/mTOR and Ras/Raf/ERK pathways implicated in NSCLC. As revealed by MTT assay (Fig. 2; Supplemental Table 1), S1, sorafenib or S1 combined with sorafenib all inhibited the proliferation of the three NSCLC cells. In particular, S1 exhibited synergistic effects with sorafenib in exerting their antiproliferation activity, as determined by CI values <1.0.

We next asked whether the antiproliferation activity of S1, sorafenib or their combination was due to the inhibition of cell clonogenicity. As shown in Fig. 3 and Supplemental Fig. 1, the number and size of cell colonies were reduced following treatment with S1, sorafenib or their combination in the three NSCLC cell lines. Moreover, S1 combined with sorafenib produced more robust effects in decreasing the number and size of cell colonies than S1 or sorafenib alone. Doses used here were according to their IC_{50} values (Supplemental Table 1).



A549		NCI-H157		95D	
Control	Control	Control	Control	Control	Control
S1 0.4μM	S1 2μM	S1 0.4μM	S1 2μM	S1 0.4μM	S1 2μM
BEZ235 2μM	Sora 2μM	BEZ235 2μM	Sora 2μM	BEZ235 2μM	Sora 2μM
S1+Sora(0.4+2μM)	S1+Sora(2+2μM)	S1+Sora(0.4+2μM)	S1+Sora(2+2μM)	S1+Sora(0.4+2μM)	S1+Sora(2+2μM)

Fig. 3 Effects of S1 and sorafenib on colony formation of NSCLC cells. The indicated cells (A549, NCI-H157 and 95D) at a density of 200 cells/well were seeded in 24-well plates and treated the next day with S1 and sorafenib. BEZ235 was used as the positive control for S1. The culture medium was replaced every 3 days. After 12 days of culturing, the cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. The picture of colonies was taken using a digital camera and colonies>10 cells were counted

In addition, we examined whether S1 alone or in combination with sorafenib can influence cell invasion and migration ability. As shown in Supplemental Fig. 2, treatment with S1 alone or combined with sorafenib significantly reduced cell invasion and migration to the lower side of the transwell. The wound healing assay further revealed that S1 alone or combined with sorafenib prevented NSCLC cell migration, as evidenced by the wider scratch width following the treatment (Supplemental Fig. 3). In similar, S1 combined with sorafenib produced stronger inhibition of cell invasion and migration when compared with S1 or sorafenib alone (Supplemental Figs. 2 and 3).

Effects of S1 alone or in combination with sorafenib on angiogenesis in vitro

Angiogenesis is known to play a facilitatory role in tumor growth, progression and metastatsis, thereby being the potential intervening target for cancer treatment [22]. We first performed in vitro angiogenesis assay by tube formation. As shown in Fig. 4a, b, the formation of vessel-like structures, characterized by the elongation and alignment of the HUVECs was effectively inhibited by S1 alone (1 or 10 μM) or in combination with sorafenib (1 μM + 10 μM). We then investigated cell migration of HUVECs, a key step for them to form new blood vessels during angiogenesis. As

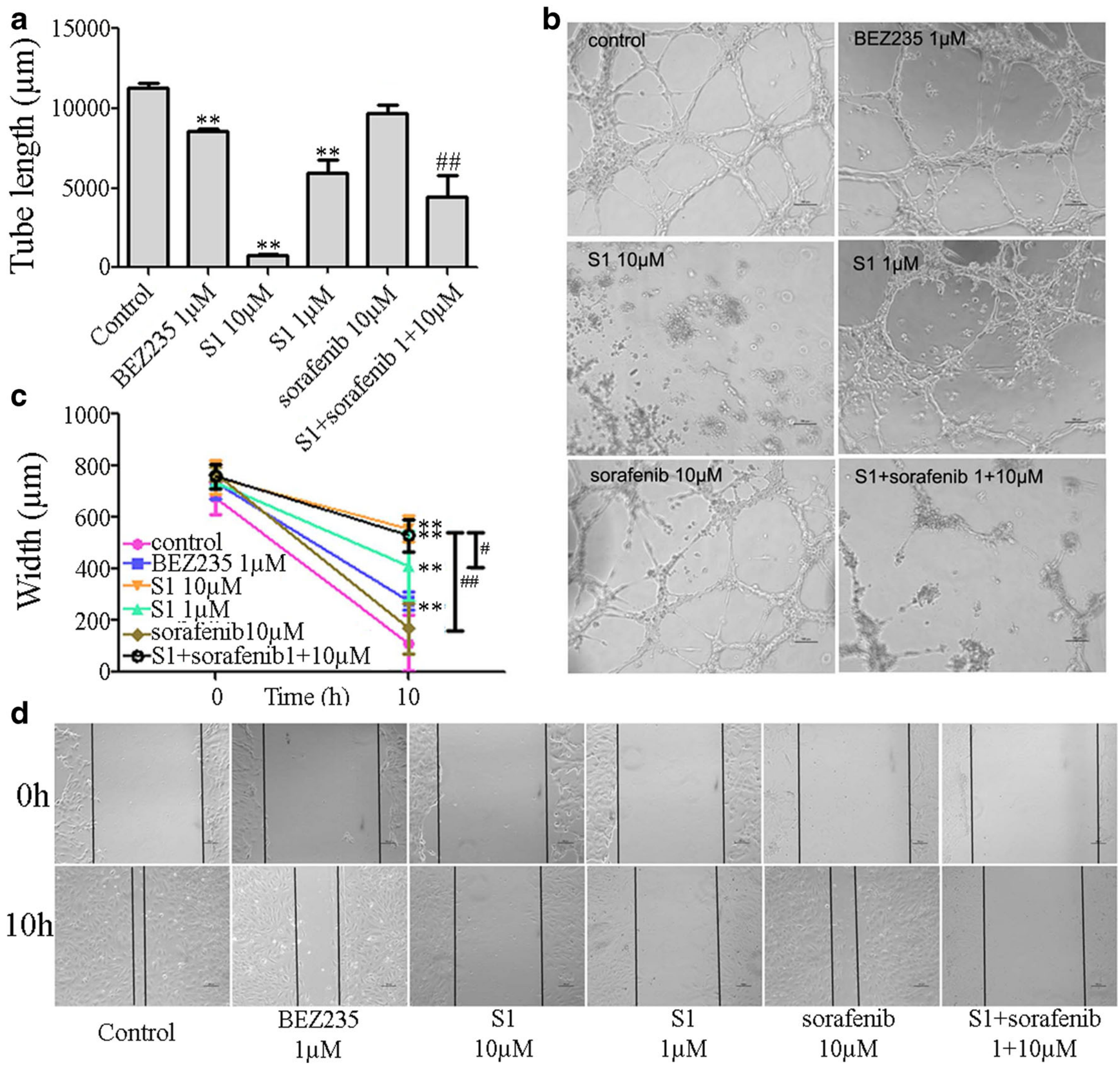


Fig. 4 Effects of S1 and sorafenib on angiogenesis in vitro. a, **b** Matrigel tube-formation assay of HUVECs. Cells were treated with various drugs at the indicated concentration for 8 h and capillary tube formation was then assessed. **c**, **d** The migration assay of HUVECs. The cell layer was scratched with a pipette tip and treated with various drugs at the indicated concentration for 10 h. Cell migration was visualized at the indicated time points under an inverted light microscope and photographed with a digital camera. Data are expressed as mean ± standard deviation from three independent experiments. *, $p < 0.05$ vs the control group; **, $p < 0.01$ vs the control group; #, $p < 0.05$ vs the S1 (1 μM) group; ##, $p < 0.01$ vs the sorafenib group. Scale bar, 100 μm

shown in Fig. 4c, d, S1 (1 or 10 μM) alone or combined with sorafenib (1 μM + 10 μM) significantly inhibited cell migration of HUVECs with a stronger inhibition by the combined treatment, as revealed by the wider scratch width. In contrast, BEZ235 only produced mild effects on the tube formation and migration of HUVECs.

Differential modulation of key signaling molecules in PI3K/Akt/mTOR and Ras/Raf/ERK cascades by S1 and sorafenib

The PI3K/Akt/mTOR and Ras/Raf/ERK cascades have been suggested to be closely associated with tumor growth, progression, metastasis and even drug resistance [5–7]. As shown in Fig. 5 and Supplemental Fig. 4, treatment with the multikinase inhibitor sorafenib (2 μM) significantly inhibited the ERK phosphorylation in 95D cells, but had no obvious effects on the phosphorylation of Akt or S6. In contrast, incubation with S1 (0.2 or 2 μM) effectively inhibited the S6 phosphorylation, downstream target of the PI3K/Akt/mTOR signaling (Fig. 5, Supplemental Fig. 4) but had no significant effects on the ERK phosphorylation in A549 and NCI-H157 cells. These data revealed a differential modulation of the ERK- and S6-related signaling cascades by S1 and sorafenib in NSCLC cells.

Effect of S1 alone or in combination with sorafenib on the growth of A549 NSCLC xenografts

To further validate the effectiveness of S1 alone or in combination with sorafenib on the growth of A549 xenografts, nude mice bearing A549 tumor xenografts were treated with S1 (30, 50 mg/kg), BEZ235 (50 mg/kg), sorafenib (30 mg/kg), and S1 + sorafenib (30 + 30 mg/kg). As shown in Fig. 6, the tumor volume was significantly decreased by S1 (30 or 50 mg/kg), sorafenib (30 mg/kg) or S1 combined with sorafenib (30 + 30 mg/kg). Moreover, the survival time was also elongated following treatment with S1 (30 mg/kg; median survival time: 76 days) or in combination with sorafenib (30 + 30 mg/kg; median survival time: 83 days), when compared with the control group (median survival time:65 days) (Fig. 6b). Taken together, these results demonstrated the anti-tumor efficacy of S1 on the growth of A549 NSCLC xenografts with an enhancement when combined with sorafenib.

To better understand the mechanism of action of S1 in vivo, the tumor xenografts were harvested and processed for western blotting. As shown in Fig. 6c, d, S1 exerted an obvious inhibition of the phosphorylation of Akt and S6, but had minimal effects on the ERK phosphorylation.

Discussion

It has been shown that tumor growth, progression, and drug resistance may involve the the interaction between the PI3K/AKT/mTOR and Ras/Raf/ERK signaling [7]. Blockade of the PI3K/Akt/mTOR pathway by rapamycin can eliminate the S6K-mediated negative feedback inhibitory loop, thereby causing the activation of the Ras/Raf/ERK pathway [23, 24]. On the other hand, the Ras/Raf/ERK pathway inhibitor sorafenib can slightly stimulate mTOR and dramatically stimulate AKT (Ser473) phosphorylation [9]. This crosstalk may underlie the resistance to the mono-targeted agents in the treatment of NSCLC, leading to the combination of different targeted agents representing a promising therapeutic strategy of NSCLC [25].

In this context, we have designed and synthesized a novel chemical compound S1 and demonstrated it to be a PI3K/mTOR dual inhibitor [13]. We have identified here that S1 alone or combined with sorafenib can inhibit the in vitro cell proliferation of NSCLC cells and tumor growth in the xenograft model, possibly through intervening the colony formation, cell migration and invasion, and angiogenesis. Furthermore, the combination of S1 and sorafenib has

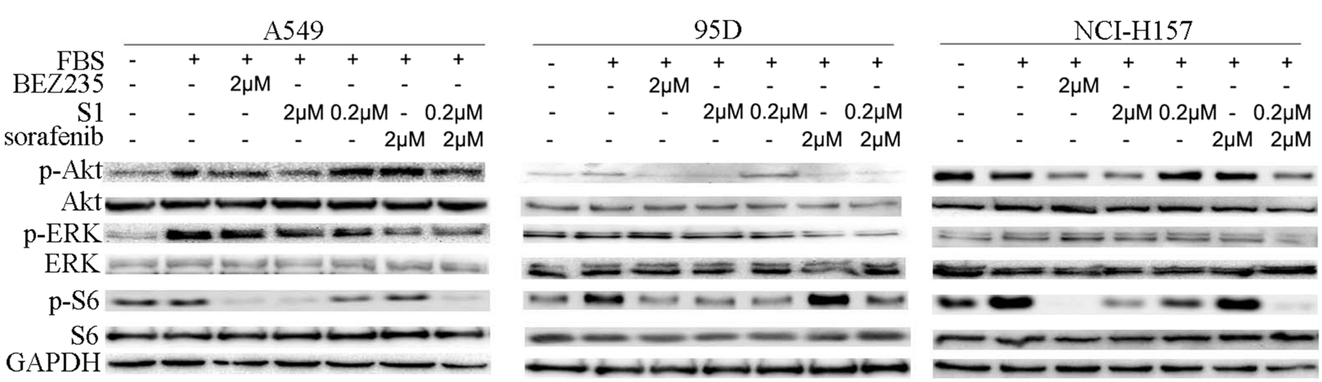


Fig. 5 **Effects of S1 and sorafenib on the phosphorylation of Akt, S6 and ERK by western blot.** Cells were subjected to serum starvation for 24 h, and then treated with BEZ235 (2 μM), S1 (2 or 0.2 μM), sorafenib (2 μM), S1 + sorafenib (0.2 + 2 μM) for 6 h. FCS (10%) was added 15 min before harvest. At 6 h post-drug, cells were harvested and lysed and cell extracts were analyzed for phospho-Akt, Akt, phospho-S6, S6, phospho-ERK and ERK levels by western blot analysis

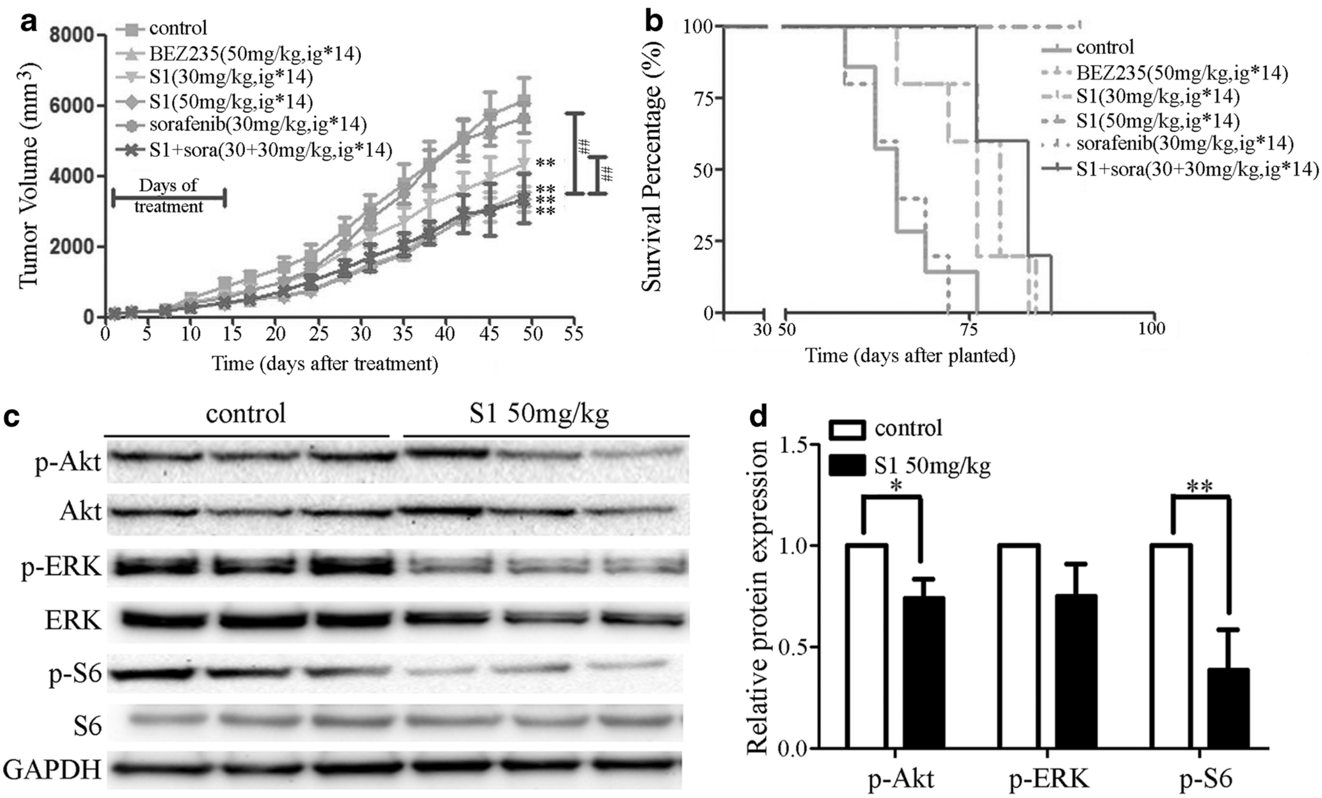


Fig. 6 Effects of S1 and sorafenib on the tumor growth of A549 NSCLC xenografts in mice. A549 xenografts were established by utilizing 3×10^6 cells inoculated s.c. into the right flank of BALB/c(Nu/Nu) mice. Treatment was initiated when tumors were palpable with a volume of ≈100 mm^3. Mice bearing A549 xenografts were treated with vehicle ($n = 8$), S1 (30, 50 mg/kg, $n = 5$ per dose), BEZ235 (50 mg/kg, n = 5), sorafenib (30 mg/kg, n = 5), or S1 + sorafenib (30 + 30 mg/kg, n = 5) by oral gavage once daily for 14 days. Tumor volume (**a**) was measured twice a week. Data are expressed as mean ± SD. **, p < 0.01 vs the control group; ##, p < 0.01 vs the S1 + sora group. **b** The Kaplan-Meier survival curve for BALB/c mice bearing A549 xenografts. Note that S1 alone (30 mg/kg; median survival time: 76 days) or combined with sorafenib (30 + 30 mg/kg; median survival time: 83 days) improved survival, when compared with the control group (median survival time:65 days). **c**, **d** Changes of p-Akt, p-S6 and p-ERK in the A549 xenografts by western blot analyses. Tumors were gathered at day 14 post-treatment ($n = 3$/group). Note that S1 (50 mg/kg) inhibited the phosphorylation of Akt and S6, but had minimal effects on the ERK phosphorylation. *$p < 0.05$ vs the vehicle control group; **$p < 0.01$ vs the vehicle control group

potentiated anti-tumor effects, possibly by their differential modulation of the PI3K/mTOR and MEK/ERK pathways.

In detail, S1 alone was observed to inhibit cell proliferation in three NSCLC cells, in which A549 cells were PTEN-positive, NCI-H157 cells were PTEN-mutant and 95D cells PTEN-null, suggesting that the prevention of the NSCLC cell proliferation by S1 was independent of their initial PTEN status. Potentiated inhibitory effects were observed on cell proliferation, invasion and migration by S1 combined with sorafenib, consistent with the viewpoint that the PI3K/Akt/mTOR and Ras/Raf/ERK singaling cascades are critical in NSCLC cell survival, proliferation, and mobility [5–7, 9].

In addition, angiogenesis is closely correlated with tumor growth, progression and aggresiveness in various cancers, including lung cancer [26, 27]. Inhibition of angiogenesis has been proposed to be a promising intervening strategy for NSCLC therapies. Our studies have demonstrated that both S1 and sorafenib can inhibit the formation of tubular structures of HUVECs as well as their migration, of which these inhibitory effects can be enhanced by the combination of S1 and sorafenib, suggesting that the anti-tumor effects by S1 and sorafenib could be ascribed, at least in part, to the inhibition of angiogenesis.

To reveal how the combination of S1 and sorafenib directly affects tumor growth at the intracellular molecular level, we have performed western blot analyses and identified differential effects on Akt/S6 phosphorylation and the phosphorylation of ERK. Specifically, FBS was previously used as an inducer of stimulating the phosphorylation of Akt and S6 and enhancing ERK phosphorylation [28]. Interestingly, S1 monotherapy strongly inhibited FBS-stimulated Akt/S6 phosphorylation with less effects on the phosphorylation of ERK. Combined with the fact that sorafenib mostly inhibits the Ras/Raf/MAPK pathway but not the PI3K/Akt/mTOR pathway [15]. It is thus reasonable to infer that the differential modulation of the Akt-S6 and ERK cascades may underlie the anti-tumor effects of S1 and sorafenib in the treatment of NSCLC.

In general, the novel PI3K/mTOR inhibitor S1 per se exhibits strong anti-tumor effects in the in vitro and in vivo models. The differential modulation of the phosphorylation of Akt and S6 in the PI3K/Akt/mTOR cascades and ERK phosphorylation in the Raf/MEK/ERK pathways may underlie the potentiated anti-tumor effects by S1 combined with sorafenib. The combination of S1 and sorafenib could be used as an additive approach in treating NSCLC in the clinic.

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Compliance with ethical standards

Conflict of interest Juan Wang declares that she has no conflict of interest. Shumei Ma declares that she has no conflict of interest. Xiuhua Chen declares that she has no conflict of interest. Sanqi Zhang declares that he has no conflict of interest. Zhiyong Wang declares that he has no conflict of interest. Qibing Mei declares that he has no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent Informed constent was obtained from all individual participants included in this study.

References

1. Hirsch FR, Scagliotti GV, Mulshine JL, Kwon R, Curran WJ Jr, Wu YL, Paz-Ares L (2017) Lung cancer: current therapies and new targeted treatments. Lancet 389(10066):299–311. https://doi.org/10.1016/S0140-6736(16)30958-8
2. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A (2015) Global cancer statistics, 2012. CA Cancer J Clin 65(2):87–108. https://doi.org/10.3322/caac.21262
3. Ray MR, Jablons D, He B (2010) Lung cancer therapeutics that target signaling pathways: an update. Expert Rev Respir Med 4(5):631–645. https://doi.org/10.1586/ers.10.64
4. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, Zhu J, Johnson DH, Eastern Cooperative Oncology G (2002) Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 346(2):92–98. https://doi.org/10.1056/NEJMoa011954
5. J E, Xing J, Gong H, He J, Zhang W (2015) Combine MEK inhibition with PI3K/mTOR inhibition exert inhibitory tumor growth effect on KRAS and PIK3CA mutation CRC xenografts due to reduced expression of VEGF and matrix metallopeptidase-9. Tumor Biol 36(2):1091–1097. https://doi.org/10.1007/s13277-014-2667-5
6. Gadgeel SM, Wozniak A (2013) Preclinical rationale for PI3K/Akt/mTOR pathway inhibitors as therapy for epidermal growth factor receptor inhibitor-resistant non-small-cell lung cancer. Clin Lung Cancer 14(4):322–332. https://doi.org/10.1016/j.cllc.2012.12.001
7. Rusconi P, Caiola E, Broggini M (2012) RAS/RAF/MEK inhibitors in oncology. Curr Med Chem 19(8):1164–1176
8. Forde PM, Ettinger DS (2013) Targeted therapy for non-small-cell lung cancer: past, present and future. Expert Rev Anticancer Ther 13(6):745–758. https://doi.org/10.1586/era.13.47
9. Heavey S, O'Byrne KJ, Gately K (2014) Strategies for co-targeting the PI3K/AKT/mTOR pathway in NSCLC. Cancer Treat Rev 40(3):445–456. https://doi.org/10.1016/j.ctrv.2013.08.006
10. Xu CX, Li Y, Yue P, Owonikoko TK, Ramalingam SS, Khuri FR, Sun SY (2011) The combination of RAD001 and NVP-BEZ235 exerts synergistic anticancer activity against non-small cell lung cancer in vitro and in vivo. PLoS One 6(6):e20899. https://doi.org/10.1371/journal.pone.0020899
11. Maira SM, Stauffer F, Brueggen J, Furet P, Schnell C, Fritsch C, Brachmann S, Chene P, De Pover A, Schoemaker K, Fabbro D, Gabriel D, Simonen M, Murphy L, Finan P, Sellers W, Garcia-Echeverria C (2008) Identification and characterization of NVP-BEZ235, a new orally available dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor with potent in vivo antitumor activity. Mol Cancer Ther 7(7):1851–1863. https://doi.org/10.1158/1535-7163.MCT-08-0017
12. Knight SD, Adams ND, Burgess JL, Chaudhari AM, Darcy MG, Donatelli CA, Luengo JI, Newlander KA, Parrish CA, Ridgers LH, Sarpong MA, Schmidt SJ, Van Aller GS, Carson JD, Diamond MA, Elkins PA, Gardiner CM, Garver E, Gilbert SA, Gontarek RR, Jackson JR, Kershner KL, Luo L, Raha K, Sherk CS, Sung CM, Sutton D, Tummino PJ, Wegrzyn RJ, Auger KR, Dhanak D (2010) Discovery of GSK2126458, a highly potent inhibitor of PI3K and the mammalian target of rapamycin. ACS Med Chem Lett 1(1):39–43. https://doi.org/10.1021/ml900028r
13. Shao T, Wang J, Chen JG, Wang XM, Li H, Li YP, Li Y, Yang GD, Mei QB, Zhang SQ (2014) Discovery of 2-methoxy-3-phenylsulfonamino-5-(quinazolin-6-yl or quinolin-6-yl)benzamides as novel PI3K inhibitors and anticancer agents by bioisostere. Eur J Med Chem 75:96–105. https://doi.org/10.1016/j.ejmech.2014.01.053
14. Wilhelm S, Carter C, Lynch M, Lowinger T, Dumas J, Smith RA, Schwartz B, Simantov R, Kelley S (2006) Discovery and development of sorafenib: a multikinase inhibitor for treating cancer. Nat Rev Drug Discov 5(10):835–844. https://doi.org/10.1038/nrd2130
15. Gridelli C, Maione P, Del Gaizo F, Colantuoni G, Guerriero C, Ferrara C, Nicolella D, Comunale D, De Vita A, Rossi A (2007) Sorafenib and sunitinib in the treatment of advanced non-small cell lung cancer. Oncologist 12(2):191–200. https://doi.org/10.1634/theoncologist.12-2-191
16. Blumenschein G Jr (2008) Sorafenib in lung cancer: clinical developments and future directions. J Thorac Oncol 3(6 Suppl 2):S124–S127. https://doi.org/10.1097/JTO.0b013e318174e085
17. Smit EF, Dingemans AM, Thunnissen FB, Hochstenbach MM, van Suylen RJ, Postmus PE (2010) Sorafenib in patients with advanced non-small cell lung cancer that harbor K-ras mutations: a brief report. J Thorac Oncol 5(5):719–720. https://doi.org/10.1097/JTO.0b013e3181d86ebf
18. Paz-Ares L, Hirsh V, Zhang L, de Marinis F, Yang JC, Wakelee HA, Seto T, Wu YL, Novello S, Juhasz E, Aren O, Sun Y, Schmelter T, Ong TJ, Pena C, Smit EF, Mok TS (2015) Monotherapy Administration of Sorafenib in patients with non-small cell lung Cancer (MISSION) trial: a phase III, multicenter, placebo-controlled trial of Sorafenib in patients with relapsed or refractory predominantly nonsquamous non-small-cell lung Cancer after 2 or 3 previous treatment regimens. J Thorac Oncol 10(12):1745–1753. https://doi.org/10.1097/JTO.0000000000000693
19. Kim ES, Herbst RS, Wistuba II, Lee JJ, Blumenschein GR Jr, Tsao A, Stewart DJ, Hicks ME, Erasmus J Jr, Gupta S, Alden CM, Liu S, Tang X, Khuri FR, Tran HT, Johnson BE, Heymach JV, Mao L, Fossella F, Kies MS, Papadimitrakopoulou V, Davis SE, Lippman SM, Hong WK (2011) The BATTLE trial: personalizing therapy for lung cancer. Cancer Discov 1(1):44–53. https://doi.org/10.1158/2159-8274.CD-10-0010
20. O'Brien PJ, Prevost N, Molino M, Hollinger MK, Woolkalis MJ, Woulfe DS, Brass LF (2000) Thrombin responses in human endothelial cells. Contributions from receptors other than PAR1 include the transactivation of PAR2 by thrombin-cleaved PAR1. J Biol Chem 275(18):13502–13509
21. Chou TC, Talalay P (1984) Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzym Regul 22:27–55

22. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144(5):646–674. https://doi.org/10.1016/j.cell.2011.02.013
23. Carracedo A, Ma L, Teruya-Feldstein J, Rojo F, Salmena L, Alimonti A, Egia A, Sasaki AT, Thomas G, Kozma SC, Papa A, Nardella C, Cantley LC, Baselga J, Pandolfi PP (2008) Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. J Clin Invest 118(9):3065–3074. https://doi.org/10.1172/JCI34739
24. Wan X, Harkavy B, Shen N, Grohar P, Helman LJ (2007) Rapamycin induces feedback activation of Akt signaling through an IGF-1R-dependent mechanism. Oncogene 26(13):1932–1940. https://doi.org/10.1038/sj.onc.1209990
25. Sosman JA, Puzanov I, Atkins MB (2007) Opportunities and obstacles to combination targeted therapy in renal cell cancer. Clin Cancer Res 13(2 Pt 2):764s–769s. https://doi.org/10.1158/1078-0432.CCR-06-1975
26. Carmeliet P, Jain RK (2000) Angiogenesis in cancer and other diseases. Nature 407(6801):249–257. https://doi.org/10.1038/35025220
27. Zhan P, Wang J, Lv XJ, Wang Q, Qiu LX, Lin XQ, Yu LK, Song Y (2009) Prognostic value of vascular endothelial growth factor expression in patients with lung cancer: a systematic review with meta-analysis. J Thorac Oncol 4(9):1094–1103. https://doi.org/10.1097/JTO.0b013e3181a97e31
28. Newell P, Toffanin S, Villanueva A, Chiang DY, Minguez B, Cabellos L, Savic R, Hoshida Y, Lim KH, Melgar-Lesmes P, Yea S, Peix J, Deniz K, Fiel MI, Thung S, Alsinet C, Tovar V, Mazzaferro V, Bruix J, Roayaie S, Schwartz M, Friedman SL, Llovet JM (2009) Ras pathway activation in hepatocellular carcinoma and anti-tumoral effect of combined sorafenib and rapamycin in vivo. J Hepatol 51(4):725–733. https://doi.org/10.1016/j.jhep.2009.03.028