



Salicylidene acylhydrazides attenuate survival of SH-SY5Y neuroblastoma cells through affecting mitotic regulator Speedy/RINGO and ERK/MAPK–PI3K/AKT signaling

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Abstract

Salicylidene acylhydrazide group synthetic compounds ME0053, ME0055 and ME0192 are known for their iron chelating properties and due to these properties they are primarily used for blocking the bacterial type 3 secretory virulence system. On the other side, targeting the metabolic pathways of iron can provide new tools for cancer prognosis and treatment. Therefore, in this study, considering their iron chelating function, the effects of the compounds ME0053, ME0055 and ME0192 were investigated in SH-SY5Y neuroblastoma cell line. Iron chelating compounds are generally known to be effective in tumor development and metastasis by targeting iron in the cell. They can exert this effect through molecules such as cyclin, CDKs, as well as signaling pathways such as PI3K/AKT and ERK/MAPK. For this reason, we analyzed the effect of the iron chelating compounds of ME0053, ME0055 and ME0192 on cell viability and proliferation rate both through ERK/MAPK and PI3K/AKT signal paths, and through the oncogenic Speedy/RINGO protein that is likely to have a regulatory effect on these two signaling pathways. Apoptosis was also investigated by measuring the amount of active caspase-3, an apoptotic marker. Along with the decrease observed in the Speedy/RINGO level, it was observed that the PI3K/AKT and ERK/MAPK signaling were decreased. This suggests that ME0053, ME0055 and ME0192 compounds significantly decrease the Speedy/RINGO expression which has a regulatory effect on the ERK/MAPK and PI3K/AKT signaling. Besides, analyzing active caspase-3 levels showed that the compounds ME0053, ME0055 and ME0192 increased its level by 218%, 60% and 175% in SH-SY5Y cells, respectively. The results of this study will pave the way for better understanding of the regulation of cancer-related ERK/MAPK and PI3K/AKT pathways and the oncogenic Speedy/RINGO which potentially affects these pathways, through synthetic salicylidene acylhydrazides and their therapeutic use in cancer.

Keywords Salicylidene acylhydrazides · Iron chelation · Neuroblastoma · ME0053 · ME0055 · ME0192 · Speedy/RINGO · PI3K/AKT · ERK/MAPK

Introduction

Salicylidene acylhydrazides (SA) are synthetic molecules used to block the prokaryotic type 3 secretory system (T3SS). These molecules block the formation of a flagella protrusion (injector-like) structure to prevent the entry of effector proteins of Gram-negative bacteria into the host cell. As a result of the researches that started with the suggestions of Slepkin et al. that SA compounds may have gene regulatory functions, it was shown that SA compounds can disrupt cellular iron stores. Furthermore, these compounds have also been shown to be effective on bacterial proliferation [1]. ME0053, ME0055 and ME0192, which are the SA compounds used in this study, were synthesized by Prof. Dr. Mikael Elofsson from Umeå University to be used as

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an alternative to antibiotics by utilizing virulence blocking and anti-proliferative properties of the compounds (Fig. 1). They showed that the iron chelating properties of these compounds had role in fulfilling both functions [2].

On the other hand, deregulation of iron homeostasis in cancer cells compared to normal cells has been widely reported. These altered iron states in malignancies are able to give cancer cells an increased phenotype of iron uptake to mediate their rapid growth which may occur with changes in iron uptake, flow and storage [3–5].

Proteins and signaling pathways in this sensitive equilibrium, which regulate cell growth, differentiation and development, undergo oncogenic changes much more frequently than other molecular groups. Signaling pathways involved in these processes and frequently mutated in cancer are phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) and extracellular signal-regulated kinases/mitogen-activated protein kinase (ERK/MAPK) pathways. In many cancers, including neuroblastoma cancer, which is one of the most common cancers in childhood and progressing very aggressively, the growth and survival of cancer cells are triggered by the abnormal function of these signaling pathways. These pathways play an important role not only in tumor development, but also in the tumor's resistance to cancer treatment

[6, 7]. However, to date, studies on the efficacy and function of these signaling pathways in neuroblastoma cancer have been very limited.

PI3K/AKT signaling is an essential pathway involved in transcription, translation, growth, survival and proliferation, also plays a role in regulating cellular functions. Abnormal activation of the PI3K/AKT pathway has been frequently shown especially in various human cancers. Recent studies have demonstrated that iron chelators target the PI3K/AKT/PTEN pathway in prostate cancer as part of their anti-tumor activities. Treatment of prostate epithelial cells and DU145 prostate cancer cell line with iron chelators DFO or Dp44mT increased expression of the AKT inhibitor PTEN and anti-metastatic protein NDRG1 [8].

In addition, abnormal activation of the ERK/MAPK signaling pathway plays a critical role in cancer development, progression, and resistance to chemotherapy [9]. In particular, the ERK/MAPK pathway activated by growth factors and mitogens is the most closely linked pathway to cancer. Cancer-related lesions leading to continuous activation of the ERK/MAPK pathway include overexpression of receptor tyrosine kinases, activating mutations in receptor tyrosine kinases, continuous autocrine or paracrine production of activating ligands, Ras and Raf mutations. This underlines that deregulation of this pathway in cancer can take place at several levels. As with the PI3K/AKT pathway, iron chelators have been used to inactivate the ERK/MAPK signal pathway and they have been shown to be able to regulate the Ras/Raf/MEK/ERK signaling by reducing ERK1/2 phosphorylation in prostate cancer cells. Given that the ERK/MAPK pathway is activated by a number of mitogenic stimuli, the ability of iron chelators to alleviate the activation of the ERK/MAPK pathway may be an important mechanism for preventing cancer [10, 11].

The usual pattern of growth factors, hormone, and cytokine receptor signal networks shows PI3K/AKT and ERK/MAPK as two independent pathways. However, there are certain cell cycle-related molecules that can act on both pathways simultaneously and also there are multiple intersecting points between these two pathways which together determine the fate of the cell [10, 11].

Considering these interactions, it is possible that PI3K/AKT and ERK/MAPK pathways affect each other both negatively or positively at different stages of signal propagation and besides, they can be affected by other proteins which are not members of these pathways [6]. However, the role of these interactions in cancer, especially in neuroblastoma is not yet known. At this point, data are available that strengthen the probability that one of the contributors of these interactions on PI3K/AKT and ERK/MAPK signaling pathways is the Speedy/RINGO protein, which is an unconventional cell cycle regulator and plays a pivotal role in cancer [12, 13].

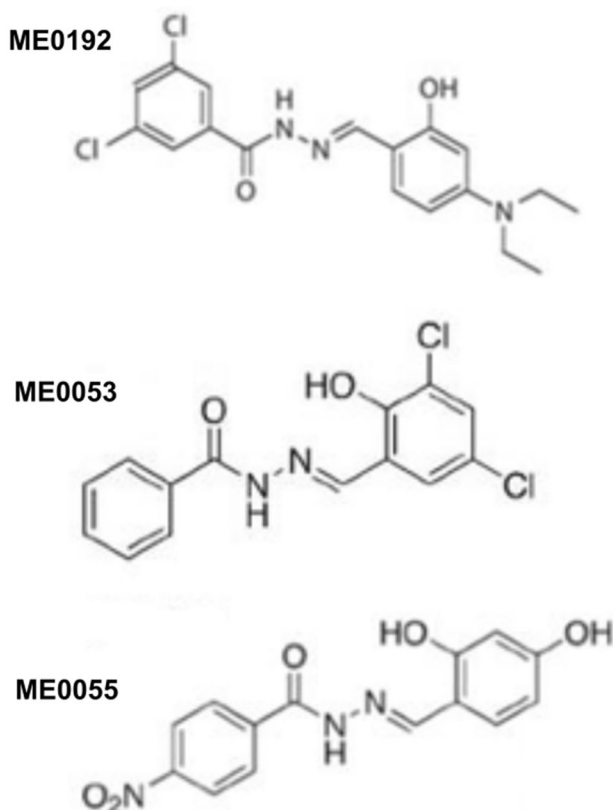


Fig. 1 Structures of ME0053, ME0055 and ME0192

Speedy/RINGO, which is a cell cycle regulatory protein that is different from classical cell cycle regulators in its mechanism of action and has been shown in *Xenopus* oocytes for the first time, controls CDK2 activity and G₁-S phase transition in the progression of the cell cycle [14]. While performing this function, it does not need phosphorylation mechanism like classical cyclins, and it is not too sensitive to inhibition by phosphorylation by inhibitor regulators such as p21Cip1 and p27Kip1. In this way, Speedy/RINGO surpasses many control points in cell cycle, including DNA damage control points, and with this feature, it is known for its oncogenic function in cancer cells by blocking apoptosis and maintaining cell division. As with ERK/MAPK and PI3K/AKT signaling pathways, overexpression of the Speedy/RINGO protein has been shown to play an important role in cancer and resistance to chemotherapy [15].

There are studies showing that Speedy/RINGO may have regulatory effects on the ERK/MAPK and PI3K/AKT signaling pathways and have a very important role in cancer [6, 10, 16]. For this reason, it is also important to examine the effect of iron homeostasis and iron chelators on the expression of Speedy/RINGO, which is seen as a highly potential and suitable molecular target for anti-cancer therapies [15].

In the light of these data, with this study, it is aimed to investigate the effects of salicylidene acylhydrazide compounds (ME0053, ME0055 and ME0192) on the proliferation of SH-SY5Y neuroblastoma cells at the molecular level through the mitotic regulator Speedy/RINGO, which is likely to be one of the remarkable effectors of the ERK/MAPK and PI3K/AKT signaling pathways. In this way, the effect of ME0053, ME0055, and ME0192 compounds on the interaction of oncogenic Speedy/RINGO with ERK/MAPK and PI3K/AKT signaling pathways will be revealed. Furthermore, this study will pave the way for more comprehensive future studies regarding the use of these synthetic salicylidene acylhydrazide compounds with iron chelating properties in combination with anti-cancer therapies.

Materials and methods

SH-SY5Y neuroblastoma cell culture

SH-SY5Y cell line were supplied by Assoc. Prof. Emin Ilker Medine from Ege University Institute of Nuclear Sciences. Within the scope of the study, Dulbecco's Modified Eagle Medium (DMEM) media containing 20% FBS (Sigma Aldrich/Merck), 1% Pen/strep and 2 Mm 1 cc L-Glutamine was used to culture SH-SY5Y cells in a humidified incubator at 37 °C and 5% CO₂. The experiments were carried out with 10 × 10⁵ cells for each of the control, ME0053, ME0055 and ME0192 groups.

Preparation of salicylidene acylhydrazide group compounds

The compounds ME0053, ME0055 and ME0192 were supplied by Prof. Dr. Mikael Elofsson from Umeå University. 10 mM stocks for ME0053 (Ma = 308.01 g/mol), ME0055 (Ma = 301.07 g/mol) and ME0192 (Ma = 379.09 g/mol) were prepared by dissolving them in Dimethyl Sulfoxide (DMSO).

Determination of half-maximal inhibitory concentration (IC50)

For this purpose, MTT (3-(4,5-dimethyl-2-thiazol-2-yl)-2,5-diphenyl-2H tetrazolium bromide) cell proliferation test, a quantitative colorimetric method, was used which determines living cells based on their mitochondrial activity by utilizing color change occurring as a result of interaction of NADH-dependent oxidoreductase enzyme with formazan salts. In this analysis, 10 × 10³ SH-SY5Y cells were plated in 96-wells. 10 mM stocks of ME0053, ME0055 and ME0192 compounds were diluted in DMEM to obtain the treatment doses of 50 μM, 100 μM, 150 μM and 200 μM. Treated- and control groups were incubated for 24 h at 37 °C and 5% CO₂. Then, 20 μl of MTS reagent (Abcam MTS Cell Proliferation Assay Kit) was added and cultures were incubated in an incubator containing 5% CO₂ at 37 °C for 3.5 h. After that, a spectrophotometric analysis was performed by measuring absorbance at 490 nm wavelength using SpectraMax i3. Data were obtained in triplicate by three independent experiments.

Western blotting

In order to analyze the expression of desired proteins upon treatment with ME0053, ME0055 and ME00192 compounds, total protein was isolated from the cells using the Whole Cell Extraction Kit (2910, Millipore). Protein concentrations were measured using the Qubit™ Protein Assay Kit (Q33211, Thermo Fisher Scientific) and the Qubit™3.0 Fluorometer. Samples loaded in equal amounts (100 μg protein/well) were separated by SDS-PAGE (12.5%) and then transferred to the nitrocellulose membrane (sc-3724, Santa Cruz Biotechnology) using the Trans-Blot Turbo Transfer System (Bio-Rad) (Tables 1 and 2). After transfer, membranes were blocked in 5% BSA/Tris Buffered Saline (TBS) for 1 h at room temperature. At the end of this period, the membranes were incubated overnight at +4 °C with primary antibodies diluted in 5% BSA/TBS. The following primary antibodies were used at 1:1000 dilutions; MEK1/2 mouse monoclonal antibody (sc-81504, Santa Cruz Biotechnology), p-Akt 1/2/3 mouse monoclonal antibody (sc-514032, Santa Cruz

Table 1 Protein concentration results

Experimental conditions	Protein concentration ($\mu\text{g/ml}$)
SH-SY5Y control	17,680
SH-SY5Y treated with ME0055	12,400
SH-SY5Y treated with ME0192	10,480
SH-SY5Y treated with ME0053	12,550

Table 2 Amount of protein to be loaded for SDS-PAGE

Experimental conditions	Protein amount (μl)	1x NEBX3 (μl)
Control SH-SY5Y	6	19
SH-SY5Y treated with ME0055	9	16
SH-SY5Y treated with ME0192	10	15
SH-SY5Y treated with ME0053	9	16

Biotechnology), p-Akt 1/2/3 mouse monoclonal antibody (sc-271966, Santa Cruz Biotechnology), Calnexin mouse monoclonal antibody (sc-80645, Santa Cruz Biotechnology) and GAPDH mouse monoclonal antibody (sc-47724, Santa Cruz Biotechnology) and at 1: 500 dilutions; SPDYA rabbit polyclonal antibody (PA1-16959, Thermo Fisher Scientific) and anti-caspase-3 rabbit recombinant antibody (ab32351, Abcam). After incubation with primary antibodies, each membrane was washed 5 times with 1X Tris Buffered Saline Tween (TBS-T) for 6 min at room temperature. Subsequently, they were incubated with 1:1000 diluted HRP-conjugated secondary antibodies (m-IgGK BP-HRP anti-mouse secondary antibody (sc-516102, Santa Cruz Biotechnology) and Mouse anti-rabbit IgG / HRP secondary antibody (bs-0295 M-HRP, Bioss Antibodies)) in 5% BSA/TBS for 1 h at room temperature. Blots were visualized using the Clarity Western ECL Substrate Kit (170-5061, BioRad). Blots were scanned and the densities of the specific bands were quantified and normalized with calnexin and GAPDH as internal loading control using ChemiDoc™ Imaging Systems (BioRad) and Lab 4.0 Software.

Integrative pixel analysis

Photoshop CS6 Software was used to analyze the relative density of protein bands in Western blot images. The relative expression value was normalized to “1” in the control cells for each protein analyzed, and then relative protein expression fold changes of the experimental groups were calculated.

Statistical analysis

Statistical Package for Social Sciences (SPSS) program was used and the results were statistically analyzed according to paired-2 tailed student's *t*-test. A *p*-value of less than 0.05 was considered significant and a *p*-value of less than 0.001 was considered extremely significant for all statistical analyses. For Western blot and MTS cell viability analysis, error bars in the graphs were generated using \pm s.d.

Results

ME0053, ME0055 and ME0192 salicylidene acylhydrazide group synthetics have cytotoxic effects on SH-SY5Y cells

MTS analysis was performed to observe the effect of ME0053, ME0055 and ME0192 treatments on the viability of SH-SY5Y neuroblastoma cells and to obtain appropriate IC50 doses for further experiments. Results showed that all treatments significantly reduced the viability of SH-SY5Y cells (Fig. 2a–c; $p < 0.001$).

When the cytotoxic effects of the compounds were examined for determining appropriate IC50 concentration, 52.35% viability rate of 50 μM of the ME0055 compound (Fig. 2a; $p = 0.0002$), 47.62% viability rate of 50 μM of the ME0053 compound (Fig. 2b; $p = 0.0000008$) and 48.85% viability rate of 50 μM of the ME0192 compound (Fig. 2c; $p = 0.000008$) were obtained and thereby, 50 μM concentration were used for further studies for all three compounds.

ME0053, ME0055 and ME0192 compounds significantly reduced Speedy/RINGO protein expression

Iron chelation is known to significantly reduce expression levels of cell cycle proteins. Considering that Speedy/RINGO is a cell cycle protein and also considering the effect of this protein on ERK/MAPK and PI3K/AKT signaling pathways, the effect of ME0053, ME0055 and ME0192 compounds with known iron chelating properties on Speedy/RINGO expression was investigated for the first time. Results of western blotting showed that ME0053, ME0055 and ME0192 compounds caused 87%, 52% and 82% decrease in Speedy/RINGO protein expression, respectively (Fig. 3; $p < 0.001$).

AKT (Thr 308) phosphorylation decreased after treatment with compounds ME0053, ME0055 and ME0192

In this study, it is aimed to observe whether the decrease observed in Speedy/RINGO protein level upon application

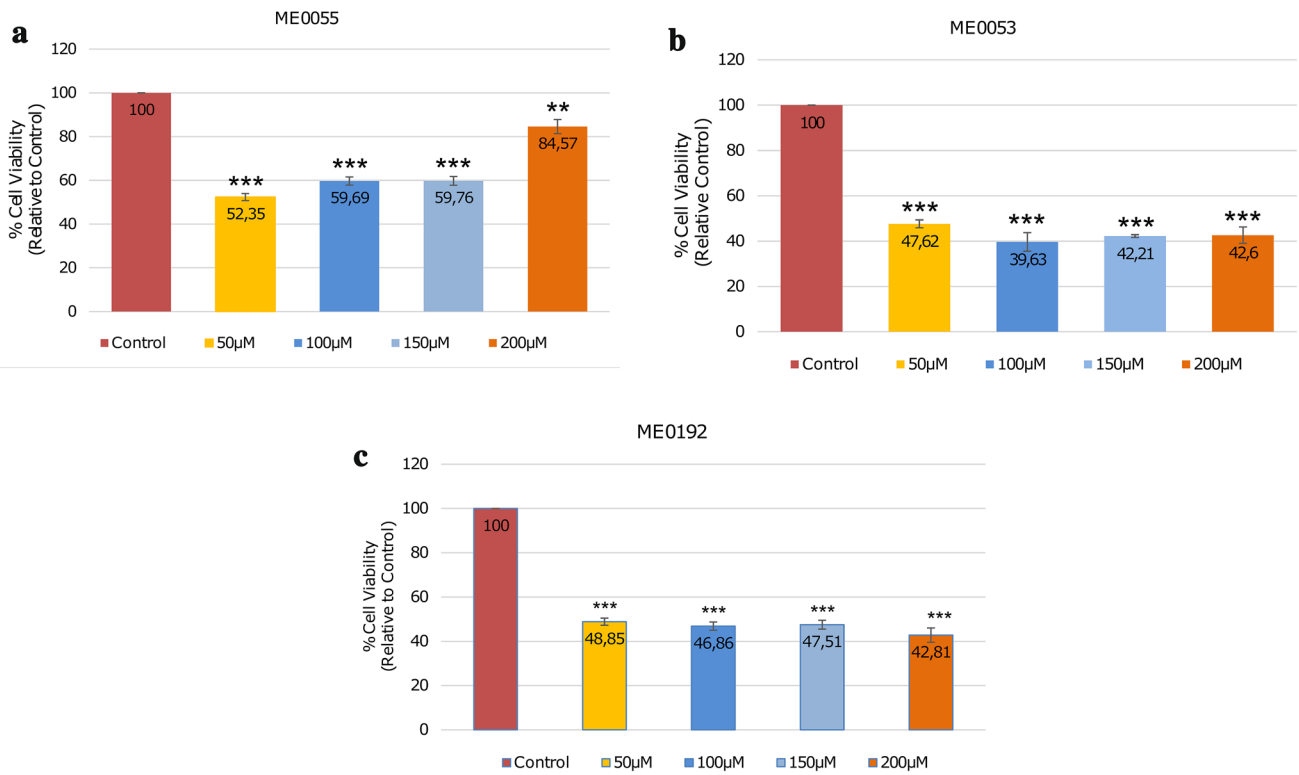
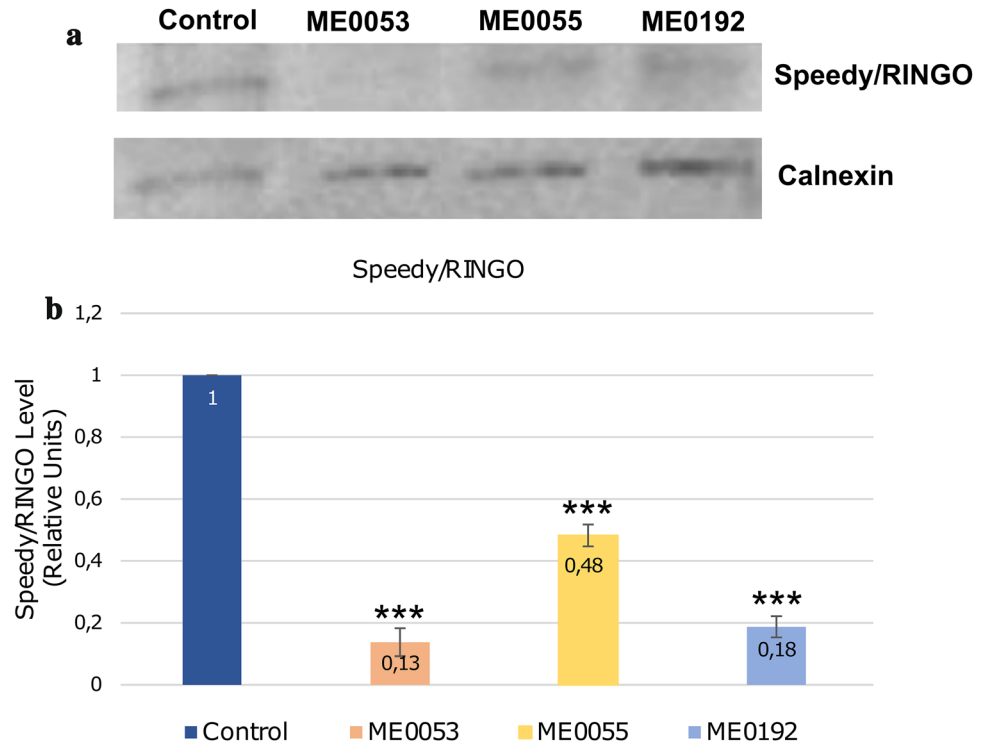


Fig. 2 a Cytotoxic effect of ME0055 compound on SH-SY5Y neuroblastoma cell line; b Cytotoxic effect of ME0053 compound on SH-SY5Y neuroblastoma cell line; c Cytotoxic effect of ME0192 compound on SH-SY5Y neuroblastoma cell line

Fig. 3 Western blotting result for Speedy/RINGO expression. a Speedy/RINGO protein level in SHSY5Y neuroblastoma cells treated with ME0053, ME0055 and ME0192. b Graph shows quantitative results for Speedy/RINGO expression. Results were presented as fold change. Error bars represent the mean value ± SD. ****p* < 0.001



of ME0053, ME0055 and ME0192 has an effect on the activity of PI3K/AKT pathway. Firstly, after treatment with ME0053, ME0055 and ME0192 compounds, Thr308 phosphorylation level, which is the first of the two phosphorylations necessary for the AKT protein to reach the fully active form in the PI3K/AKT pathway, was examined. The results showed that applied compounds significantly reduced phosphorylation compared to the control group. The compounds ME0053, ME0055 and ME0192 provided 79%, 66% and 93% decrease in Thr308 phosphorylation of AKT, respectively (Fig. 4a, b; $p < 0.001$).

ME0053, ME0055 and ME0192 treatments have variable effects on AKT (Ser473) phosphorylation

The second phosphorylation site required for the transition of the AKT protein to the fully active form is Ser473. Examining the phosphorylation amount on this site showed that there was varying amounts of phosphorylation changes depending on the compound used in SH-SY5Y cells compared to the control (Fig. 4c, d). The compounds ME0053 and ME0192 caused a significant reduction of 39% and 90% on Ser473 phosphorylation, respectively ($p < 0.001$), while

the compound ME0055 caused a significant increase of 20% compared to control ($p < 0.05$).

The effects of the compounds ME0053, ME0055 and ME0192 on MEK1/2 protein expression are variable

In order to analyze how the ERK/MAPK signal was affected in the cells after the ME0053, ME0055 and ME0192 treatments, the amount of MEK1/2 protein expression was examined. The data obtained showed that the compounds ME0053 and ME0055 reduced the MEK1/2 level by 26% and 13% respectively, and that ME0192 caused an increase of 3%, indicating a varying effect according to the compound used (Fig. 5a, b).

ME0053, ME0055 and ME0192 compounds increased the level of Active Caspase-3 to varying extents

In this experiment, considering the data obtained about the effects of ME0053, ME0055 and ME0192 on PI3K/AKT and ERK/MAPK signal pathways and mitotic Speedy/RINGO protein in SH-SY5Y neuroblastoma cells, caspase-3 activity was investigated to find out the apoptotic state of the

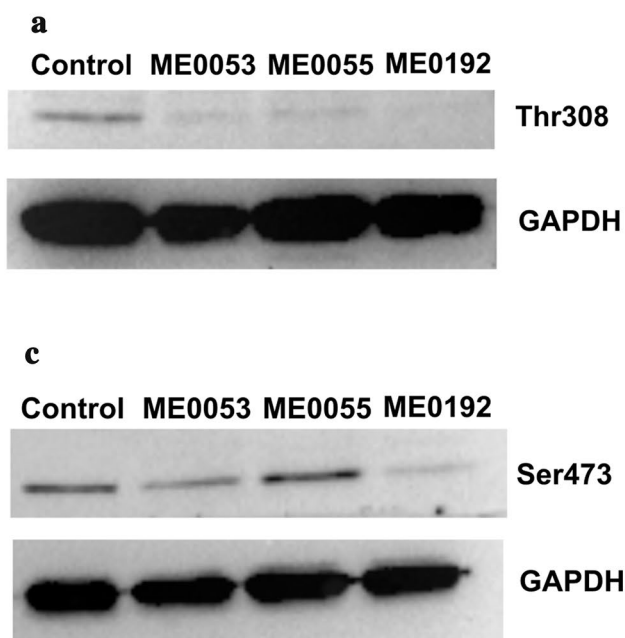
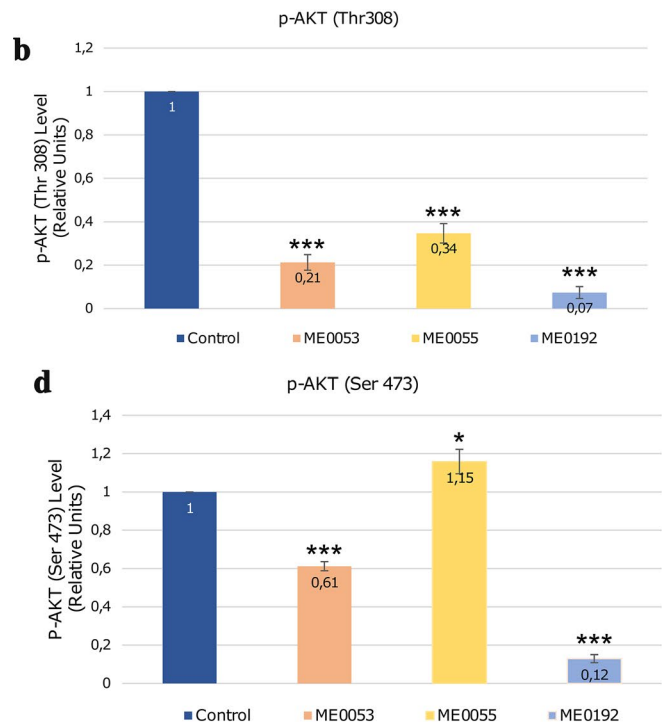


Fig. 4 Western blotting result for Akt (Thr308); **a** Graph shows quantitative results for Akt (Thr308) phosphorylation levels. Results were presented as fold change. Error bars represent the mean value \pm SD. *** $p < 0.001$. **b** p-Akt Thr 308 protein level in SH-SY5Y neuroblastoma cells treated with ME0053, ME0055 and ME0192. Western



blotting result for Akt (Ser473); **c** Graph shows quantitative results for Akt (Ser473) phosphorylation levels. Results were presented as fold change. Error bars represent the mean value \pm SD. *** $p < 0.001$. **d** p-Akt Ser473 protein level in SH-SY5Y neuroblastoma cells treated with ME0053, ME0055 and ME0192

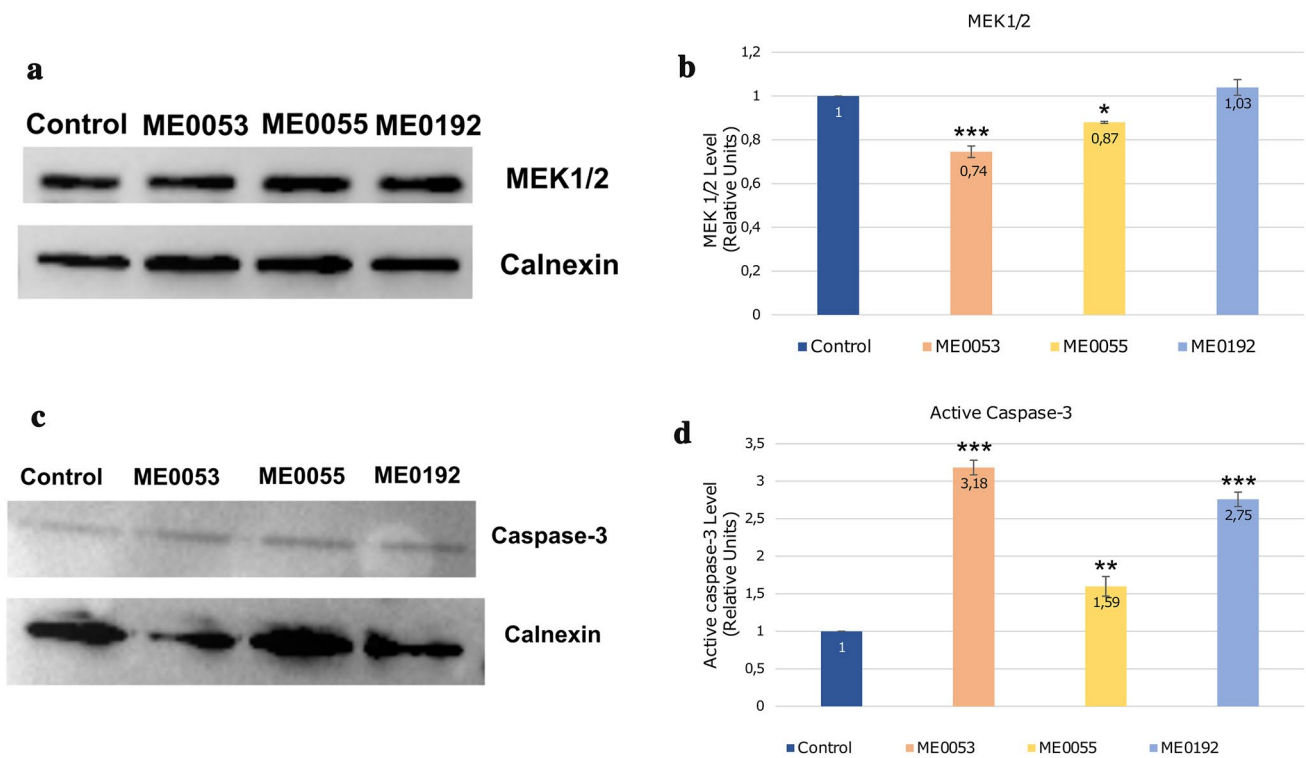


Fig. 5 Western blotting result for MEK1/2; **a** Graph shows quantitative results for MEK1/2 expression. Results were presented as fold change. Error bars represent the mean value \pm SD. *** p < 0.001. **b** MEK1/2 protein level in SHSY5Y neuroblastoma cells treated with ME0053, ME0055 and ME0192. Western blotting result for cas-

pase-3 activity; **c** Graph shows quantitative results for active caspase-3 levels. Results were presented as fold change. Error bars represent the mean value \pm SD. *** p < 0.001. **d** Active Caspase-3 level in SH-SY5Y neuroblastoma cells treated with ME0053, ME0055 and ME0192

cells. As a result, the amount of active caspase-3 showed a significant increase of 218%, 60% and 175% in the cells treated with the ME0053, ME0055 and ME0192, respectively (p < 0.001), indicating that the cells may have entered the apoptotic pathway (Fig. 5c, d).

Discussion

Salicylidene acylhydrazide group synthetic compounds ME0053, ME0055 and ME0192 are known for their iron chelating functions mostly on bacterial T3SS virulence system. However, iron chelating compounds are also known to be effective in tumor development and metastasis by targeting iron in the cell. They can fulfill this function through mitotic molecules such as cyclin, CDKs, as well as mitotic signal pathways such as PI3K/AKT and ERK/MAPK. Therefore, we analyzed the effect of the iron chelating compounds ME0053, ME0055 and ME0192 on cell viability and apoptosis through ERK/MAPK and PI3K/AKT signaling pathways, and through the unconventional mitotic protein Speedy/RINGO which is likely to have a regulatory effect on these two signal pathways.

With this study, it is demonstrated for the first time that ME0053, ME0055 and ME0192 salicylidene acylhydrazide compounds caused a significant decrease in the level of oncogenic Speedy/RINGO protein (87% for ME0055, 52% for ME0053, 82% for ME0192). Considering the regulatory effect of Speedy/RINGO on PI3K/AKT and ERK/MAPK signal pathways [17, 18], it was estimated that this decrease in Speedy/RINGO amount may also affect the activity of PI3K/AKT signal pathway. For this reason, AKT phosphorylation level was measured upon treatment with each compound. It is known that in order for the PI3K/AKT signal pathway to be activated, the AKT protein have to be sequentially phosphorylated through two different sites, Thr308 and Ser473 [6, 19]. Firstly, AKT protein cis transformed to semi-active form by phosphorylation on Thr308, and then a sequential phosphorylation on Ser473 forms fully active AKT protein. Compounds with iron chelating properties are known to generally have inhibitory functions in AKT phosphorylation mechanism [20]. In this study, the compounds ME0053 and ME0192 with iron chelating properties showed a significant reducing effect on the phosphorylation and thus the activity of the AKT on both phosphorylation sites (Thr308 and Ser473). However, the ME0055 compound caused a 66% reduction on Thr308 phosphorylation

while causing a 15% increase on Ser473 phosphorylation. Although ME0055 caused an increase in Ser473 phosphorylation, this increase does not make sense and the Akt protein does not gain activity since ME0055 significantly decreased Thr308 phosphorylation which is the first phosphorylation step mandatory for AKT to become fully active. Therefore, it is possible to say that all three compounds provide an effective inhibition on the PI3K/AKT pathway through decreasing AKT phosphorylation. In order to better understand the molecular mechanism of the effects of these compounds on the PI3K/AKT pathway, the expression levels of the proteins that have regulatory effect on this signal pathway should be investigated in case these compounds may indirectly act on the PI3K/AKT through these proteins. The decrease observed in the expression level of the Speedy/RINGO, one of these regulatory proteins, suggests that this may be the reason for the decrease in AKT phosphorylation. However, since iron chelators such as DFO, which are used in cancer treatment today, are effective on many points in cell signaling pathways [21, 22], it is very difficult to clearly demonstrate the effect of iron chelators by current technologies. Therefore, we have a long way to clearly reveal the intracellular targets of the compounds used in this study. However, given that this study is the first in which these compounds were used in neuroblastoma cancer cells, it can obviously be seen that valuable clues have been reached about the effect of the compounds on two mitogenic signal pathways, as well as on the oncogenic protein Speedy/RINGO.

Studies on the ERK/MAPK signaling utilizing frequently used iron chelators have shown that iron chelators reduce ERK1/2 phosphorylation, which is one of the key elements of this signaling pathway [23, 24]. In this study, upon treatment with ME0053, MEK1/2 expression, one of the key members of this signaling pathway, decreased significantly (26%) ($p < 0.0001$), while ME0055 caused a 19% decrease ($p = 0.02$) in MEK1/2 expression. Conversely, ME0192 treatment resulted in an insignificant 3% increase in MEK1/2 protein expression ($p > 0.05$, not significant). Since the multiple interactions and multiple effects of iron chelating compounds in the cell are known, [25, 26] and the compounds used in the study were primarily studied in bacterial cells [27, 28] but not frequently studied in eukaryotic cells, the mechanisms of action in eukaryotic cells, moreover, in cancerous cells with abnormal cellular behavior are not well known. In addition, the results obtained with MEK1/2 were not surprising, considering that frequently used iron chelators may give results contrary to what is expected in different cancer tissues or under stress conditions [29, 30]. Besides, even though the ME0192 compound showed an increasing effect on the MEK1/2 expression, this result is not statistically significant and it still reduced the viability of the cells with its inhibition effect on the PI3K/AKT signal pathway and caused an increase in caspase-3 activity, which is an

apoptotic marker. This result reveals the degree of interaction of proteins and signaling pathways associated with the growth and division of the cell and, especially the versatility of cancer cells.

Lastly, active caspase-3 quantification was performed to understand how the changes made by ME0053, ME0055 and ME0192 compounds on the cell's mitogenic and survival signaling pathways, as well as on the anti-apoptotic Speedy/RINGO protein, affect the apoptotic states of the cells. ME0053, ME0055 and ME0192 caused an increase in the amount of active caspase-3 by 218%, 60% and 175%, respectively, a strong finding indicating that SH-SY5H neuroblastoma cells may have entered the apoptotic pathway.

The general purpose of cancer treatments targeting iron metabolism is to control the proliferation rate of the cells in a controlled manner and prevent metastasis. Therefore, it is very important to examine the effect of ME0053, ME0055 and ME0192 compounds with iron chelating properties on proliferation and vital activities of neuroblastoma cells especially through mitotic signaling pathways and oncogenic Speedy/RINGO protein.

Conclusion

With this study, it is presented for the first time that salicylidene acylhydrazide group synthetic compounds ME0053, ME0055 and ME0192 have anti-proliferative effects in SH-SY5Y neuroblastoma cells by analyzing the effects of these compounds on ERK/MAPK, PI3K/AKT activities and Speedy/RINGO expression. This study is also important because it is the first time that compounds with iron chelating properties are shown to be effective on Speedy/RINGO protein expression, which is a critical mitotic regulator and frequently overexpressed in many cancers.

This study will shed light for further studies that will allow better understanding of the mechanisms underlying the development of various types of cancer, thereby allowing choosing the right targets in diagnosis and treatment. In the event that such studies are advanced and versatile in vitro and in vivo analyzes are carried out, the use of iron chelating salicylidene acylhydrazide-based preparations for the purpose of supporting the cancer treatment will be made more widespread.

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SH-SY5Y neuroblastoma cell line and to Prof. Dr. Mikael Elofsson from Umeå University for providing all the salicylidene acylhydrazide compounds used in this study.

Compliance with ethical standards

Conflict of interest We certify that all of our affiliations with or without financial involvement, within the past 5 years and foreseeable future and, any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript are completely disclosed (e.g., employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, and royalties). “No conflicts of interest”.

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