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Cytotoxic and Apoptogenic Properties of 2-Phenylthiazole-4-Carboxamide Derivatives in Human Carcinoma Cell Lines

Hanifeh Nazari Tarhan[a], Leila Hosseinzadeh[b]*, Alireza Aliabadi[c], Babak Gholamine[b], Alireza Foroumadi[d]

[a]Students Research Committee, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran.
[b]Department of Toxicology and Pharmacology Medical services, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran.
[c]Department of Medicinal Chemistry, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran.
[d]Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran

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Apoptosis, or programmed cell death, is an essential physiological process that plays a critical role in development and tissue homeostasis. Caspases, a family of cysteine-dependent aspartate-directed proteases, play a critical role in the initiation and execution of apoptosis. In this study, cytotoxicity and apoptogenic effect of 2-phenyl 4-carboxamide derivatives were evaluated in SKNMC (human neuroblastoma), MCF-7 (human breast adenocarcinoma) and HT-29 (human colon cancer) cell lines. Cell viability was determined by MTT assay. Also, activation of caspase-3 was evaluated by spectrophotometry. The overall cytotoxicity profiles of derivatives demonstrated that the HT-29 cell line has more sensitivity respect to other cell lines. Moreover, our observations indicated that 3-F and 2-F derivatives and 4-Cl derivative increased caspase-3 activation in three carcinoma cell line compared to control. Collectively, these findings suggest that these derivatives are able to induce apoptosis in cancer cell lines.

***Corresponding author: Leila Hosseinzadeh**, **E-mail:** lhoseinzadeh@kums.ac.ir

Introduction

Cancer is one of the most studied worldwide, the killer disease that touches every region and socioeconomic level. The induction of apoptosis in tumor cells is considered very useful in the management and therapy as well as in the prevention of cancer [1-4]. Hence, while the primary goal of chemotherapy is to kill cancer cells, a secondary goal is to have those cells die by apoptosis [5]. So, that they may be cleared quickly and "quietly" by neighboring phagocytic cells.

2-phenyloxazole-4-carboxamide derivatives synthesized by Vincent in bioorganic and medicinal chemistry letters [6]. Study of derivatives ′s biological effects showed that they degenerated poly-ADP-Ribose polymerase enzyme by induction of caspases enzymes. Poly-ADP- Ribose polymerase enzyme plays important role in repair process of genetical information and structure of DNA. In the previous study, a series of substituted 2-phenylthiazole-4-carboxamide derivatives were synthesized as potential cytotoxic agents and evaluated against some human cancer cell lines (**Figure 1**) [7]. The aim of this study was to learn about the events associated with the anti-proliferative actions of 2-phenylthiazole-4-carboxamide derivatives in three human cancer cell lines consist of HT-29 (human colon cancer), MCF-7 (human breast adenocarcinoma), SKNMC (human neuroblastoma). Hence, we explored the role of apoptosis in 2-phenylthiazole 4-carboxamide derivatives induced cytotoxicity in human cancer cell lines.

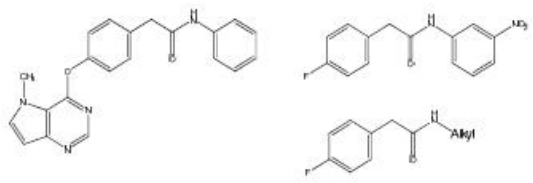


Fig. 1. Structures of some phenylacetamide derivatives with anticancer properties.

Materials and Methods

Materials

3-F and 2-F, 4-Cl and 2-Cl, 4-Br 2-Phenylthiazole-4-carboxamide derivatives, 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazoli- umbromide (MTT), BIO-RAD protein assay kit and Caspase-3 colorometric detection kit were obtained from Sigma Aldrich (St Louis, MO, USA). Cell culture medium, Trypsin, penicillin–streptomycin, and fetal bovine serum (FBS) were obtained from Gibco (Gibco, Grand Island, NY, USA).

Cell Culture conditions

HT-29, SKMNC and MCF-7 were obtained from Pasteur Institute (Tehran, Iran) and maintained at 37 º C in a humidified atmosphere (90%) containing 5% CO_2. Cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) with 5% (v/v) fetal bovine serum, $100Uml^{-1}$ penicillin and 100 $mgml^{-1}$ streptomycin. Cells were seeded overnight and then incubated with various concentrations of different extracts in 95% CO_2 humified incubator. The medium was changed 2–3 days and subcultured when the cell population density reached to 70–80% confluence. Cells were seeded at an appropriate density according to each experimental design [8].

Viability assay

The cytotoxic effects of derivatives were determined against cell lines by a colorimetric assay using 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) and compared with the untreated control [9]. Cells were plated onto 96-well plates at a density of 2.0×10^4 cells/well and in a volume of 200 µl. Stock solutions of of 3-F,2-F,4-Cl and 2-Cl, 4-Br 2-Phenylthiazole-4-carboxamide derivatives were prepared in dimethyl sulfoxide (DMSO). The final concentration of the vehicle in the medium was always 0.5%. One day after seeding, 2 µl of the DMSO containing of 3-F and 2-F,4-Cl and 2-Cl ,4-Br 2-Phenylthiazole-4-carboxamide derivatives at different concentrations were added to each well. At appropriate time intervals, the medium was removed and replaced by 100 µl of 0.5 mg/ml of MTT in growth medium and then the plates transferred to a 37 °C incubator for 3-4 hr. Supernatants were removed and the reduced MTT dye was solubilized with DMSO (100 µl /well). Absorbance was determined on an ELISA plate reader (Biotek, H1M.) with a test wavelength of 570 nm and a reference wavelength of 630 nm to obtain sample signal (OD570–OD630). Percentage of proliferation was calculated using the following formula:

Percent of control proliferation= (OD test/ODcontrol) ×100

IC_{50} values were calculated by plotting the log10 of the percentage of proliferation versus drug concentration.

Detection of caspase-3 activation

Apoptosis can be assessed by measuring the caspase activity in thetreated cells [10].The activity of caspase-3 was determined by the sigma colorimetric caspase-3 kit according the treated tomanufactur's instrument. This assay is based on spectrophotometric detection of the chromo-phore p-nitroanilide (pNA) after cleavage from the la-beled substrate DEVD-pNA in equal amount of cells pro-tein lysate. Briefly, 1×106 cells were collected and lysed with 50 µL of chilled lysis buffer and incubated on ice for 10 min. Cell lysates were centrifuged at maximum speed for 5 min at 4°C, after which 50 µL of 2× reaction buf-fer/DTT mix and 5 µL of 1 mM caspase-3 substrate (DEVD-pNA) were added to each reaction and incubated at 37°C for 1 h. The pNA light emission was quantified using a microplate reader at 400 or 405-nm. Comparison of the absorbance of pNA from an apoptotic sample with an un-induced control allowed determination of the fold increase in caspase-3 activity.

Protein assay

The concentration of protein was measured by BIO-RAD protein assay kit following manufacture procedure. Briefly, several dilution of protein standard (1-25 µg) was prepared, then 0.8 mL of standard and appropriately diluted samples were placed in clean dry test tubes. Next, 0.2 mL Dye Reagent Concentrate was added to tubes and mixed several times by gentle inversion of them. The absorbance was measured at 590 nm versus reagent blank after a period of from 5 min to one hour. Finally the absorbance against concentration of standards was plotted.

Statistical analysis

Each experiment was performed at least three times, and the results were presented as mean ± S.E.M. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare the differences between means. A probability value of $p < 0.05$ was considered to be statistically significant.

Results and discussion

Inhibition of Cell Viability

The cytotoxicity of 3-F and 2-F,4-Cl and 2-Cl, 4-Br 2-Phenylthiazole-4-carboxamide derivati- ves were examined on malignant cell lines. First, malignant cells were incubated with various concentrations of 3,2-F,3,2-Cl and 4-Br 2-Phenylthiazole-4-carboxamide (2.5-40 µM) for 24 h. The result showed this derivatives decreased cell viability of cells in a concentration-dependent manner. The toxicity started at a concentration as little as 30 μm and the dose inducing 50% cell growth inhibition (IC_{50}) against SKNMC, HT-29, MCF-7 was calculated (**Figure2, 3 and 4**).

3- F derivative. Our findings about the 3-F derivatives (2.5–40 µM) also showed anticancer effects on these cell lines. The IC_{50} of the 3-F derivatives against SKNMC, MCF-7, HT-29 was 17, 16, 1.75 (µM) for 24 h, respectively.

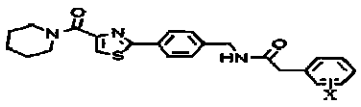
2-F derivative. The IC_{50} values of this dreivates against SKNMC, MCF-7,HT-29 cell lines after 24 h were determined, 5, 4.5, 4.25 µM, respectively 3-F and 2 –F derivatives were most sensitive derivatives against 3 cell lines.

4-Cl *derivative*. In order to ,for SKNMC, MCF-7,HT-29 cell lines, The growth inhibitory concentrations of this analoge was 21, 16, >>>1000, 2.75 µM for 24 h respectively. This derivatives showed good cytotoxic activity against HT-29 cells.

2-Cl *derivative*. The IC_{50} values of this dreivate against SKNMC, MCF-7,HT-29 cell lines after 24 h were determined, 24, 28, >>>1000 µM respectively. 4-Cl and 2-Cl analogs showed good cytotoxic activity profile against SKNMC cell line with IC_{50} values less than 25 µM.

Br *derivative*. These derivatives could not significantly improve the cytotoxicity in comparison with other derivative, but showed good cytotoxicity profile against HT-29 cell line. The IC_{50} values of this dreivate against SKNMC, MCF-7, HT-29 cell lines after 24 h were determined >>>1000, >>>1000, 1.75 µM, respectively. The overall activity profiles of derivatives demonstrated that the HT-29 cell line has more sensitivity respect to other cell lines.

Table 1. Cytotoxicity activity (IC_{50} , µM) of 2-phenyl 4-carboxamide derivates against HT-29 (colon cancer), MCF-7 (breast cancer), SKNMC (neuroblastoma) cell lines.



X: F, Cl, Br

x	SKNMC	MCF-7	HT-29
3-F	17	16	1.75
4-Br	>>>500	>>>500	1.75
4-Cl	21	>>>500	2.75
2-Cl	24	28	>>>500
2-F	5	4.5	4.25
Doxorubicin	8.13	4.76	5.25

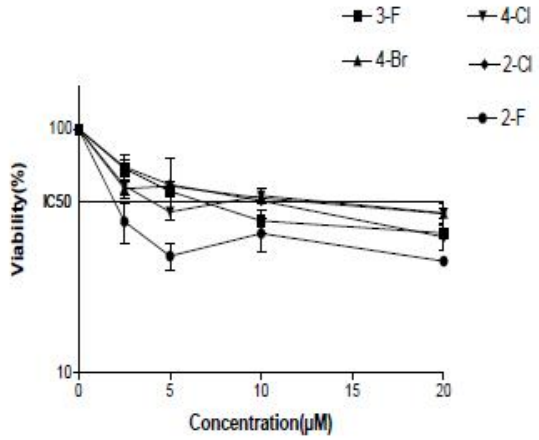


Fig. 2. Cell viability of SKNMC cells after exposure to 3-F and 2-F,4-Cl and 2-Cl, 4-Br 2-Phenylthiazole-4-carboxamide derivatives. Cells were treated with different concentrations of derivatives (2.5-20 µM) for 24 hr. The cell viability was determined by MTT assay. Data are expressed as the mean± SEM of three separate experiments (N= 6).

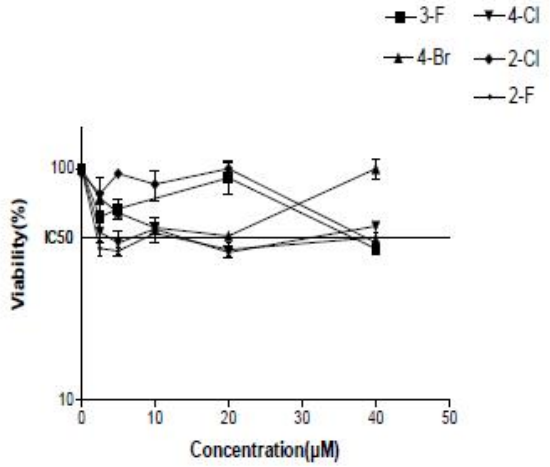


Fig. 3. Cell viability of HT-29 cells after exposure to 3-F and 2-F,4-Cl and 2-Cl, 4-Br 2-Phenylthiazole-4-carboxamide derivatives. Cells were treated with different concentrations of derivatives (2.5-40 µM) for 24 hr. The cell viability was determined by MTT assay. Data are expressed as the mean±SEM of three separate experiments (N= 6).

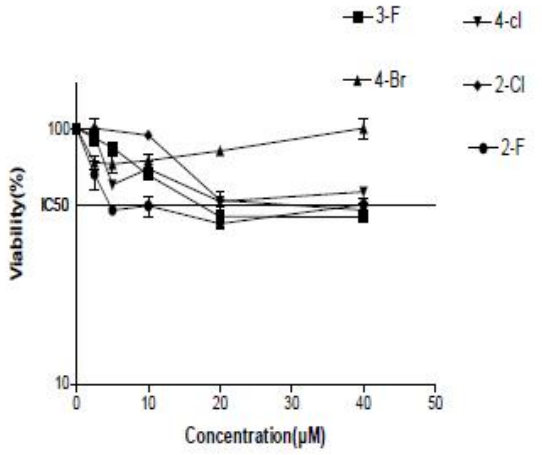


Fig. 4. Cell viability of MCF-7 cells after exposure to 3-F and 2-F,4-Cl and 2-Cl, 4-Br 2-Phenylthiazole-4-carboxamide derivatives. Cells were treated with different concentrations of derivatives (2.5-40 µM) for 24 hr. The cell viability was determined by MTT assay. Data are expressed as the mean±SEM of three separate experiments (N= 6).

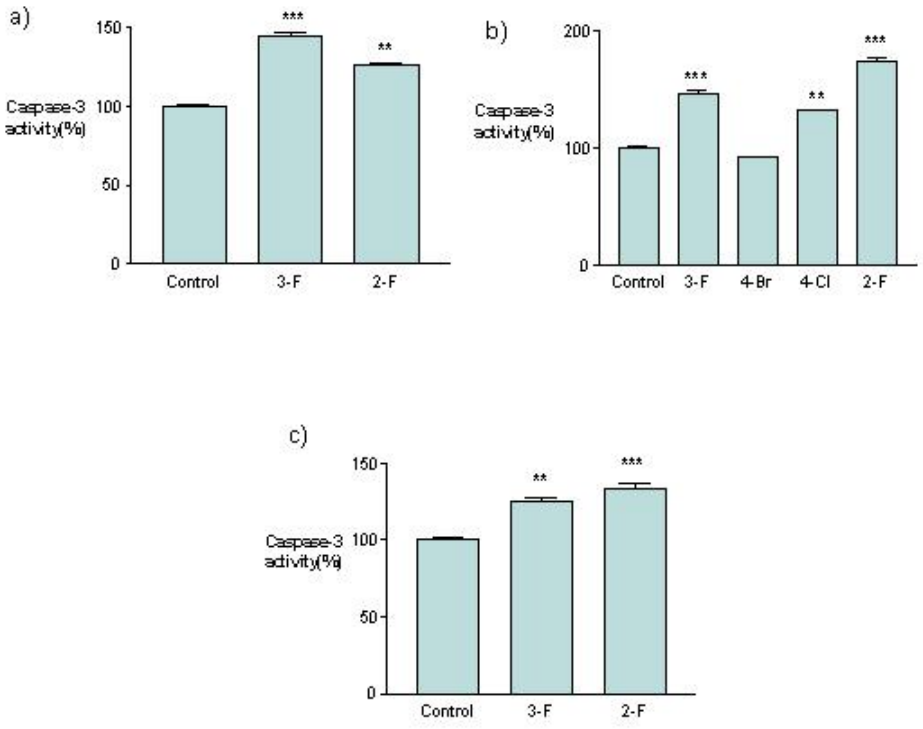


Fig. 5. The effect of 3,2- F derivatives on caspase-3 activity in (a: SKNMC cells, b: HT-29, c: MCF-7) Cells pretreated with different concentrations of 3,2-F derivatives. Caspase-3 activity measured by colorimetric detection of p-nitroanilide and expressed as percentage of control. Data are expressed as the mean±SEM of three separate experiments .**p<0.01, ***p<0.001.

Effect of 2-Phenylthiazole-4-carboxamide derivates on caspase-3 activation:

Caspase, a family of cysteine-dependent aspartate-directed proteases, play a critical role in the initiation and execution of apoptosis. Among this family of caspases, caspase 3, in particular, is believed to be one of the most commonly involved caspases in the execution of apoptosis in various cell types [11]. Therefore, we assayed caspase-3 activation for charact- erizing the nature of cell death that occurred in response to the pretreatment with 2-phenylthiazole-4-carboxamide Derivatives.Our result showed that 3-F and 2-F derivatives increased caspase-3 activation in three carcinoma cell line compare to control. 4-Cl derivate increased enzyme activity only in HT-29 cell line (**Figure 5**). Collectively, these findings suggest that these derivatives are able to induce apoptosis in cancer cell lines through activation of caspase-3.

Conflict of interest

Authors certify that no actual or potential conflict of interest in relation to this article exists.

Acknowledgment

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