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PRECLINICAL STUDIES

Inhibition of SHP2 by new compounds induces differential effects on RAS/RAF/ERK and PI3K/AKT pathways in different cancer cell types

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Summary

Kinases and phosphatases are important players in growth signaling and are involved in cancer development. For development of targeted cancer therapy, attention is given to kinases rather than phosphatases inhibitors. Src homology region 2 domain-containing protein tyrosine phosphatase2 (SHP2) is overexpressed in different types of cancers. We investigated the SHP2-inhibitory effects of two new 5-aminosalicylate–4-thiazolinones in human cervical (HeLa) and breast (MCF-7 & MDA-MB-231) cancer cells. *In-silico* molecular docking showed preferential affinity of the two compounds towards the catalytic over the allosteric site of SHP2. An enzymatic assay confirmed the docking results whereby 0.01 μM of both compounds reduced SHP2 activity to 50%. On cellular level, the two compounds significantly reduced the expression of SHP2, KRAS, p-ERK and p-STAT3 in HeLa but not in the other two cell lines. Phosphorylation of AKT and JNK was enhanced in HeLa and MCF7. Both compounds exhibited anti-proliferative/anti-migratory effects on HeLa and MCF7 but not in MDA-MB-231 cells. These results indicate that inhibition of SHP2 and its downstream pathways by the two compounds might be a promising strategy for cancer therapy in some but not all cancer types.

Keywords SHP2 · RAS/MAPK · AKT · STAT3 · Phosphatase inhibitors

Introduction

Discovery of novel compounds that preferentially inhibit tumor cell proliferation/migration by targeting proteins involved in survival signaling such as kinases and phosphatases appear to be an effective tool in cancer therapy [1] . Many tyrosine kinase inhibitors are already approved as targeted therapeutics for different types of cancers. On the other hand, to the best of our knowledge, no tyrosine phosphatase inhibitors are approved although they are also important signal transducers. Src homology 2 domain-containing protein tyrosine phosphatase 2 (SHP2) is one such protein that is widely involved in transcriptional regulation, cytokine signaling, cellular differentiation and tumor cell proliferation and migration [2]. SHP2 is encoded by PTPN11 gene. Germline /somatic mutations in this gene are reported to associate with syndromes such as Noonan and LEOPARD as well as with tumor development such as lung, breast, neuroblastoma, skin and cervical cancers [3]. An elevated expression level of SHP2 has been observed in many of these cancers and is considered as prognostic and predictive marker [4–6].

Phosphorylation of SHP2 activates many cellular signaling pathways mainly RAS-MAPK, mTOR, and PI3K-AKT [2]. Moreover, its role associated with RAS-MAPK pathway in proliferating tumor cells is widely recognized and accepted [7, 8]. Protein tyrosine phosphatases (PTPs) and protein tyrosine kinases (PTKs) are required to activate these pathways and are often overactivated in cancer cells [9]. Recent studies have shown that the phosphatase activity of SHP2 regulates RAS-MAPK pathway with the co-ordinated expression of down-stream factors like c-Myc, signal transducer and activator of transcription-3 (STAT3) and zinc finger E-box binding homeobox 1 (ZEB1) along with checkpoint kinases in breast and cervical cancers [2, 10]. SHP2 expression was shown to

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be negatively regulated by STAT3 in some types of tumors [9, 11]. Moreover SHP2 mediates tumor invasion and metastasis in breast cancer cells. Therefore targeting SHP2 by developing potent inhibitors may reduce tumor cell growth and metastasis [12].

Few synthetic compounds have been reported previously for their SHP2 inhibiting potentials. Those compounds target either the catalytic site (such as: NSC 87877 and some thiazolidinone derivatives), or the allosteric site (such as: SHP099) of SHP2 (Fig. 1a). The recently developed compound, SHP099, inhibits SHP2 and suppresses RAS-MAPK signaling in tumor cells and xenograft models as well [12].

Salicylic acid derivatives are used in various ailments and are known to have some anti-cancer properties as well [13]. We have previously synthesized and tested novel derivatives of 5-aminosalicylate–4-thiazolinone for their anti- cancer activities. Two of these derivatives showed good anti-cancer effects on different cancer cell lines [14]. In the present study, we investigated the effects of these two compounds on the phosphatase activity of SHP2 and its downstream targets and how this may modulate the proliferation and the migration of cancer cells.

Materials and Methods

Chemicals, antibodies and cell culture

Dulbecco's Modified Eagles Medium (DMEM) and RPMI 1640 medium were purchased from Sigma Aldrich (Darmstadt, Germany). For western blot, the antibodies; SHP2, RAS and p-MAPK, Akt, p-Akt, JNK, p-JNK, STAT3 and p-STAT3 and their respective mouse/rabbit secondary antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). For SHP2 inhibition assay, the IRSI peptide was purchased from Peptide 2.0 Inc, (VA, USA). The SHP2 protein and DiFMUP were purchased from Thermo Fisher Scientific (Waltham, MA, USA). BpV was purchased from Sigma Aldrich (Darmstadt, Germany). The synthesis of novel HH compounds were elaborated previously [13, 14]. All the stock solutions of HH compounds were made in 100% DMSO and working solutions for HH compounds treatment never exceeded 1% DMSO.

The cell lines were gifted from Radiobiology and Experimental Radio Oncology lab, University Cancer

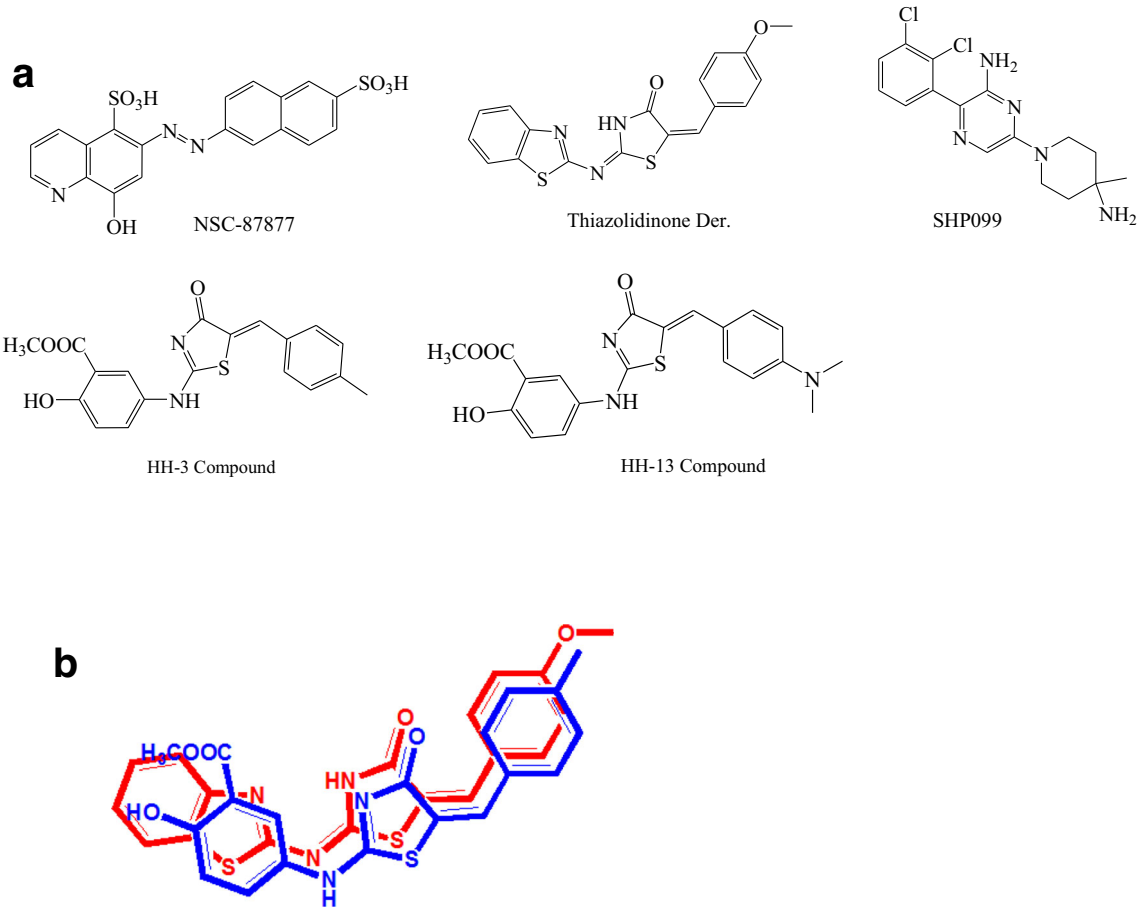


Fig. 1 SHP-2 inhibitors. a Chemical structure of some previously reported SHP-2 inhibitors and our HH compounds. **b** Two dimensional (2D) overlay between the thiazolidinone derivative (red) and our newly synthesized HH-3 compound (blue)

Center, Hamburg University, Hamburg, Germany. HeLa and F180 cells were cultured in DMEM while MCF-7 and MDA-MB-231 cells were cultured in RPMI medium along with 10% FBS and 5% CO_2 in a humidified incubator at 37° C. The cells were grown on polystyrene T75 ($75cm^2$) culture flasks and all the experiments were performed at ~70% cell confluency.

Molecular docking

The *In-silico* molecular docking study was performed employing the program Autodock Vina [15]. The X-ray crystal structures of SHP2 enzyme bound to catalytic inhibitor (PDB ID: 4RDD, 1.6 Å), and to allosteric inhibitor (PDB ID: 5EHR, 1.7 Å) were obtained from the RSCB protein data bank (http://www.rcsb.org). The complexed inhibitors (coded as 3LU and 5OD) were extracted from the initial X-ray structures followed by removal of water molecules. Polar hydrogens and Gastieger charges were added and the corresponding charge files were generated using the MGL Tools. The compounds under study were drawn using the software ChemDraw Ultra 8.0 (Cambridge Soft Corporation, USA) and were optimised for energy and geometry using MMFF94 force field. All the compounds were treated employing the same preparation procedure mentioned earlier. Later, a grid box of the size (20 $Å^3$) was established to cover the SHP-2 catalytic site and was centered toward the coordinates of (27.47, 7.11, -0.39). For the SHP-2 allosteric site, a grid box sized (28 $Å^3$) was established and centered toward the coordinates of (22.41, 41.18, 4.70). In both cases the spacing between grid points was set to 1.0 Å. The exhaustiveness and the number of poses were set to 12 and 10 respectively. The 3D-best docked poses were visualized using PyMOL molecular viewer (Schrödinger Inc., New York, NY, USA).

Sulforhodamine-B (SRB) assay

SRB assay was performed on HeLa, MCF-7 and MDA-MB-231 cells in order to find out the sub-lethal concentration of HH compounds with minor modifications from an already established protocol [16]. Briefly, $1X10^4$ cells were seeded in 96-well plate for overnight and were treated with different concentrations of HH3 or HH13 and incubated further for 48 hrs. Control cells were treated with DMSO. At the end of the incubation period, cells were fixed with 50% trichloroacetic acid (TCA) at 4°C for 1 hr. Plates were washed, dried and stained with SRB dye for 30 min at room temperature. After washing the excess dye, two hundred micro liter of 10 mM Tris base solution was used to solubilize the dye. The OD was read at 492 nm by using a Multiskan™ GO (Thermo Scientific, USA) microplate Spectrophotometer.

Cell migration assay

Inhibition of cell migration by HH compounds was analyzed using the wound healing assay [17]. $8X10^5$ cells were seeded in 6-well plate for 24 hrs. *In vitro* 'scratch' was created by scrapping the cells off from the monolayer by using a micro tip end and washed with PBS to remove dislodged cells. The wells were then treated with different concentrations of HH compounds and incubated for 72 hrs. The area of migrated cells were then monitored at different time points (0, 6, 24, 48 and 72 hrs).

Cell-free Enzymatic SHP-2 inhibition assay

The assay was performed to evaluate the potential inhibitory effect of HH compounds on SHP2 activity as described previously with minor modifications [12]. A 96-well, black polystyrene flat bottom, non-binding surface plate was used to perform the assay with final volume of HEPES buffer (60 mM HEPES, 75 mM NaCl, 75mM KCl, 1 mM EDTA, 0.05% T-20, 5 mM DTT, pH 7.2) not exceeding 25 μl/well. 0.5 nM of SHP2 protein was co-incubated at 25°C with 0.5 μM of bisphosphorylated IRSI peptide (H2N-LN(pY)IDLDLV(dPEG8)LST(pY)ASINFQK-amide) along with different concentrations of HH compounds for 1 hr. The plate was further incubated with 0.2 mM of DiFMUP for 30 min at 25°C. Five micro liter of 160 μM bpV was then added to each well to quench the reaction and the fluorescence signal was measured at 450 nm using a Multiskan™ GO (Thermo Scientific, USA) microplate Spectrophotometer.

Western blot

HeLa, MCF-7 and MDA-MB-231 cell lysates were prepared after 24 hrs treatment with HH compounds by using 1X laemmli buffer. Total protein concentration was estimated for each sample and 15-40 μg of protein lysate were loaded on 10-12% SDS-PAGE gel in order to detect various proteins expression levels as previously described [18]. In short, the membrane was incubated with SHP2, RAS, p-MAPK, STAT3, p-STAT3, p-JNK, Akt or p-AKT primary antibodies (1:1000) for overnight after blocking with 5% non-fat milk solution for 1 hr. The membrane was then re-probed with respective secondary mouse/rabbit antibodies (1:2000) for 1 hr, and developed by enhanced chemiluminescence (ECL) method by using a Chemidoc MP (BioRad, Germany). The expression levels of various proteins were then quantified with respect to their internal actin level and compared with untreated control cells.

Statistical analysis

All experiments were carried out in triplicates and for three independent times and were analysed by two-tailed Student's

t-test and ANOVA by using Graph-Pad Prism software (GraphPad Software Inc., San Diego, CA, USA). The data for SRB and SHP2 enzymatic assay are presented as mean ± standard deviation (SD) and p values ≤ 0.05 were considered as significant between control and treated groups.

Results

Docking analysis

A number of molecular docking experiments were carried-out in order to forecast the affinity of our compounds in targeting the SHP2 sites (catalytic and allosteric), and to determine the most probable binding site. For this purpose two X-ray crystal structures were adapted to serve as templates for the study; the first structure representing an inhibitor (3LU) bound to the catalytic site of SHP2, while the second one representing an inhibitor (5OD) bound allosterically to SHP2 enzyme. In the first set of experiments, our HH compounds plus the thiazolidinone catalytic inhibitor (Fig. 1) were docked into the catalytic site. Later, the HH compounds and the SHP099 allosteric inhibitor were docked into the allosteric site. Benchmarking of the docking experiments was achieved by utilizing known inhibitors to serve as basis for comparison. The binding affinity scores in kcal/mol are presented in Table 1. Based on the docking results, our compounds achieved higher scores compared to the thiazolidinone catalytic inhibitor, while achieved inferior scores compared to the SHP099 allosteric inhibitor, which suggests the preferential affinity of our compounds in targeting the catalytic site over the allosteric.

The detailed binding modes of the HH compounds within the SHP2 catalytic site are illustrated in Fig. 2. In case of HH3-SHP2 complex (Fig. 2a), the compound was able to form a number of important H-bond interactions within the catalytic site. The first set of interactions was observed between the thiazolinone ring carbonyl with the backbone amides of Ile-463 and with the residue Gly-464 of the corresponding P-loop. The 5-aminosalicylate moiety was engaged in another set of interactions with the Trp-423 residue and with the Gly-427 amide backbone of the corresponding WPD-loop. Additionally, it was able to form H-bond with the Gln-510 residue of the Q-loop.

On the other hand, the HH13-SHP2 complex showed similar network of interactions within the catalytic site (Fig. 2b), where the thiazolinone ring carbonyl was able to form H-bond interaction with the corresponding backbone amides of Gln-361. The 5-aminosalicylate moiety was able to establish a number of H-bond interactions with Arg-465 of the P-loop, Trp-423 of the WPD-loop and with the Gln-510 of the Q-loop.

Table 1 Binding affinity scores (kcal/mol) for the HH-compounds against the corresponding SHP2 enzyme catalytic and allosteric sites compared to other SHP2 inhibitors

Compound	Docking Score Kcal/mol	
	Catalytic site	Allosteric site
Thiazolidinone Der.	-6.6	----
SHP-099	----	-10.8
HH-3	-7.7	-9.4
HH-13	-7.4	-9.3

Inhibition of SHP2 enzyme activity by HH compounds (cell-free assay)

To validate the docking results, an enzymatic assay was performed to investigate the inhibitory potentials of HH compounds on SHP2 activity and the results are shown in Fig. 3a. A high affinity for bistyrosylphorphorylated peptides (SHP2 substrate) to Src Homology 2 (SHP2) domains were observed in control wells as later activation showed maximum SHP2 activity. However, the addition of HH compounds reduced SHP2 activity toward its substrate as they might mask the active site of SHP2 for substrate recognition. Both HH3 and HH13 inhibits SHP2 activity significantly ($p<0.05$) with respect to control samples. An equal or more potent inhibitory activity for HH compounds was observed when compared to NSC 87877 (positive control) at lower concentrations. However, we did not observe any concentration-dependent SHP2 inhibitory effect either for HH3 or HH13 compounds.

HH3 and HH13 inhibits SHP2 phosphatase in HeLa cells

The effect of HH compounds on cellular SHP2 was analyzed. The baseline level of SHP2 was analyzed in one normal fibroblast strain (F180) and three cancer cell lines (HeLa, MCF-7 and MDA-MB-231). As depicted in Fig. 3b, the three cancer cell lines express significantly higher baseline level of SHP2 than the normal fibroblasts indicating that cancer cells might be more sensitive to SHP2 inhibitors than normal cells.

Treatment of cancer cells with HH3 or HH13 reduced SHP2 expression in HeLa cells where the level of reduction was comparable to that induced by the positive control NSC87877. On the other hand, neither of the two compounds reduced the expression of SHP2 in MCF-7 or in MDA-MB-231 cells (Fig. 3c).

HH compounds inhibit cell proliferation and migration

The proliferation inhibitory effects of HH compounds on three cancer cell lines along with doxorubicin as internal standard were analyzed by SRB assay and the results are depicted in Table 2. The compound HH3 showed an IC50 values of 0.30

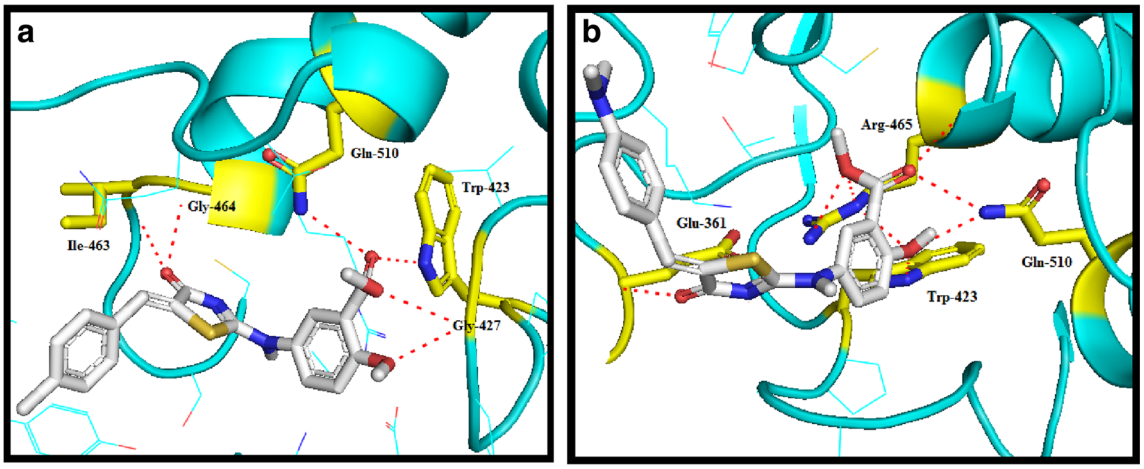
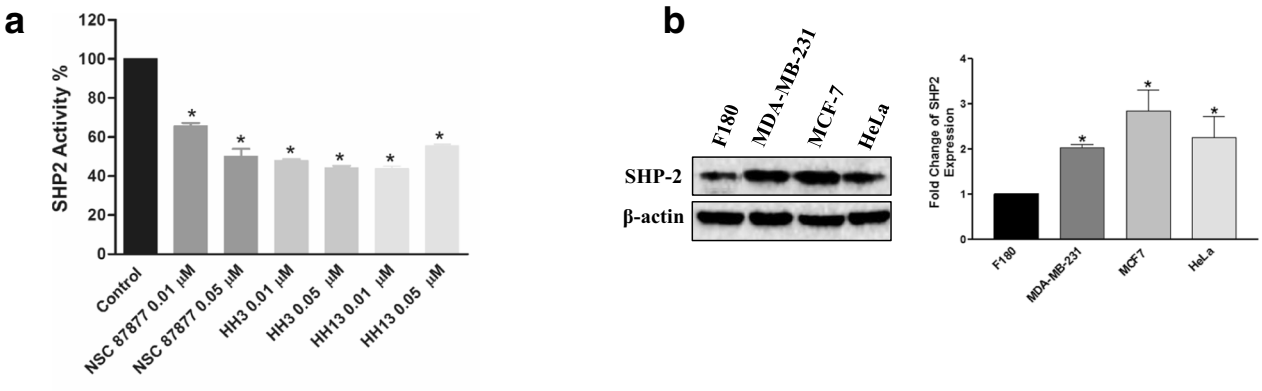


Fig. 2 Preferential affinity of HH compounds towards the catalytic site of SHP2 enzyme. Best docked pose of compound HH-3 (**a**), and compound HH-13 (**b**) within the catalytic site of SHP2 enzyme. Important amino acid residues (highlighted yellow) are in sticks rendering; while hydrogen bond interactions are shown as red dashed lines

±0.099, 0.31±0.056 and >10 μM in HeLa, MCF-7 and MDA-MB-231 cells respectively. HH13 exhibited IC50 values of 0.48±0.02, 0.30±0.045 and 2.5± 0.24 μM in HeLa, MCF-7 and MDA-MB-231 cells respectively. Based on these, we have selected two concentrations of HH compounds (0.5 and 1 μM) for our further molecular studies. We then analyzed the effect of the two compounds on migration of cancer cells. Results of this assay revealed significant reduction in cell



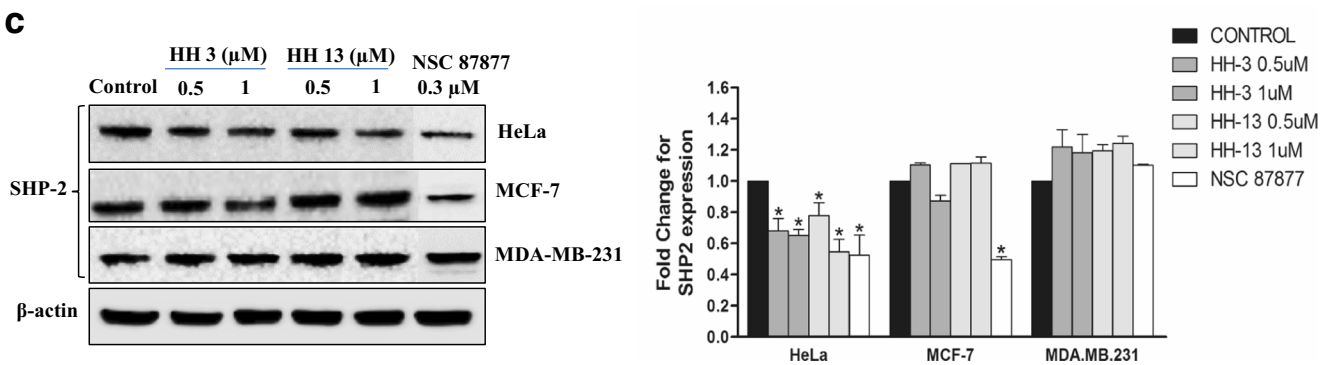


Fig. 3 SHP2 phosphatase inhibiting potential of HH compounds. a cell-free enzymatic assay showing inhibition of SHP2 activity for HH compounds by masking the active site of SHP2 for substrate recognition. **b** Baseline level of SHP2 in F180 normal fibroblasts in comparison with its level in three cancer cell lines (HeLa, MCF-7 and MDA-MB-231 cells) analyzed by western blot. **c** HH compounds and the positive control (NSC87877) SHP2 inhibitor significantly ($p<0.05$) reduce SHP2 expression in HeLa but not in MCF7 nor MDA-MB-231 cells

Table 2 IC50 values of HH compounds for HeLa, MCF-7 and MDA-MB-231 cells compared with doxorubicin as internal reference

Compounds	HeLa IC50 (μM)	MCF-7 IC50 (μM)	MDA-MB-231 IC50 (μM)
HH3	0.30 ± 0.009	0.31 ± 0.065	>10
HH13	0.48 ± 0.02	0.30 ± 0.045	2.5 ± 0.24
DOX	0.21± 0.009	0.07± 0.006	0.15± 0.03

Data are shown as mean ± SD

migration after treating HeLa or MCF-7 cells with HH compounds up to 72 hrs compared with untreated cells. No inhibition of cell migration was observed upon treatment of MDA-MB-231 cells with either of the two compounds (Fig. 4a**)**. Many cancer cells phosphorylate their JNK protein to overcome stressful conditions, therefore increased level of p-JNK is a marker of cellular stress. Analysis of p-JNK in the three cell lines after treatment with HH compounds revealed an enhanced level of p-JNK in HeLa and MCF-7 more than MDA-MB-231 cells (Fig. 4b).

RAS/MAPK regulatory effects of HH compounds

To further investigate the effect of HH compounds on SHP2 down-stream targets, we analyzed the activity of members of the RAS/MAPK and PI3K-AKT pathways in HeLa, MCF-7 and MDA-MB-231 cells after treatment with HH compounds and NSC 87877 (Fig. 5)**.** Both HH compounds down-regulated the expression of RAS and p-MAPK in HeLa cells significantly. In contrast, the two compounds did not show any effect on the expression of these proteins in MCF-7 cells,

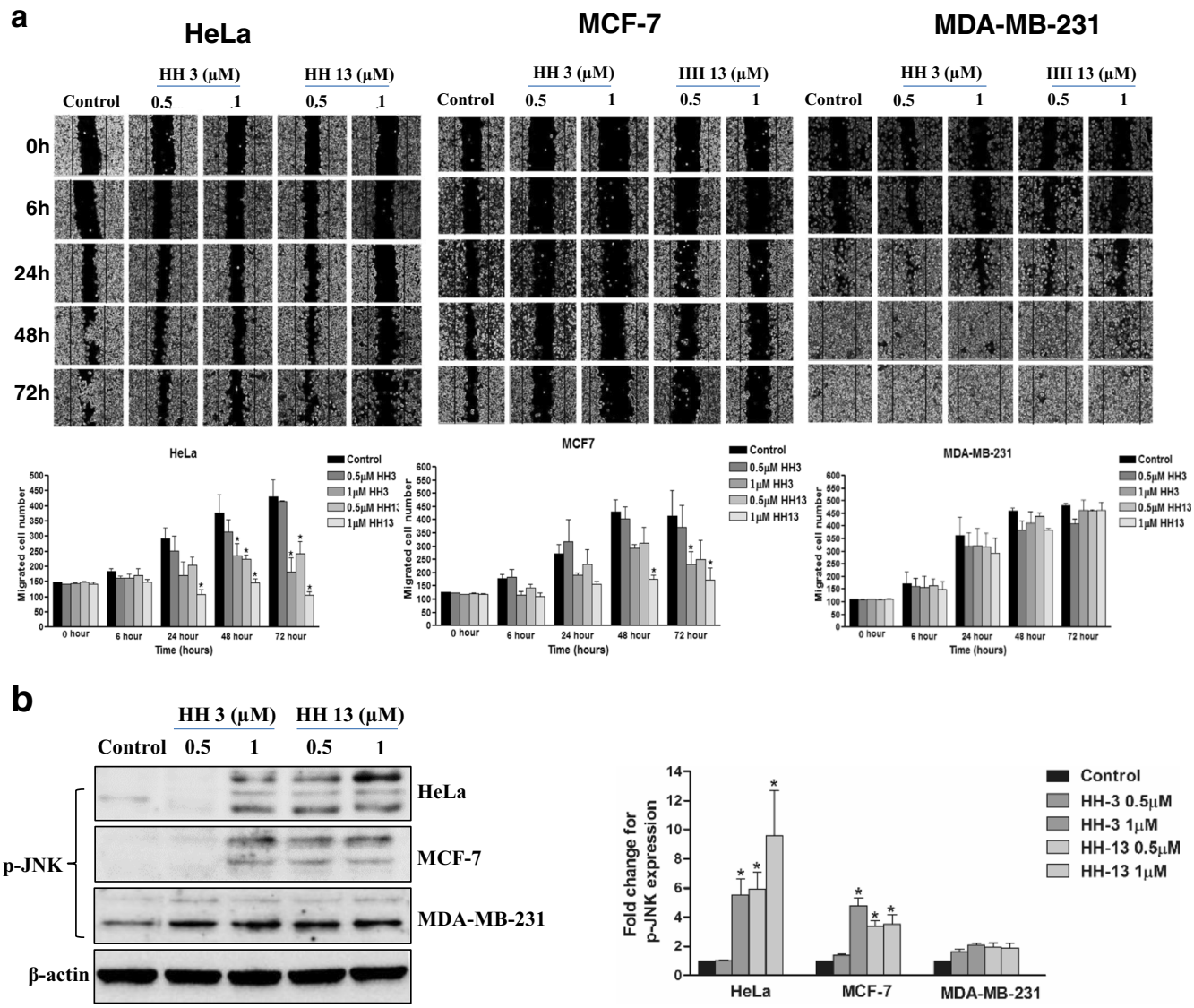


Fig. 4 HH compounds inhibits cell proliferation and migration. a Significant ($p<0.05$) inhibition of cell migration was observed in HeLa and MCF-7 cells after treatment with HH compounds at different time intervals. **b** HH compounds treatment enhances p-JNK expression in HeLa and MCF-7 cells

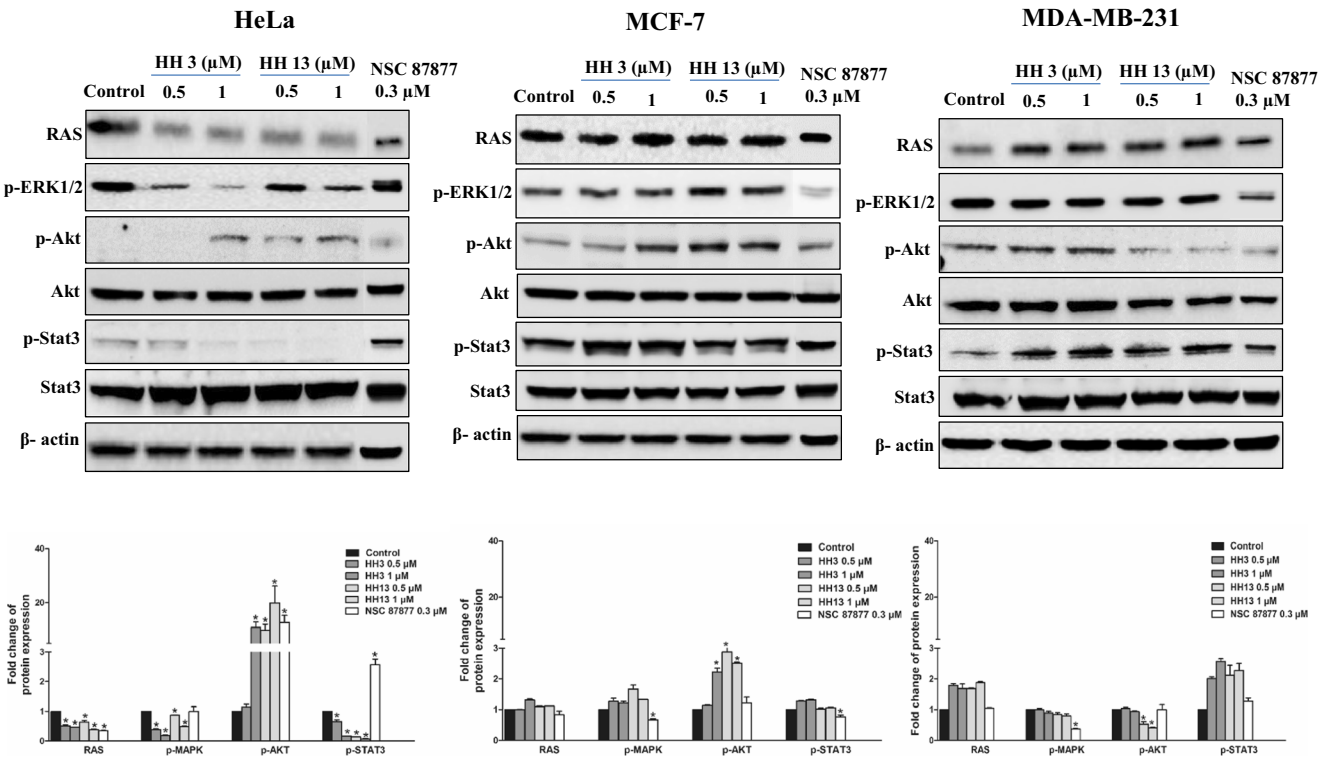


Fig. 5 Effect of HH compounds on RAS/MAPK and PI3K/AKT pathways. a Treatment with HH compounds significantly ($p<0.05$) reduced RAS/MAPK and activate AKT signaling pathways in HeLa cells. STAT3 phosphorylation was also reduced upon treatment of HeLa cells with HH compounds. **b** HH compounds did not affect the RAS/MAPK pathway, but stimulated the AKT phosphorylation in MCF-7 cells or (**c**) HH compounds did not affect the RAS/MAPK pathway, but reduced the AKT phosphorylation and enhanced STAT3 phosphorylation in MDA-MB-231 cells

while MDA-MB-231 cells exhibited an increased expression of RAS in response to HH compounds treatment. An increased p-Akt level was observed in HeLa and MCF-7 cells treated with HH compounds. Treatment with HH compounds also reduced the level of phosphorylated STAT3 in HeLa cells. An increased level of p-Stat3 was evident in MCF-7 and MDA-MB-231 cells after treatment with HH compounds compared with untreated cells. These results collectively suggest that HH compounds regulate the expression of RAS/MAPK along with Akt and STAT3 in HeLa cells.

Discussion

Targeting SHP2, which is ubiquitously expressed in tumor cells is gaining increased attention not only to develop potent inhibitors but to establish new biomarkers for tumor diagnosis as well [19]. We report here about two new SHP2 inhibitors. Initial molecular docking study suggested clear affinity of the new compounds to SHP2 catalytic site. This is evident because, 1) they achieved superior affinity scores over the reference catalytic inhibitor; 2) they share considerable structural and electronic similarity with other SHP2 catalytic inhibitors such as the thiazolidinone derivatives (Fig. 1b). The incorporation of 5-aminosalicylate moiety within our structures proved to be vital for their catalytic affinity. The salicylate (-COOH and –OH) groups were able to initiate a network of important hydrogen bond interactions with residues of the catalytic site. Furthermore, the carboxylic acid could be viewed as an isosteric replacement for sulfonic acid found in many earlier reported inhibitors such as the NSC 87877.

In-vitro cell free enzymatic inhibition assay confirmed the docking results whereby very low concentrations (0.01 and 0.05 μM) of both compounds reduced the SHP2 activity down to 50% of its initial value. The reduction in SHP2 activity by both compounds at 0.01 μM was even higher than the reduction induced by the positive control NSC87877. The SHP2 inhibitory activity of HH3 and HH13 encouraged us to evaluate their anti-SHP2 activity inside the cells. We utilized three different cancer cell lines with different sensitivity to the two compounds (HeLa ~ MCF-7>>MDA-MB-231). Previous reports have shown that receptor tyrosine kinase driven cancer cells depend on SHP2 for their survival and inhibition of SHP2 results in death or at least stop proliferation of these cells [20]. RAS/ERK signaling pathway is a known downstream target of SHP2 and an intact RAS/ERK pathway is a prerequisite for cell growth inhibition induced by SHP2 inhibitors [21]. The insensitivity of MDA-MB-231 cells to SHP2 inhibition by our two compounds (IC50>10 for HH3 and 2.5 μM for HH13) is mainly due to the mutant KRAS status of these cells [22]. This is

consistent with previous results showing that KRAS or BRAF mutant cells are generally resistant to SHP2 inhibition. On the other hand, treatment of HeLa cells, which have an intact RAS/ERK pathway [23] with the new compounds resulted in down-regulation of SHP2 and its downstream targets KRAS and ERK proteins, which explains cell death and migration inhibition in these cells by HH3 and HH13.

Another pathway that orchestrate with RAS-ERK to support cell survival is PI3K-AKT. Treatment with HH compounds induced differential effects on AKT phosphorylation in the three cell lines whereby enhanced AKT phosphorylation was observed in HeLa and MCF-7 cells while reduced AKT phosphorylation was seen in MDA-MB-231 cells. For HeLa cells, increased phosphorylation of AKT is consistent

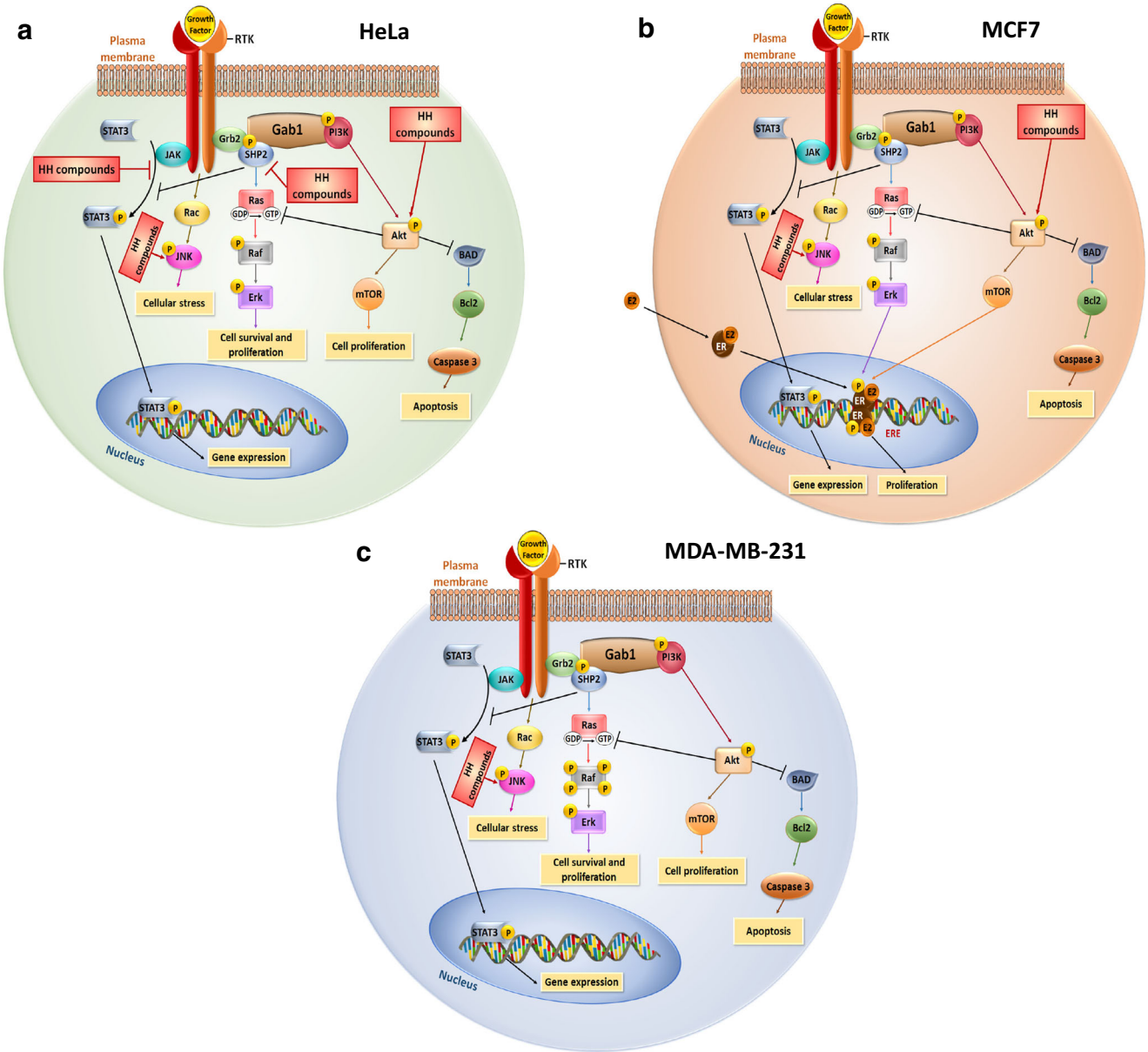


Fig. 6 Schematic representation of the differential effects of HH compounds on SHP2-mediated RAS/MAPK, PI3K-AKT and STAT3 signaling pathways in HeLa, MCF7 and MDA-MB-231 cells. a Proliferation of HeLa cells is driven by membrane receptor tyrosine kinase. Treatment with HH compounds inhibits SHP2 and its downstream KRAS/BRAF/MAPK pathway and thereby inhibits tumor cell survival/proliferation. Reduction of KRAS/BRAF signaling inhibits the negative crosstalk with PI3K-AKT pathway leading to activation of AKT (by phosphorylation). HH compounds also reduced the STAT3 phosphorylation. **b** MCF7 are estrogen receptor (ER) positive breast cancer cells and their proliferation is mainly estrogen-dependent. Treatment with HH compounds did not affect the KRAS/BRAF/MAPK pathway. Activation of AKT was observed which might be to overcome the stress induced by treatment with HH compounds. **c** MDA-MB-231 cells have BRAF mutation (observe the over-phosphorylation of BRAF compared to the other two cell lines), therefore their growth is not driven by membrane receptor tyrosine kinases and treatment with HH compounds did not affect the KRAS/BRAF/MAPK pathway while reduced AKT activity and enhanced STAT3 activity were observed

with earlier reports that RAS-ERK and PI3K-AKT pathways negatively regulate each other's activity [24]. Chemical blocking of RAS-ERK pathway by HH compounds in HeLa cells released the cross-inhibition and activates the PI3K-AKT pathway.

Increased phosphorylation of AKT in MCF-7 cells upon treatment with the two compounds despite the lack of effect on SHP2 expression and RAS/ERK pathway is in line with many previous results showing direct increased phosphorylation of AKT in some cells upon exposure to some stressful conditions such as treatment with anti-cancer drugs (Taxol, 5-FU and doxorubicin) and ionizing radiation independent on SHP2-RAS-ERK pathway [25]. This enhanced AKT phosphorylation may help the cells to overcome death signals induced by such agents. The reduced phosphorylation of AKT in MDA-MB-231 cells after treatment with HH compounds is also consistent with earlier results [25] and it could be concluded that activation/inhibition of AKT under stressful conditions may be a direct effect or a result of cross-talk between RAS-ERK and PI3K-AKT pathways depending on the genetic background of the affected cells (Fig. 6).

STAT3 protein is a transcriptional regulator of cell growth and its activation by phosphorylation leads to dimerization and translocation to the nucleus where it binds to DNA and induces transcription of survival proteins such as survivin, cyclin D1 and Bcl-xl [26]. The reduced level of p-STAT3 in HeLa cells after treatment with HH compounds is consistent with the reduced survival of those cells upon treatment with the two compounds, whereas the minor increase in the level of p-STAT3 in MCF-7 or the great increase in MDA-MB-231 cells is another indicator that the response of these two cell lines to treatment with HH compounds and/or SHP2 inhibition is different from the HeLa cells.

For MCF-7 cells, treatment with HH compounds reduced cell migration and cell survival. However, neither SHP2 expression nor activation of RAS-ERK pathway were affected by the two compounds, only the level of p-AKT and p-JNK were increased. The most probable explanation for these effects is that survival of MCF-7 cells which are estrogen receptor (ER) positive is dependent mainly on activation of ER rather than receptor tyrosine kinase pathway in which SHP2 is involved. It seems that HH compounds exert effects on MCF-7 cells other than inhibition of SHP2. These other effects involve activation of AKT and JNK which have been reported previously to be activated in cells undergoing stressful conditions to enable them to survive these conditions [25, 27] .

Conclusion

In conclusion, we report here two salicylate - thiazolinone derivatives that inhibit the activity of SHP2 enzyme *in-vitro*. On the cellular level, the two compounds induce differential effects and stimulate different pathways in three different cell lines. The present results indicate that the two compounds reported here reduce survival of protein tyrosine kinase (TK) driven malignancies through inhibition of SHP2 and down-regulation of RAS/ERK pathway and reduce the survival of non-TK driven cancers by other mechanisms with little or no effect on malignancies harboring KRAS-BRAF mutations. Clinically, selected tumours with intact KRAS/ERK pathway may benefit from SHP2 inhibition therapy.

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Compliance with Ethical Standards

Conflict of Interest Author CV declares that he has no conflict of interest. Author ES declares that he has no conflict of interest. Author WR declares that he has no conflict of interest. Author VM declares that he has no conflict of interest. Author AA declares that he has no conflict of interest. Author HT declares that he has no conflict of interest. Author HA declares that he has no conflict of interest. Author AE declares that he has no conflict of interest. Author RE declares that he has no conflict of interest

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent For this type of study, formal consent is not required.

References

1. Ventura J-J, Nebreda AR (2006) Protein kinases and phosphatases as therapeutic targets in cancer. Clin Transl Oncol 8:153–160. https://doi.org/10.1007/s12094-006-0005-0
2. Zhang J, Zhang F, Niu R (2015) Functions of Shp2 in cancer. J Cell Mol Med 19:2075–2083. https://doi.org/10.1111/jcmm.12618
3. Zhao S, Sedwick D, Wang Z (2014) Genetic alterations of protein tyrosine phosphatases in human cancers. Oncogene:1–10. https://doi.org/10.1038/onc.2014.326
4. Han T, Xiang D-M, Sun W, Liu N, Sun H-L, Wen W, Shen W-F, Wang R-Y, Chen C, Wang X, Cheng Z, Li H-Y, Wu M-C, Cong W-M, Feng G-S, Ding J, Wang H-Y (2015) PTPN11/Shp2 overexpression enhances liver cancer progression and predicts poor prognosis of patients. J Hepatol 63. https://doi.org/10.1016/j.jhep.2015.03.036
5. Grossmann KS, Rosário M, Birchmeier C, Birchmeier W (2010) The tyrosine phosphatase Shp2 in development and cancer. Adv Cancer Res 106:53–89. https://doi.org/10.1016/S0065-230X(10)06002-1
6. Zhou XD, Agazie YM (2008) Inhibition of SHP2 leads to mesenchymal to epithelial transition in breast cancer cells. Cell Death Differ 15:988–996. https://doi.org/10.1038/cdd.2008.54
7. Dance M, Montagner A, Salles JP, Yart A, Raynal P (2008) The molecular functions of Shp2 in the Ras/Mitogen-activated protein kinase (ERK1/2) pathway. Cell Signal 20:453–459. https://doi.org/10.1016/j.cellsig.2007.10.002

8. Butterworth S, Overduin M, Barr AJ (2014) Targeting protein tyrosine phosphatase SHP2 for therapeutic intervention. Future Med Chem 6:1423–1437. https://doi.org/10.4155/fmc.14.88
9. Liu Z, Zhao Y, Fang J, Cui R, Xiao Y, Xu Q, Liu Z, Zhao Y, Fang J, Cui R, Xiao Y, Xu Q (2017) SHP2 negatively regulates HLA-ABC and PD-L1 expression via STAT1 phosphorylation in prostate cancer cells. Oncotarget 8:53518–53530. https://doi.org/10.18632/oncotarget.18591
10. Aceto N, Sausgruber N, Brinkhaus H, Gaidatzis D, Martiny-Baron G, Mazzarol G, Confalonieri S, Quarto M, Hu G, Balwierz PJ, Pachkov M, Elledge SJ, van Nimwegen E, Stadler MB, Bentires-Alj M (2012) Tyrosine phosphatase SHP2 promotes breast cancer progression and maintains tumor-initiating cells via activation of key transcription factors and a positive feedback signaling loop. Nat Med 18:529–537. https://doi.org/10.1038/nm.2645
11. Wu D, Pang Y, Ke Y, Yu J, He Z, Tautz L, Mustelin T, Ding S, Huang Z, Feng GS (2009) A conserved mechanism for control of human and mouse embryonic stem cell pluripotency and differentiation by Shp2 tyrosine phosphatase. PLoS One 4. https://doi.org/10.1371/journal.pone.0004914
12. Chen Y-NP, LaMarche MJ, Chan HM, Fekkes P, Garcia-Fortanet J, Acker MG, Antonakos B, Chen CH-T, Chen Z, Cooke VG, Dobson JR, Deng Z, Fei F, Firestone B, Fodor M, Fridrich C, Gao H, Grunenfelder D, Hao H-X, Jacob J, Ho S, Hsiao K, Kang ZB, Karki R, Kato M, Larrow J, La Bonte LR, Lenoir F, Liu G, Liu S, Majumdar D, Meyer MJ, Palermo M, Perez L, Pu M, Price E, Quinn C, Shakya S, Shultz MD, Slisz J, Venkatesan K, Wang P, Warmuth M, Williams S, Yang G, Yuan J, Zhang J-H, Zhu P, Ramsey T, Keen NJ, Sellers WR, Stams T, Fortin PD (2016) Allosteric inhibition of SHP2 phosphatase inhibits cancers driven by receptor tyrosine kinases. Nature 535:148–152. https://doi.org/10.1038/nature18621
13. Abdu Allah HH, El Shorbagi ANA (2016) 5-Aminosalyclic Acid (5-ASA): A Unique Anti-Inflammatory Salicylate. Med Chem (Los Angeles) 6. https://doi.org/10.4172/2161-0444.1000361
14. Abdu-Allah HHM, Abdel-Moty SG, El-Awady R, El-Shorbagi ANA (2016) Design and synthesis of novel 5-aminosalicylate (5-ASA)–4-thiazolinone hybrid derivatives with promising antiproliferative activity. Bioorg Med Chem Lett 26:1647–1650. https://doi.org/10.1016/j.bmcl.2016.02.073
15. Trott O, Olson A (2010) AutoDock Vina: inproving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem 31:455–461. https://doi.org/10.1002/jcc.21334.AutoDock
16. Saleh EM, El-awady RA, Eissa NA, Abdel-Rahman WM (2012) Antagonism between curcumin and the topoisomerase II inhibitor etoposide: A study of DNA damage, cell cycle regulation and death pathways. Cancer Biol Ther 13:1058–1071. https://doi.org/10.4161/cbt.21078
17. Justus CR, Leffler N, Ruiz-Echevarria M, Yang LV (2014) In vitro cell migration and invasion assays. J Vis Exp 752:e51046. https://doi.org/10.3791/51046
18. El-Awady RA, Saleh EM, Dahm-Daphi J (2010) Targeting DNA double-strand break repair: Is it the right way for sensitizing cells to 5-fluorouracil? Anti-Cancer Drugs 21:277–287. https://doi.org/10.1097/CAD.0b013e328334b0ae
19. He R, Yu Z, Zhang R, Zhang Z (2014) Protein tyrosine phosphatases as potential therapeutic targets. Acta Pharmacol Sin 35:1227–1246. https://doi.org/10.1038/aps.2014.80
20. Prahallad A, Heynen GJJE, Germano G, Willems SM, Evers B, Vecchione L, Gambino V, Lieftink C, Beijersbergen RL, Di Nicolantonio F, Bardelli A, Bernards R (2015) PTPN11 Is a Central Node in Intrinsic and Acquired Resistance to Targeted Cancer Drugs. Cell Rep 12:1978–1985. https://doi.org/10.1016/j.celrep.2015.08.037
21. Schneeberger VE, Ren Y, Luetteke N, Huang Q, Chen L, Lawrence HR, Lawrence NJ, Haura EB, Koomen JM, Coppola D, Wu J (2015) Inhibition of Shp2 suppresses mutant EGFR-induced lung tumors in transgenic mouse model of lung adenocarcinoma. Oncotarget 6:6191–6202. https://doi.org/10.18632/oncotarget.3356
22. Eckert LB, Repasky GA, Ulkü AS, McFall A, Zhou H, Sartor CI, Der CJ (2004) Involvement of Ras activation in human breast cancer cell signaling, invasion, and anoikis. Cancer Res 64:4585–4592. https://doi.org/10.1158/0008-5472.CAN-04-0396
23. Perera D, Venkitaraman AR (2016) Oncogenic KRAS triggers MAPK-dependent errors in mitosis and MYC-dependent sensitivity to anti-mitotic agents. Sci Rep 6. https://doi.org/10.1038/srep29741
24. Mendoza MC, Er EE, Blenis J (2011) The Ras-ERK and PI3K-mTOR pathways: Cross-talk and compensation. Trends Biochem Sci 36:320–328. https://doi.org/10.1016/j.tibs.2011.03.006
25. Li X, Lu Y, Liang K, Liu B, Fan Z (2005) Differential responses to doxorubicin-induced phosphorylation and activation of Akt in human breast cancer cells. Breast Cancer Res 7:R589–R597. https://doi.org/10.1186/bcr1259
26. Li X, Ma H, Li L, Chen Y, Sun X, Dong Z, Liu JY, Zhu W, Zhang JT (2018) Novel synthetic bisindolylmaleimide alkaloids inhibit STAT3 activation by binding to the SH2 domain and suppress breast xenograft tumor growth. Oncogene. https://doi.org/10.1038/s41388-017-0076-0
27. Magi S, Tashiro E, Imoto M (2012) A chemical genomic study identifying diversity in cell migration signaling in cancer cells. Sci Rep 2. https://doi.org/10.1038/srep00823