Original Article

The effect of thiosemicarbazone compounds on MCF-7 breast cancer cells

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ABSTRACT

Background : Todays, using the various derivatives of thiosemicarbazone is increasing for cancer treatment. Due to the increasing rate of cancer, and in particular breast cancer in developing countries, the use of nanoparticles such as iron oxide magnetic nanoparticles to enhance the effect, as well as the targeted effect of various anticancer compounds such as thiosemicarbazone is of particular importance. **Methods:** In this study, the anticancer activity of thiosemicarbazone pyrazole derivative alone (compound P) and in conjunction with iron oxide nanoparticles (compound PL) was investigated on MCF-7 cell line. Following MTT assay to evaluate the toxicity of the P and PL compounds on MCF-7 cells, the RT-qPCR reaction was performed to measure the expression of BAK-1, EIF4E and hTERT genes in 24-hr treatment of MCF-7 cells with the above mentioned compounds. **Finding:** The results showed more strong anticancer activity of the PL compound on MCF-7 cells compared to the P compound. The IC_{50} values were obtained 15.780 and 13.184 μg/well for the P and PL compounds, respectively. Also, the effect of PL compound on the expression of targeted genes was Meaningfully more than P compound. **Conclusion:** Due to the high penetration capability of iron oxide nanoparticles, as well as the high toxicity of these compounds, the synthesis of magnetic P nanoparticles is used in the purposeful transfer of these compounds, in addition to increasing the toxicity of these compounds on cancer cells.

Keywords: Breast Cancer, Pyrazole, Iron oxide nanoparticles, Carbazone, Gene expression

Introduction

Cancer is an important cause of mortality. As the most common type of women's cancer, breast cancer accounts for %23 of all cancer categories. Since the onset of cancer age is much lower in developing than developed countries, implementation of cancer controlling strategies and actions is felt necessary [1]. As promising scaffolds for medicinal chemistry, Pyrazole derivatives are key compounds used in cancer therapy with a variety of biological effects including anti-inflammatory, antimicrobial and anticancer properties that have made them widely known as anticancer agents [2, 3]. They usually function through being attached to ATP binding sites in such kinases as Aurora Kinase and deactivating cyclin-dependent kinase (CDKs) [4, 5]. Auroraa Kinase inhibition in cancer cells is crucial for elevating cellular apoptosis [6]. As a vital nonsurgical cancer treatment, apoptosis is highly targeted in all varieties of cancers meaning that it is not type-specific [7-9]. Considering the central role of protein kinase in cell signaling and its impact on malignant lesions, it has been attempted to develop specific protein kinase inhibitors to be used in the treatment of various cancers [10]. The significant role of diverse pyrazole forms, e.g. pyrazole-4-yl-urea, has been confirmed in the inhibition of Aurora-A and Aurora-B that ultimately suppresses cancer cell growth and survival [11]. The

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insensitivity of tumor cells to chemotherapical medications necessitates the use of magnetic nanoparticles as a drug delivery system for enhancing the drug release property [12, 13]. A study has accentuated the increased solubility of Fe3o4-bound drugs [14]. The present study intended to investigate the anticancer effect of 2((pyrazol-4-yl) methylene) hydrazinecarbothioamide (P) and CPTES-functionalized Fe3o4 nanoparticles[1] (PL) synthetic compounds on the changes in the expression of BAK-1, EIF4E and hTERT genes in the breast cancer cell line MCF-7 based on Real Time PCR.

Materials and Methods

Cell Culture

This study used the cell line MCF-7 procured from Iranian Biological Resource Center (IBRC). Once the cells were cultured in a 0.2-gr NaHco3-containing RPMI 1640 medium, an amount of 1ml antibiotic pen/strep was added per 100ml of cellular culture medium to avoid any cell culture medium contamination. The cells were incubated in a 25-cm^3 flask at 37°C under a humid atmosphere containing %5 CO2 and %95 humidity. Once cell density increased at the due time, the cells were gently scraped off the flask bottom by 1ml Trypsin-EDTA. As the unicellularity was assured, a %10-FBS containing medium was added. Subsequently, after a minimum of 6 cell passages to reach cellular growth phase, the percentage of live cells was determined by an optical microscope using neobar lam and Trypan blues dye.

MTT Assay for Cell Viability

The inhibitory effect of P and PL-related derivatives on cell line MCF-7 was investigated using MTT assay. Once cell MCF-7 culture was fulfilled at 1×10^5 density, in 96-well plates under the conditions of 37°C and %5 CO2, it was treated with various concentrations (1.562, 3.125, 6,25, 12.5, 25 and 50 (100 λ) µg/well) of P and PL-related compounds for 24 hours in order to reach cytotoxicity and IC50 properties of P and PL-related compounds. After adding MTT solution, a 24-hour incubation was carried out to form Formazan purple crystals. Besides, after removing cell culture contents, an amount of 100-µl dimethyl sulfoxide (DMSO) was added to each well so as to dissolve insoluble crystals. Nearly 15 minutes later, the optical absorption was read at 570 nm by ELISA.

RNA Extraction

RNA extraction was performed using Transgen Biotech ER101-01. As the 24-hour treatment of cells with P and PL-related compound was accomplished, MCF-7 adhesive cells were isolated using trypsin enzyme to prepare cellular suspension. Afterwards, the obtained cellular suspension was centrifuged at 12000g RPM in an RNase-free microtube for 5 minutes and the supernatant was discarded. In order to dissolve the formed cellular precipitate, an amount of 600-µl lubricant was added to the microtube and re-centrifuged at 12000g RPM for 5 minutes. Next, 600-µl ethanol %70 was added to the solution to be put into vertex. Once CB4 and WB4 solutions were added and the processes were re-administered, about 30-µl RNase-free water was added to the column, incubated at room temperature for 1 minute and the column was centrifuged to rinse the RNA from the column.

Purification of the Quality of Extracted RNA

This stage determined the purification and quality of extracted RNA using spectrophotometer. The optical absorption of extracted RNA at a 260/280-nm wavelength was measured, and samples whose 260/280 ratio ranged from 1.8 to 2.2 were used for cDNA synthesis.

Complementary DNA (cDNA) Synthesis

For cDNA synthesis, Transgen Biotech ER101-01 was used. To this end, amounts of 4-µl total RNA (50 ng-5 µg / 5-500 ng), 1-µl Oligo (dT) primer, 1-µl reverse transcriptase enzyme, 4-µl RNase-free water and 10-µl 2×reaction mix were mixed up and placed in the thermocycler (TC-96/g/Hcb/C. Hangzhou Bioer Technology) at temperatures of 45°C for 15 minutes and 85°C for 5 seconds to synthesize cDNA.

Primer Design

In the present study, BAK-1, EIF4E and hTERT were selected as target genes GAPDH as reference gene. The intended gene sequencing was retrieved from http://www.ncbi.nlm.nih.gov. Primer Express was used to design the specific primers for the foresaid genes. The sequences of reverse and forward primers was equal to Reverse 5'-CGTGGTTTCTGTGTGGTGTC-3' and Forward 5'-CGTGGTTTCTGTGTGGTGTC-3' at 58°C for the target hTERT gene, Reverse 5'-CTGTAGGGGATGTTTAATATAGTGTTC-3' and Forward 5'-AACCACCCCTACTCCTAATCC-3' at 35°C for the target EIF4E gene and Reverse 5'-GGTGGCAATCTTGGTGAAGT-3' and Forward 5'- TTTTCCGCAGCTACGTTTTT-3' at 56°C for the target BAK-1 gene. The intended primers for the housekeeping GAPDH gene included Reverse 5'-TTGATTTTGGAGGGATCTCG-3' and Forward 5'-TCACCAGGGCTGCTTTAAC-3' at 56°C. Finally, BLAST was followed (http://www.ncbi.nlm.nih.gov/blast) to ensure the nonspecific binding of primers in genome,

Real-Time PCR

This study used a 3-phased thermal-temporal cycling program to perform Real-time PCR. The first stage included initial denaturation at 95°C for 10 minutes. The second stage involved the 3-phased program at 40 cycles: Denaturing phase at 95°C for 30 seconds; Annealing phase at 58°C, 56°C and 56°C respectively for BAK-1, EIF4E and hTERT genes for 45 seconds; Extending phase at 72°C for 45 seconds. The temperature zone

[1] Fe3o4 nanoparticle functionalized by *3-(Chloropropyl)Triethoxysilane (CPTES)*

ranged from 56°C to 95°C to draw a melting curve. For Real-Time PCR in a final volume of 20 µl, the amounts of 1×10-µl SinaGreen Qpcr Mix,2x under Cat. No.: MM2041, 1-µl forward primer, 1-µl reverse primer and 1.5-µl cDNA were used to obtain a 20-µl volume by the means of PCR H_2O grade. Each test was replicated twice. Afterwards, CT concentration was measured on the basis of gene copy number through standard curve method and then normalized based on the mean score of the housekeeping GAPDH gene expression (as internal control). Finally, the formula $2^{-\Delta\Delta Ct}$ was used to assess the expression of target BAK-1, EIF4E and hTERT genes versus reference GAPDH gene.

Statistical Data Analysis

The observed findings of this study was the result of two replications. The intended changes were estimated using the indices of mean and standard deviation. Based on ANOVA, the p-value was considered at <0.05 significance level.

Results

Results of MTT Assay for cell Viability

MTT assay was performed to measure the amount of IC50 in P and PL-related compounds after the 24-hour treatment of cell line MCF-7 with the intended compounds. As displayed in Figures (1) and (2), MTT assay resulted in 15.780 and 13.183 (100 λ) µg/well, respectively for the amount of IC50 in P and PL compounds. The higher the P and PL concentrations, the higher the growth inhibition. Accordingly, the growth inhibition was concentration-dependent in both compounds. Noteworthy point was the lower IC50 dosage in PL than P compound, while fatality rate was higher in P than PL compounds in the 500-µg concentration.

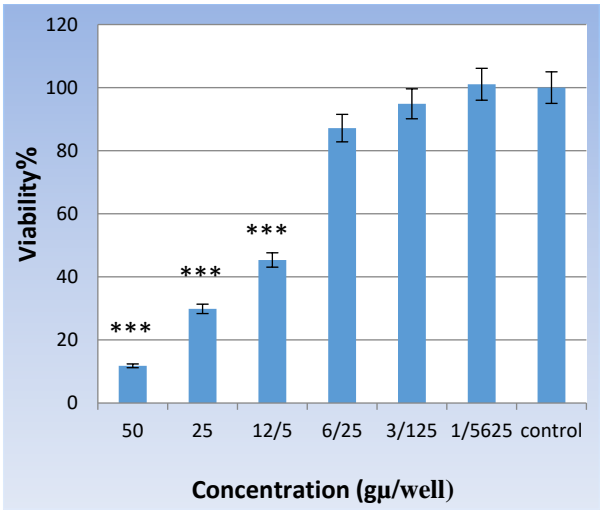

Figure 1. Results of cell viability after the 24-hour treatment of cell line MCF-7 with P compound (P<0.05) compared with the control group

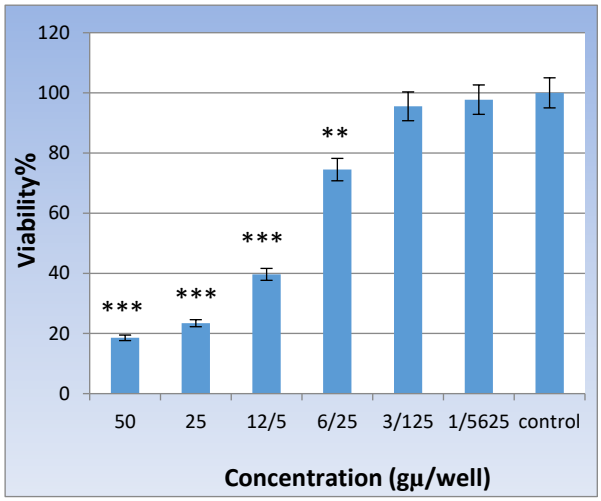

Figure 2. Results of cell viability after the 24-hour treatment of cell line MCF-7 with PL compound (P<0.05) compared with the control group

Analysis of Real-Time PCR Based on Amplification Curve

Real-Time PCR was used to analyze the expression of mRNA in BAK-1, EIF4E and hTERT genes. The results indicated that mRNA significantly overexpressed in BAK-1 gene after the 24-hours treatment of MCF-7 with P and PL compounds. The mRNA overexpression of P and Pl-treated MCF-7 in BAK-1 gene was equal to 1.48 and 1.53, respectively, as compared with the control group with a significant p-value for both P and PL compounds (P<0.05).The changes of mRNA overexpression of P and Pl-treated MCF-7 in EIF4E gene was 0.97 and 0.89 respectively, which was solely significant for PL compound. Moreover, the mRNA overexpression of P and Pl-treated MCF-7 in hTERT gene was equal to 1.01 and 1.70 respectively, compared with the control group. The expression results of BAK-1, EIF4E and hTERT genes have been presented in Figures (3) and (4).

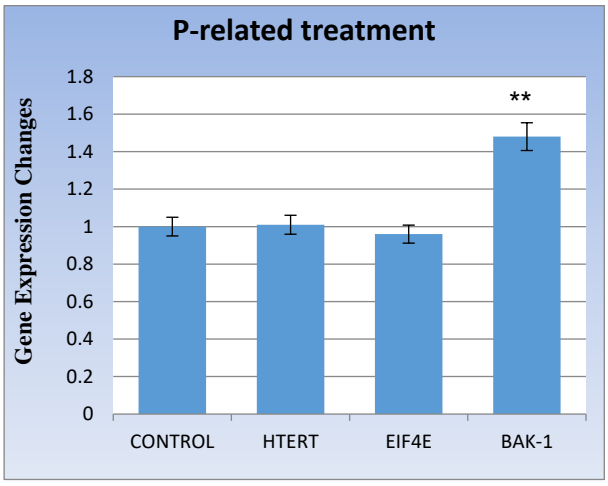

Figure 3: Expression of mRNA in BAK-1, EIF4E and hTERT genes at P-related IC50 concentration based on Real-Time PCR, normalized by GAPDH expression (P<0.05) compared with the control group

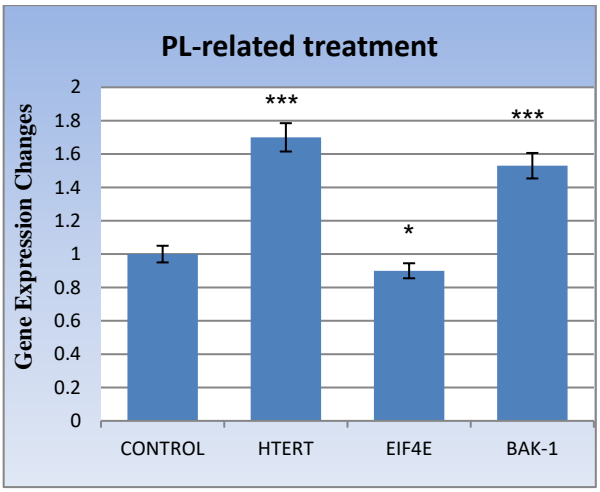

Figure 4. Expression of mRNA in BAK-1, EIF4E and hTERT genes at PL-related IC50 concentration based on Real-Time PCR, normalized by GAPDH expression ($P<0.05$) compared with the control group

Discussion and Conclusion

The present study investigated the effect of 24-hour P and PL-related treatment on cell line MCF-7. To this end, Real-Time PCR was carried out to assess the mRNA expression in BAK-1, EIF4E and hTERT genes once MTT assay had been performed for reaching the cytotoxicity property of P and PL-related compounds.

Pyrazole derivatives are important class of heterocyclic compounds in medicinal chemistry. Such heterocyclic scaffolds are widely used for cancer treatment. Not only does they have anticancer effect but also they are antimicrobial and anti-inflammatory [2, 3]. The significant role of pyrazole compounds has been proven in the deactivation of aurora kinase [4]. Furthermore, the heterocyclic compounds are prominently potential in deactivating cyclin-dependent kinases [5]. In their mechanism of action, Pyrazole compounds possess a structurally inhibitory impact on kinases blockage; this inhibition occurs through being attached to the ATP binding sites. Considering the central role of protein kinase in cell signaling and its impact on malignant lesions, it has been attempted to develop specific protein kinase inhibitors to be used in the treatment of various cancers [10]. Aurora Kinase inhibition in cancer cells is crucial for elevating cellular apoptosis [6]. As a vital nonsurgical cancer treatment, apoptosis is highly targeted in all varieties of cancers meaning that it is not type-specific [7-9]. Due to the key role of kinases in cell division esp. in mitosis stage, Aurora kinase can be effectively targeted in chemotherapy and cancer treatment [15]. Thio-semi-carbazones and their metal complexes have extensive antifungal, antiviral, antibacterial and anticancer properties. These compounds are known as polydentate ligands due to the presence of amide, imine and thione groups in their structure [16]. The inhibitory function of thio-semi-carbazones complexes in the treatment of various cancers operates through the blockage of ribonucleotide diphosphate reductase (rNDP) that is considered as a vital enzyme in DNA biosynthesis and cell division [17]. The upmost problem in tumour treatment is the sensitivity of tumour cells to cytotoxic drugs. Therefore, the use of nanoparticle delivery system, including body-specific drug delivery, for enhancing the efficiency of anticancer drugs seems to be essential [12]. Magnetic nanoparticles, such as Fe3o4, are foremost compounds with high biocompatibility, increased drug release, appropriate chemical stability, targeted drug delivery and drug solubility properties [13, 14]. Iron oxide nanoparticles can produce considerable toxicity in cancer cells by generating reactive oxygen species [18].

Srinivasa T. et al. (2015) studied the effect of three distinct pyrazole derivatives on the expression of Bcl-2, Bax and Caspase3 genes in cell line MCF-7. The results were indicative of the under-expression of Bcl-2 and overexpression of Bax and Caspase3 in all three derivatives [19], which was consistent with the overexpression of BAK-1 in the treatment of cell line MCF-7 with pyrazole derivatives in the present study. Mihai Nitulescu G. et al. (2015) investigated the effect of 8 diverse pyrazole derivatives on the expression pf cyclin-A, Cyclin-B, cdkl and cdc20 genes in cell line HT29. The results were representative of an ascending and descending asymmetric expression pattern in pyrazole-treated cells [11]. In their study of the effect of magnetic nanoparticle Fe3o4 binding to chemotherapeutic compounds on the expression of Bcl-2, Bax, and Caspase3 genes, Ren Y. et al. (2013) showed that Bcl-2 under-expressed while Bax and Capase3 overexpressed in the treated cells [20].

Gene EIF4E is a eukaryotic transcriptional primer that plays a critical role in directing ribosomes to the cap structure in mRNA. Almost all cellular mRNAs require EIF4E to be transcribed to protein. The excessive EIF4E overexpression leads to the growth of down-regulation and malignancy of a variety of cell lines [21, 22]. Besides, high EIF4E levels are usually evident in breast, colon, brain and neck cancers [23]. The transcription of EIF4E elevates in response to mitogenic stimulation that may be mediated by C-myc [24]. The present study assessed the mRNA expression of EIFE4 gene after the 24-hour treatment of cell line MCF-7 with IC50 concentration of P and PL-related compounds. Accordingly, the expression changes were equal to 0.97 in P-treated cells and 0.89 in PL-treated cells. The former, i.e. expression in P-treated cells, was insignificant, while the latter exhibited a significant descending expression of cellular mRNA level in PL-treated cells. EIF4E under-expression is an important factor for decreasing cellular carcinogenesis. Its overexpression is associated with the expression of coding VEGF and CyclinD1 gene that are effective in the carcinogenicity process [25]. EIF4E under-expression leads to decreased metastasis in cancer cells [26]. Since Aurora kinase activation is involved in the activation of map kinase that has a main role in EIF4E expression [27], P and PL-related compounds can be expected to significantly reduce EIF4E expression by affecting Aurora Kinase activation and deactivation.

Telomerase is a ribonucleo protein that maintains telomere`s length and prevents cellular aging process. This enzyme binds telomeric replication to chromosome ends. Telomerase are mainly active in somatic cells and inactive on cancer cells.

Telomerase hyperactivity has been observed in nearly %85 of the most prevalent cancer types like the breast cancer [28]. The mRNA overexpression of P and Pl-treated MCF-7 in hTERT gene was equal to 1.01 and 1.69 respectively, compared with the control group. The former, i.e. expression in P-treated cells, was insignificant, while the latter exhibited a significant ascending hTERT expression in PL-treated cells. The increased expression of activating genes may be due to the negative and positive feedback in the cell following its under-expression that can neutralize the effect of intended compounds used for its under-expression having a reverse impact on its expression level. Iron oxide nanoparticles in PL-related compound and increased drug penetration can allow its effect in shorter time. Subsequently, negative feedback mechanisms had a longer time to affect PL-treated cells. Thus, gene expression was higher in PL-treated than P-treated cells. The under-expression response of the intended gene may be witnessed in shorter time e.g. 8 or 12 hours. Consequently, it is recommended to study the anticancer effect of P and PL compounds in shorter times to reach more comprehensive information on gene expression [29]. Bcl-2 homologous antagonist is a protein encoded by BAK-1 gene. This gene is located on Chromosome 6 [30]. The encoded protein belongs to the Bcl-2 family. Bcl-2 family members are composed of oligomers or heterodimers with an anti-apoptotic and pro-apoptotic role in various cell activities. This protein is centered in mitochondria and functions as an apoptosis inducer. In general, BAK-1 is overexpressed in neurologic and autoimmune diseases, while the same is under-expressed in different cancers [31]. BAK-1-regulating drugs can be used as a promising medications against cancer [32]. The overexpression of BAK-1 gene after being treated with P and PL-related compounds was equal to 1.48 and 1.53 respectively, compared with the control group with a significant p-value for both P and PL compounds ($P<0.05$).

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References

1. Kavithaa K, Paulpandi M, Ponraj T, Murugan K, SJKIJoMS S. Induction of intrinsic apoptotic pathway in human breast cancer (MCF-7) cells through facile biosynthesized zinc oxide nanorods. 2016;2(1):46-55.
2. Keter FK, Darkwa JJB. Perspective: the 2. potential of pyrazole-based compounds in medicine. 2012;25(1):9-21.
3. Çalışkan B, Yılmaz A, Evren İ, Menevşe S, Uludag O, EJMCR B. Synthesis and evaluation of analgesic, anti-inflammatory, and anticancer activities of new pyrazole-3 (5)-carboxylic acid derivatives. 2013;22(2):782-93
4. Howard S, Berdini V, Boulstridge JA, Carr MG, Cross DM, Curry J. Fragment-based discovery of the pyrazol-4-yl urea (AT9283), a multitargeted kinase inhibitor with potent aurora kinase activity. 2008;52(2):379-88.
5. Pevarello P, Brasca MG, Orsini P, Traquandi G, Longo A, Nesi M. 3-Aminopyrazole inhibitors of CDK2/cyclin A as antitumor agents. 2. Lead optimization. 2005;48(8):2944-56
6. Bebbington D, Binch H, Charrier J-D, Everitt S, Fraysse D, Golec J. The discovery of the potent aurora inhibitor MK-0457 (VX-680). 2009;19(13):3586-92
7. Liu Y, Zhu XJTn. Endoplasmic reticulum-mitochondria tethering in neurodegenerative diseases. 2017;6(1):21
8. Villa-Pulgarín JA, Gajate C, Botet J, Jimenez A. ,8. Justies N, Varela-M RE, et al. Mitochondria and lipid raft-located FOF1-ATP synthase as major therapeutic targets in the antileishmanial and anticancer activities of ether lipid edelfosine. 2017;11(8):e0005805
9. Bao H, Zhang Q, Zhu Z, Xu H, Ding F, Wang M. BHX, a novel pyrazoline derivative, inhibits breast cancer cell invasion by reversing the epithelial-mesenchymal transition and down-regulating Wnt/β-catenin signalling. 2017;7(1):9153
10. Chahrour O, Cairns D, ZJMrimc O. Small molecule kinase inhibitors as anti-cancer therapeutics. 2012;12(5):399-411
11. Nitulescu GM, Matei L, Aldea IM, Draghici C, Olaru OT, CJAJoC B. Ultrasound-assisted synthesis and anticancer evaluation of new pyrazole derivatives as cell cycle inhibitors. 2015
12. Kouassi GK, JJJon I. A nanoparticle-based immobilization assay for prion-kinetics study. 2006;4(1):8
13. Lin B-L, Shen X-D, SJBM C. Application of nanosized Fe3O4 in anticancer drug carriers with target-orientation and sustained-release properties. 2007;2(2):132
14. Pfeffer C, AJIjoms S. Apoptosis: a target for anticancer therapy. 2018;19(2):448.14
15. SJCoiid K. AT-9283, a small-molecule multi-targeted kinase inhibitor for the potential treatment of cancer. 2010;11(12):1442-9
16. Abou-Hussen A, El-Metwally N, Saad E, AJJoCC EL. Spectral, magnetic, thermal and electrochemical studies on phthaloyl bis (thiosemicarbazide) complexes. 2005;58(18):1735-49
17. Gokhale N, Padhye S, Newton C, RJM-bd P. Hydroxynaphthoquinone metal complexes as antitumor agents x: synthesis, structure, spectroscopy and in vitro antitumor activity of 3-methyl-phenylazo lawsone derivatives and their metal complexes against human breast cancer cell line mcf-7. 2000;7(3):121-8
18. Vinardell M, MJN M. Antitumor activities of metal oxide nanoparticles. 2015;5(2):1004-21.18
19. Reddy TS, Kulhari H, Reddy VG, Bansal V, Kamal A, RJEjomc S. Design, synthesis and biological evaluation of 1, 3-diphenyl-1H-pyrazole derivatives containing benzimidazole skeleton as potential anticancer and apoptosis inducing agents. 2015;101:790-805

20. Ren Y, Zhang H, Chen B, Cheng J, Cai X, Liu R. Multifunctional magnetic Fe3O4 nanoparticles combined with chemotherapy and hyperthermia to overcome multidrug resistance. 2012;7:2261
21. De Benedetti A, Joshi B, Graff J, SJMCD Z. CHO cells transformed by the translation factor eIF-4E display increased c-myc expression, but require overexpression of Max for tumorigenicity. 1994;2:347-71
22. Lazaris-Karatzas A, Sonenberg NJM, biology c. The mRNA 5'cap-binding protein, eIF-4E, cooperates with v-myc or E1A in the transformation of primary rodent fibroblasts. 1992;12(3):1234-8
23. Sorrells DL, Meschonat C, Black D, BDJJoSR L. Pattern of amplification and overexpression of the eukaryotic initiation factor 4E gene in solid tumor. 1999;85(1):37-42
24. Jones RM, Branda J, Johnston KA, Polymenis M, Gadd M, Rustgi A. An essential E box in the promoter of the gene encoding the mRNA cap-binding protein (eukaryotic initiation factor 4E) is a target for activation by c-myc. 1996;16(9):4754-64
25. Yang SX, Hewitt SM, Steinberg SM, Liewehr DJ, SMJOr S. Expression levels of eIF4E, VEGF, and cyclin D1, and correlation of eIF4E with VEGF and cyclin D1 in multi-tumor tissue microarray. 2007;17(2):281-7
26. Gao M, Zhang X, Li D, He P, Tian W, BJO Z. Expression analysis and clinical significance of eIF4E, VEGF-C, E-cadherin and MMP-2 in colorectal adenocarcinoma. 2016;7(51):85502
27. Korneeva NL, Song A ,27. Gram H, Edens MA, REJJoBC R. Inhibition of mitogen-activated protein kinase (MAPK)-interacting kinase (MNK) preferentially affects translation of mRNAs containing both a 5′-terminal cap and hairpin. 2016;291(7):3455-67
28. Tian N, Wilson JG, FBJH Z, place. Female breast cancer mortality clusters within racial groups in the United States. 2010;16(2):209-18
29. Cui J, Chen C, Lu H, Sun T, PJPO S. Two independent positive feedbacks and bistability in the Bcl-2 apoptotic switch. 2008;3(1):e1469
30. Chittenden T, Harrington EA, O'connor R, Remington C, Lutz RJ, Evan GI. Induction of apoptosis by the Bcl-2 homologue Bak. 1995;374(6524):733.
31. Cartron P-F, Petit E, Bellot G, Oliver L, FMJCs V. Metaxins 1 and 2, two proteins of the mitochondrial protein sorting and assembly machinery, are essential for Bak activation during TNF alpha triggered apoptosis. 2014;26(9):1928-34
32. Westphal D, Kluck RM, Dewson G. "Building blocks of the apoptotic pore: how Bax and Bak are activated and oligomerize during apoptosis".2014; 21 (2): 196–205.