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Chalcone Derivative 15 Induces Apoptosis in Human HOS, HCT116, and MCF-7 Cancer Cells

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Abstract

Cancer is one of the most life threating diseases requiring the search of novel treatment strategies. Our study aims to investigate the anticancer effect of new chalcone derivatives on human cancer HOS, HCT116, and MCF-7 lines. Both 2- and 3-D spheroid cell culture models were constructed, MTT / acid phosphatase cytotoxic assays were applied, and GraphPad PRISM was used to for IC50 values estimation. RT-qPCR analysis for apoptotic genes was conducted and followed by analysing of the cell cycle via flow cytometry. Screening results showed that exceeding Doxorubicin, a concentration of 200 µM of the Compound 15 exerted a significant in vitro cytotoxic activity of 96.50, 90.56, and 81.43 % with IC50 values of 32.73, 25.60, 22.65 µM on MCF-7, HCT116, and HOS cell lines, respectively. Compound 15 indicated a very high selectivity against tested lines of cancer relative to the normal one (hTERT RPE-1). Data from RT-qPCR reported that Compound 15 induces apoptosis in MCF-7 through upregulating P53 & BAX proapoptotic genes while simultaneously downregulating BCL-2 antiapoptotic gene. It also arrests the cellular cycle at the G0/G1 phase (85.36 %). Applied on 3-D spheroid culture model of MCF-7, Compound 15 induced an anti-cancer effect reaching 69.30 % with an IC_{50} value of 59.86 µM ±8.97. Hence, Compound 15 represents a potential synthetic chalcone-based drug against MCF-7 breast cancer, HCT116 colorectal carcinoma and HOS osteosarcoma human cancer cell lines.

Keywords: Chalcone derivative 15, MCF-7, HCT116, HOS, Drug repurposing, P53, 3-D spheroid culture

1. Introduction

Cancer is the status where the genetic makeup is altered due to mutations which either activate oncogenes or supress antiapoptotic genes [1-2].

The World Health Organization (WHO) has declared that men prevalent cancers include liver, stomach, lung, colorectal, lung, and prostate. While women most prevalent ones are thyroid, colorectal, lung, breast, and cervix [3]. Their incidence are expected to rise over the next decades [4]. The most type of cancer that develops in women globally is breast cancer due to many factors [5-6]. The most common malignancy in women is breast cancer. Whose risk factors are hormone usage, alcohol consumption, obesity, and history of neoplastic disease. Moreover, carrying BRCA mutation can raise the risk from 3 to 200 in premenopausal women [7-10].

Globally, colorectal cancer is ranked as the third prevalent type and the second one in death leading [11].The most frequent type of cancer in bone is osteosarcoma, originating from mesenchymal tissue. Its therapy is difficult and calls for a comprehensive strategy [12]. Synthetic compounds represent a favourable choice to innovate potential anti-cancer drugs. Moreover, the advantage of compounds synthesis is to solve the restraints of natural products, mostly low yield and time consumption, and ability of the production of new compound derivatives [13-15].

Chalcones, natural flavonoids, demonstrate wide biological potentials against leishmania, tuberculosis, hyperglycemia, fungi, malaria, and inflammation [16]. Additionally, chalcones, either natural or synthetic, have shown effective antitumor properties inhibiting numerous lines of cancerous cells [17]. Our work aims to determine the potential cytotoxicity of some chalcone derivatives [18] against MCF-7, HCT116, and HOS cell lines.

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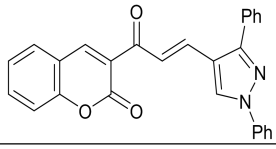
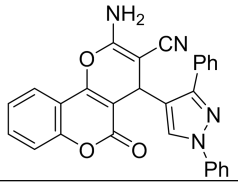
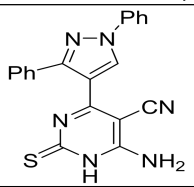
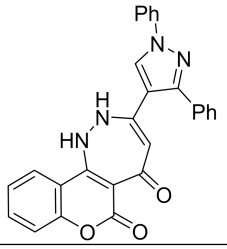
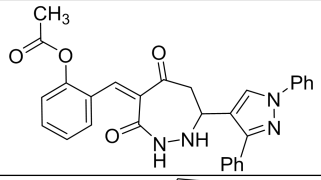
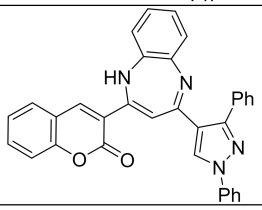
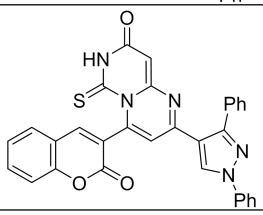
2. Experimental

2.1. Chemistry

Seven chalcone derivatives are synthesized according to [18] and recorded in Table 1.

Table 1 Chalcone derivative compounds subjected to cytotoxicity bioassay on human cell line

Code	Chemical structure	IUPAC
3		**E - 3 - [3- [1,3 - di-phenyl - 1 H - pyrazol - 4 - yl] acryloyl] - 2 H - chromen - 2 - one**
6		**2 - amino - 4 - [1,3 – di-phenyl - 1 H – pyrazol - 4 - yl]- 5 – oxo – 4 H ,5 H - pyrano [3,2 - c] chromene - 3 - carbonitrile**
7		**6 - amino - 4 - [1,3 – di-phenyl - 1 H - pyrazol - 4 - yl] - 2 - thioxo - 1,2 - di-hydro-pyrimidine - 5 - carbonitrile**
9		**3 - [1,3 - diphenyl -1 H - pyrazol - 4 -yl] - 1,2 - di-hydrochromeno [4,3 c] [1,2] di-azepine - 5,6 - di-one**
10		**Z - 2 - [(7 - (1,3 - di-phenyl - 1 H - pyrazol - 4 - yl) - 3,5 - di-oxo - 1,2 - di-azepan - 4 - ylidene) methyl] phenyl acetate**
15		**3 - [4 - (1,3 – di-phenyl – 1 H – pyrazol - 4 - yl)- 1 H - benzo [b] [1,4] di-azepin – 2 – yl] – 2 H – chromen - 2 - one**
17		**2 - [1,3 - di-phenyl - 1 H - pyrazol - 4 - yl] - 4 - [6 - methyl - 2 - oxo - 5 - vinyl - 2 H -pyran - 3 - yl] - 6 - thioxo - 6,7 - dihydro - 8 H - pyrimido [1 , 6 – a] pyrimidin - 8 - one**

2.2. Two-Dimensional cell culture of MCF-7, HCT116 and HOS

All the utilized cell lines were kindly provided by Prof. Dr. Stig Linder (Karolinska Institute – Sweden). DMEM media supplemented with fetal bovine serum (10 %) was used for culturing of human cancer cells (i.e., MCF-7 breast cancer, HCT116 colorectal carcinoma, and HOS osteosarcoma), while DMEM-F12 medium (Life Technologies) supplemented with fetal bovine serum (10 %) in addition to streptomycin/penicillin (1 %) and l-glutamine was used for culturing of hTERT RPE-1 human retinal pigment epithelial-1, at 37 °C, 95 % humidity, and 5 % CO_2 [19-20]. The tested samples were prepared in a stock solution of 40 mM. Normal and cancer cells were seeded at a concentration of 60.000 and 200.000 cells/ml, respectively. After a 24-h incubation period, different concentrations of tested compounds (200; 100; 50; 25 µM) have been applied to the cells in triplicates. Then, a 72-h incubation period was done [19].

2.3. MTT assay

According to Mosmann [21], MTT [3- [4, 5- dimethylthiazol - 2 - yl] - 2,5 - diphenyltetrazolium bromide] (Bio Basic, New York, USA) was added after media discarding, and a 4-h incubation at 37 °C was followed. Formed crystals were dissolved using 10 % sodium dodecyl sulfate. Measurement of absorbance was performed at 595 nm using 690 nm as reference [22].

2.4. Determination of IC_{50}

IC_{50} values were computed for the compounds by means of the Software [GraphPad PRISM 6.0] (San Diego - USA).

Subsequently, MCF-7 treated with compound 15 was evaluated by RT-qPCR for gene expression and flow cytometry for cell cycle analysis, as cells were seeded in 6-well plates 200.000 cells/ml and were incubated for 72 h with the concentrations corresponding to the IC_{50} of compound 15, using Doxorubicin as the positive control.

2.5. RT-qPCR analysis for apoptotic genes evaluation (p53, BAX, and BCL-2 genes)

The extraction of RNA from cell pellets was performed by utilizing RNeasy Mini Kit (Qiagen - Hilden - Germany). Reverse transcription was done via RevertAidTM First Strand cDNA Synthesis Kit (Fermentas - Germany). Estimation of gene expression (p53; BAX; BCL-2) was determined via StepOne™ Real-Time PCR System (Applied Biosystems - Thermo Fisher Scientific – Waltham - USA). The sequences of specific primers for MCF-7 cell line were designed (Table 2), and the housekeeping β-actin gene was taken as a reference [23-24].

Table 2 Sequences of qRT-PCR primers of MCF-7 cell line

Gene	Primer sequence	GenBank (accession no)
P53	F: TTGTAATGCAGGGCTGAGGA R: CTGCTCCCACCTCCTGTTAA	U94788.1
BAX	F: GATGACCCTCTGACCCTAGC R: CGGGCATTAAAGAGCTGGAC	NM_001291430.2
Bcl-2	F: CAAGTGTTCCGCGTGATTGA R: CAGAGGAAAAGCAACGGGG	KY098818.1
β-actin	F: CATGGAATCCTGTGGCATCC R: CACACAGAGTACTTGCGCTC	HQ154074.1

2.6. Flow cytometry analysis for cell cycle phase determination

Analysis of MCF-7 cell cycle has been performed using flow cytometric technique, by incubating both untreated (negative control) and compound 15-treated cells for 72 h. This is followed by cell suspension in 1x DPBS, 70 % ethanol fixation, and storing for 2 h on ice before a 5-min centrifugation at 300 ×g. After a careful decantation of supernatant, a 30-sec resuspension of cell pellets using 1x DPBS (5 ml), followed by a 5-min centrifugation at 300 ×g. After resuspension of cell pellets was done using PI (1 ml), staining solution, a 30-min dark incubation at room temperature was done. Eventually, cell fluorescence is estimated, and cell cycle is analysed by transferring cells into CytoFLEX Flow Cytometer [Beckman Coulter Life Sciences – USA; Beckman Coulter Inc. – Cairo –

Egypt (Cat. No. 4238055-CB)]. Cells % in the cycle phases (i.e., G0/G1; S; G2/M) has been computed by utilizing the CytExpert [25].

2.7. Spheroid cell model of MCF-7 for in vitro cytotoxicity investigation

Poly-HEMA was used for the coating of 96-well plates. The MCF-7 cells seeding was set at 10.000 cells per well. [26]. The plate with cells underwent a 10-min centrifugation (1000 ×g / 4 °C) [27]. It was then incubated overnight for 7 days in the CO_2 incubator [28]. Compound 15 was applied as in case of 2-D method. Subsequently, they were incubated for 72 h at concentrations of 200, 100, 50, and 25 µM with each concentration applied in a triplicate. Negative and positive controls were untreated MCF-7 and Cisplatin, respectively.
After 72 h, the method of acid phosphatase was applied for cytotoxicity assessment [29].

Spheroids were washed 2 times using PBS buffer (250 µl), then a lysis buffer pH 5 (sodium citrate 100 µl 0.1 M, Triton X-100 0.1 %, p-nitrophenyl phosphate 2 mg/ml; Santa Cruz-Germany) was utilized, followed by a 90-min incubation at 37 °C. Eventually, 1N NaOH (10 µl) stop solution was added and the absorbance at 405 nm was measured [30].

3. Results and discussion

3.1. In vitro cytotoxic assay

The anticancer effect of seven chalcone derivative compounds against human cancer cell lines of MCF-7, HCT116 and HOS were investigated. The Cultures of different cell lines were treated with the compounds at an initial screening at 200 µM (Figure 1). Percentage cytotoxicity of the tested compounds at different concentrations against MCF-7, HCT116, and HOS (Tables 3-5).
The most active compounds have been further tested for their cytotoxicity against human normal cell hTERT RPE-1 (Table 6 and Figure 1) to calculate their SI values (Table 7).

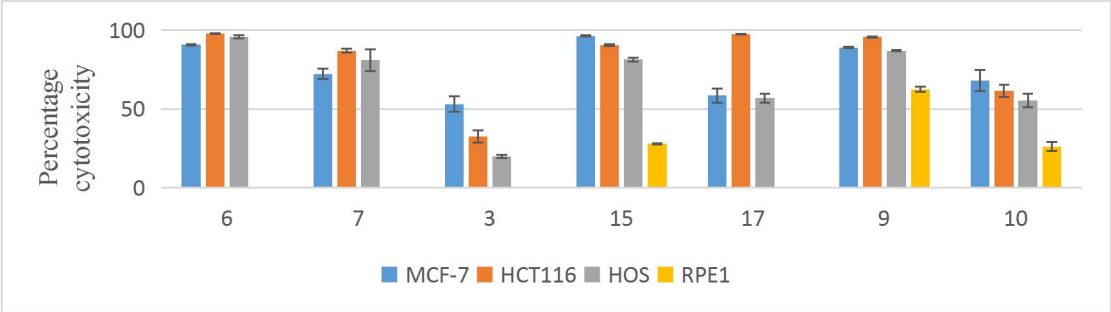


Figure 1: Cytotoxicity of the compounds at 200 µM on human cancer and cell lines and normal human cell line

Table 3: Cytotoxicity % of synthetic chalcones on MCF-7

Compound	200 µM	100 µM	50 µM	25 µM
6	90.86 ± 0.51	20.56 ± 1.32	0 ± 0.005	0 ± 0.005
7	72.36 ± 3.25	66.03 ± 4.98	22.73 ± 1.55	15.10 ± 0.65
3	53.16 ± 4.80	35.83 ± 3.70	36.26 ± 2.82	30.30 ± 1.69
15	96.50 ± 0.30	95.9 ± 0.30	84.30 ± 2.60	25.90 ± 1.80
17	58.60 ± 4.52	60.66 ± 4.05	53.29 ± 3.06	16.00 ± 0.51
9	89.06 ± 0.50	87.60 ± 1.13	78.40 ± 4.24	47.45 ± 2.01
10	68.20 ± 6.64	65.50 ± 5.37	55.95 ± 0.91	40.70 ± 2.37

The results are shown as average ± standard deviation

Table 4: Cytotoxicity % of synthetic chalcones on HCT116

Compound	200 µM	100 µM	50 µM	25 µM
6	97.94 ± 0.08	65.41 ± 1.68	14.05 ± 1.61	0.10 ± 0.01
7	86.97 ± 1.25	43.00 ± 1.53	36.62 ± 2.03	28.90 ± 2.95
3	32.56 ± 3.78	19.96 ± 1.49	10.11 ± 0.24	2.73 ± 0.11
15	90.56 ± 0.46	87.86 ± 0.92	79.40 ± 1.80	46.73 ± 2.85
17	97.46 ± 0.15	43.50 ± 2.57	22.41 ± 2.77	13.70 ± 1.21
9	95.73 ± 0.30	94.53 ± 0.46	59.13 ± 1.40	13.70 ± 1.21
10	61.65 ± 3.78	44.60 ± 0.78	31.73 ± 1.55	10.75 ± 0.16

The results are shown as average ± standard deviation

Table 5: Cytotoxicity % of synthetic chalcones on HOS

Compound	200 µM	100 µM	50 µM	25 µM
6	95.90 ± 0.98	74.85 ± 7.56	0 ± 0.005	0 ± 0.005
7	81.14 ± 6.95	35.76 ± 3.87	26.16 ± 1.40	18.76 ± 1.77
3	19.93 ± 1.02	21.76 ± 1.87	16.36 ± 0.20	9.59 ± 0.86
15	81.43 ± 1.07	78.04 ± 1.90	68.40 ± 1.55	49.60 ± 2.12
17	57.00 ± 2.82	38.50 ± 2.16	30.20 ± 1.11	25.35 ± 1.02
9	87.00 ± 0.45	82.86 ± 1.55	75.56 ± 2.35	61.20 ± 3.38
10	55.50 ± 4.24	39.96 ± 1.66	32.76 ± 1.63	24.78 ± 0.41

The results are shown as average ± standard deviation

Table 6: Cytotoxicity % of the most active synthetic chalcones on hTERT RPE-1 cell line

Compound	200 µM	100 µM	50 µM	25 µM
6	0 ± 0.005	0 ± 0.005	0 ± 0.005	0 ± 0.005
15	27.90 ± 0.25	0 ± 0.005	0 ± 0.005	0 ± 0.005
9	62.48 ± 1.63	25.10 ± 1.70	14.42 ± 0.74	12.70 ± 0.27
10	26.23 ± 2.82	0 ± 0.005	0 ± 0.005	0 ± 0.005

The results are shown as average ± standard deviation

Table 7: Selectivity indices (SI)

Compound	SI on MCF -7	SI on HCT116	SI on HOS
6	>10	>10	>10
7	>10	>10	>10
3	>10	>10	>10
15	>10	>10	>10
17	>10	>10	>10
9	6.42	3.61	13.63
10	>10	>10	>10

All tested compounds reported very high selectivity on cancer cells over the normal cells hTERT RPE-1 cells as they showed almost no cytotoxicity on hTERT RPE-1, except for compound 9.

Table 8: IC50 (µM) values of chalcone compounds

Compound	MCF -7	HCT116	HOS
6	129.3±1.35	83.39±1.72	88.97±3.45
7	85.43±9.35	78.22±15.64	106.5±23.81
3	210.8±23.10	-	-
15	32.73±1.19	25.60±3.69	22.65±4.47
17	79.36±6.34	94.53±17.34	157.3±23.55
9	25.01±2.75	44.42±1.45	13.63±2.74
10	39.86±5.97	122.6±13.90	158.1±20.45
Doxorubicin	3.90±0.06	1.55±0.04	0.30±0.01

The results are represented as average of IC_{50} ± SD

3.3. RT-qPCR analysis for apoptotic genes evaluation (p53;BAX;BCL-2)

Expression of MCF-7 apoptotic genes (p53; BAX;BCL-2) in response to compound 15 is estimated (Figures 2-4). Figures 2 & 3 illustrated that the proapoptotic genes (p53;BAX) expression intensity were increased progressively in ascending in the MCF-7 exposed to Doxorubicin, and reaching the highest up-regulation in cells that were treated with compound 15. Each of these expression levels were significantly higher compared to untreated MCF-7 cell lines.Meanwhile, as shown in Figure 4, levels of expression of BCL-2, antiapoptotic gene, gave the higher value of the gene expression in the untreated MCF-7 cells compared with the treated ones. As The expression levels of BCL-2 gene were reduced significantly in response to Doxorubicin and reached the lowest level of down-regulation with compound 15.

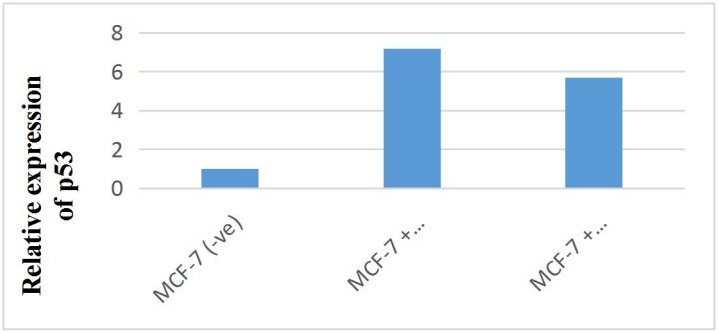


Figure 2: Expression quantity of p53 gene in MCF-7 cells by RT-qPCR using the 2−ΔΔCT method.The values represent the mean ± SD (P<0.05).

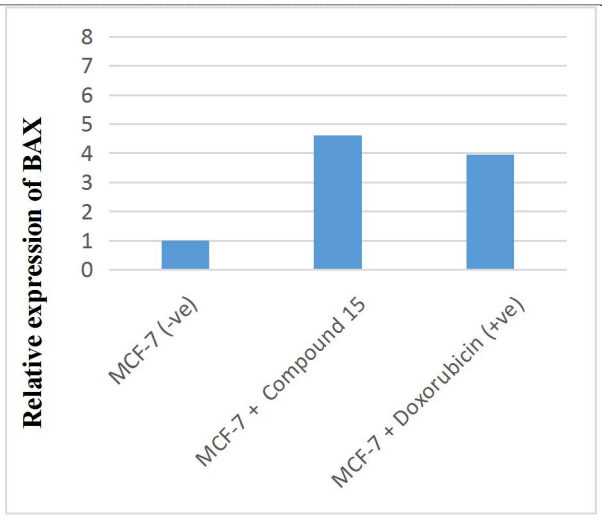


Figure 3: Expression quantity of BAX gene in MCF-7 cells by RT-qPCR using the 2−ΔΔCT method. The values represent the mean ± SD (P<0.05).

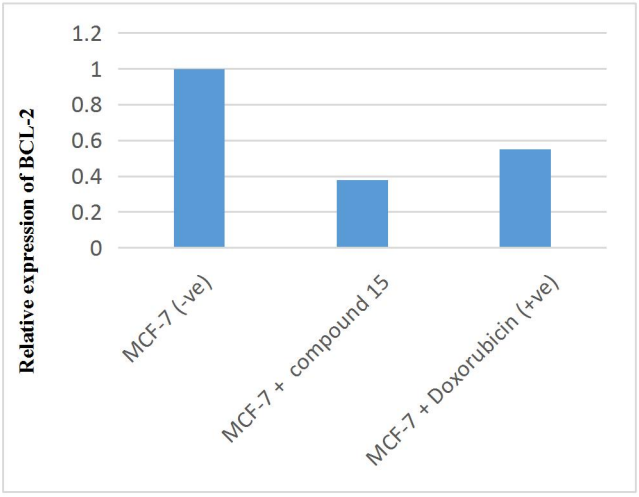


Figure 4: Expression quantity of BCL-2 gene in MCF-7 cells by RT-qPCR using the 2−ΔΔCT method.The values represent the mean ± SD (*P*<0.05).

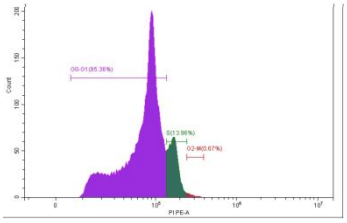


Figure 5: Flow cytometry diagram represents the distribution of cell cycle stages in MCF-7 cells treated with compound 15. The diagram highlights the cells % in cells % in (G0/G1; S; G2/M) phases.

3.4. Analysis of cell cycle via flow cytometry

MCF-7 treated with compound 15 induced a cell cycle arrest at G0/G1 phase with an increased percentage of cells (85.36 %) compared to the negative control (83.30 %) and positive control (81.98 %), as illustrated in Table 9 and Figures 5-7.

Table 9 :Cell cycle analysis of compound 15

Sample	Phase		
	G0/G1 %	S %	G2/M %
compound 15-treated MCF-7	85.36 %	13.96 %	0.67 %
Untreated MCF-7	83.30 %	15.62 %	1.14 %
Doxorubicin treated MCF-7	81.98%	17.53%	0.49%

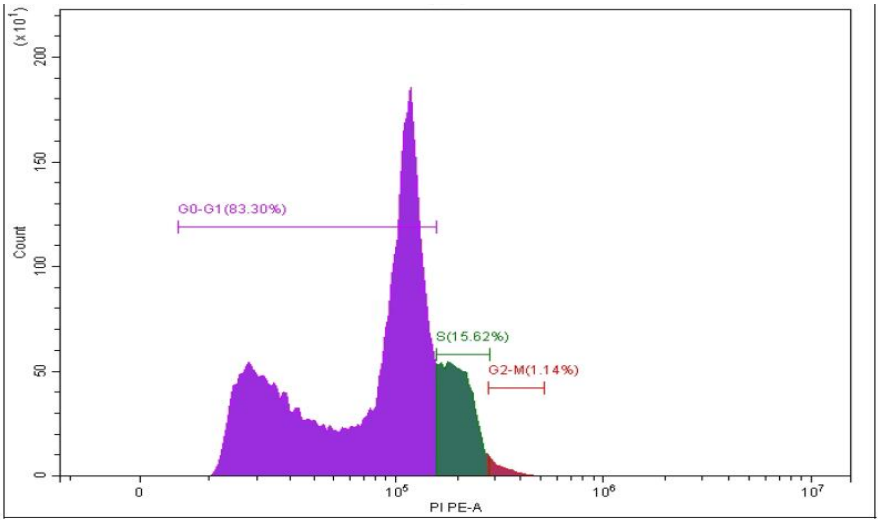


Figure 6: Flow cytometry diagrams represent the distribution of cell cycle stages in untreated MCF-7 as negative control. The diagram highlights the cells % in (G0/G1; S; G2/M) phases.

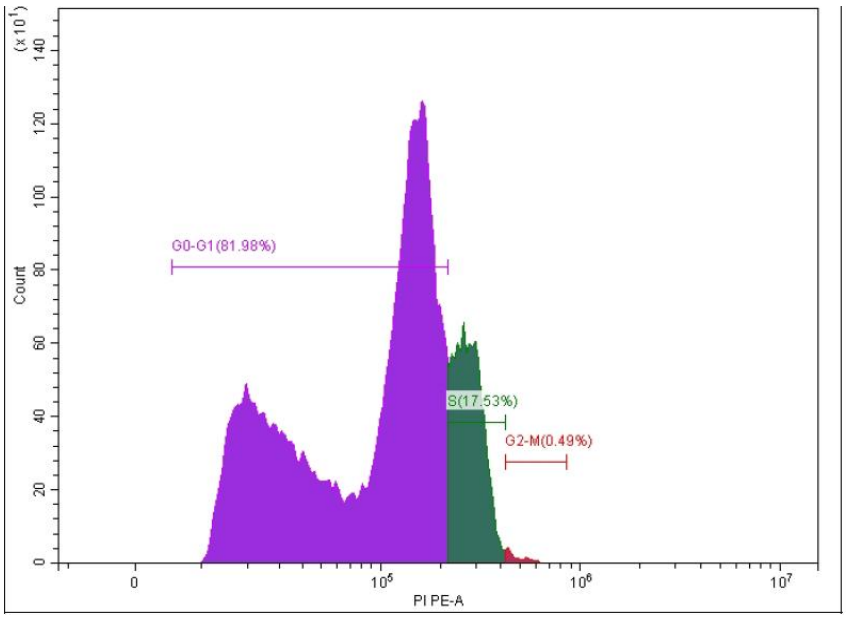


Figure 7: Flow cytometry diagram represents the distribution of cell cycle stages in MCF-7 cells treated with Doxorubicin as positive control. The diagram highlights the cells % in cells % in (G0/G1; S; G2/M) phases.

3.5. Effect of compound 15 on 3-D spheroid culture model

MCF-7 spheroids were developed within a week, then the tested compound 15 was applied at 4 concentrations (200, 100, 50, and 25 µM), with Cisplatin as a positive control, and incubated for 72h. Monitoring was performed every day for 3 days for the treated MCF-7 spheroids.
Along the 3 days, and comparing with the negative control, the treated spheroids showed irregular or fragmented shape where cells died, in addition the spheroids became less dense and more loosely organized, as cellular connections got disrupted (Figures 8-9).

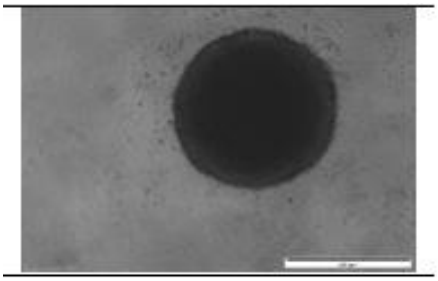


Figure 8: Negative control of untreated MCF-7 spheroid cell

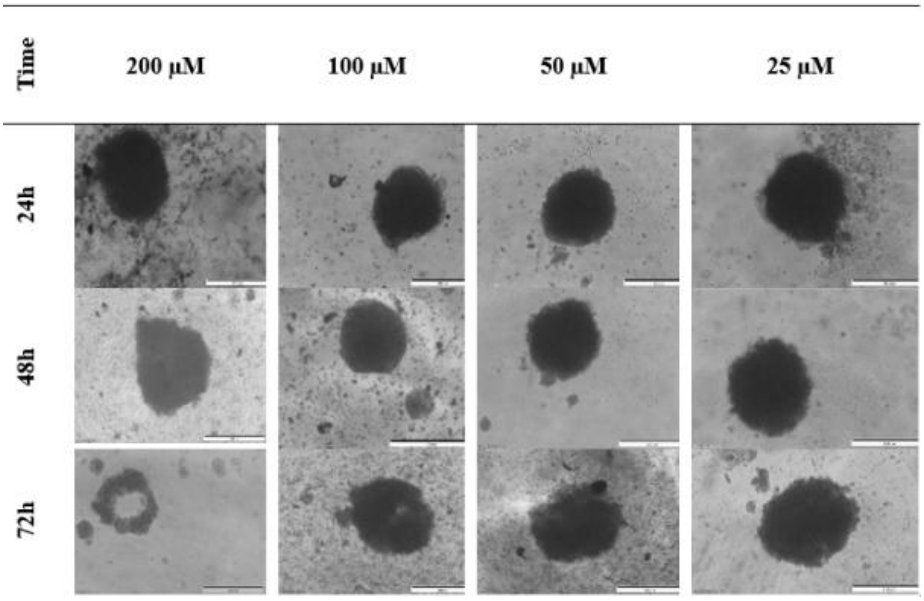


Figure 9: Anti-cancer effect of compound 15 at different concentrations on MCF-7 spheroids

The acid phosphatase cytotoxicity assay revealed a cytotoxicity of 69.30 % at 200 µM for compound 15 while the positive control Cisplatin was utilized at concentration of 50 µM (Table 10). IC_{50} value of compound 15 against MCF-7 spheroids was calculated as 59.86 µM ±8.97.

Table 10 Percentage cytotoxicity of compound 15 at different concentrations on MCF-7 spheroids

Compound	200 µM	100 µM	50 µM	25 µM
15	69.30	64.60	56.00	21.10
Cisplatin	-	-	25.11	-

4. Conclusion

New synthetic chalcone derivatives were investigated for their anticancer potentials. Compound 15 was very promising when tested against cancer cell lines in both 2- and 3-D culture models. In the 2-D models, compound 15 exerted a notable cytotoxic effect against MCF-7, HCT116 and HOS (96.50 %, 90.56 % and 81.43 % with IC 50 values 32.73, 25.60, and 22.65 µM, respectively), giving a significant selectivity index. Furthermore, RT-qPCR analysis revealed that compound 15 induces apoptosis through regulating P53 & BAX (proapoptotic) and BCL-2 (antiapoptotic) up and down, respectively, in MCF-7 cells in which about 85.36 % were arrested at G0/G1 phase. In 3-D spheroid culture model of MCF-7, Compound 15 induced an anti-cancer effect reaching 69.30 % with an IC_{50} value of 59.86 µM ±8.97. Therefore, Compound 15 could be a potential anticancer therapeutic agent against MCF-7, HCT116 and HOS human cell lines.

5. Conflicts of interest

No conflict of interest could be declared by the authors.

6. Formatting of funding sources

Not applicable.

7. Acknowledgments

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8. References

[1] Mroz, E. A. and Rocco, J. W. (2017) 'The challenges of tumor genetic diversity', Cancer, 123(6), pp. 917–927. doi: 10.1002/cncr.30430.

[2] Ujvari, B. et al. (2018) 'Genetic diversity, inbreeding and cancer', Proceedings of the Royal Society B: Biological Sciences, 285(1875). doi: 10.1098/rspb.2017.2589.

[3] Bray, F., Ferlay, J. and Soerjomataram, I. (2018) 'Global Cancer Statistics 2018 : GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries', pp. 394–424. doi: 10.3322/caac.21492.

[4] Torre, L. A. et al. (2015) 'Global cancer statistics, 2012', CA: A Cancer Journal for Clinicians, 65(2), pp. 87–108. doi: 10.3322/caac.21262.

[5] Aizaz, M., Khan, M., Khan, F. I., Munir, A., Ahmad, S., & Obeagu, E. I. (2023). Burden of Breast Cancer: Developing Countries Perspective. Internaltional Journal of Innovative and Applied Research, 11(01), 31–37. https://doi.org/10.58538/IJIAR/2008

[6] Obeagu, E. I., Babar, Q., Vincent, C. C. N., Udenze, C. L., Eze, R., Okafor, C. J., Ifionu, B. I., Amaeze, A. A., & Amaeze, F. N. (2021). Therapeutic Targets In Breast Cancer Signaling: A Review. Journal of Pharmaceutical Research International, 33, 82–99. https://doi.org/10.9734/jpri/2021/v33i56a33889

[7] Faryabi, R., Daneshi, S., Davarani, E. R., Yusefi, A. R., Arabpour, M., & Ezoji, K. (2023). The assessment of risk factors and risk perception status of breast cancer in Northern Iran. 1–7.

[8] Singletary, S. E. (2003). Rating the Risk Factors for Breast Cancer. 237(4), 474–482.

[9] Stanisławek, A. (2021). Breast Cancer—Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies— An Updated Review. 1–30.

[10] Veneroni, S., Boracchi, P., Cappelletti, V., Dn, P., Ce, H., Galea, M., Ja, B., Ra, N., & Cw, E. (1996). Re: Risk Factors for Breast Cancer According to Family History of Breast Cancer. 88(14), 1003–1004.

[11] Roshandel, G., Ghasemi-kebria, F., & Malekzadeh, R. (2024). Colorectal Cancer : Epidemiology , Risk Factors , and Prevention. 0–22.

[12] Pilavaki, P., Ardakani, A. G., Gikas, P., & Constantinidou, A. (2023). Osteosarcoma : Current Concepts and Evolutions in Management Principles.

[13] Rattanaburee, T. et al. (2019) 'Biomedicine & Pharmacotherapy Anticancer activity of synthetic (±) -kusunokinin and its derivative (±) -bursehernin on human cancer cell lines', Biomedicine & Pharmacotherapy. Elsevier, 117(May), p. 109115. doi: 10.1016/j.biopha.2019.109115.

[14] Badawy E, Abel-Azim N, Shams K, Abdur-Rahman M, Mahmoud K, Abd-Rabou A, Fahmi A E, & Saleh I. 2023. Chemical Composition and Bioactivities of Egyptian *Opuntia ficus–indica* Seeds Oils Obtained by

Conventional and Ultrasound-assisted Extraction Techniques. *Egyptian Journal of Chemistry 66*(3): 91-101. (doi: 10.21608/ejchem.2023.181098.7358)

[15] Omar S, Fahmi A, Abdur-Rahman M, Ghareeb M, Abdelaziz M. 2023. Biological and Chemical Evaluation of Secondary Metabolites from Endophytic Fungi Isolated from Egyptian Ornamental Plants. *Egyptian Journal of Chemistry* 66(8), 267-282 (doi: 10.21608/ejchem.2022.173108.7160)

[16] Zhuang, C. et al. (2018) 'Chalcone: A Privileged Structure in Medicinal Chemistry', 117(12), pp. 7762–7810. doi: 10.1021/acs.chemrev.7b00020.Chalcone.

[17] Mirzaei, H. and Emami, S. (2016) 'Recent advances of cytotoxic chalconoids targeting tubulin polymerization: Synthesis and biological activity', European Journal of Medicinal Chemistry. Elsevier Ltd. doi: 10.1016/j.ejmech.2016.05.067.

[18] Kaddah, M. M. et al. (2021) 'Synthesis, characterization, computational chemical studies and antiproliferative activity of some heterocyclic systems derived from 3-(3-(1,3-diphenyl-1H-pyrazol-4-yl)acryloyl)-2H-chromen-2-one', Synthetic Communications. Taylor & Francis, 51(12), pp. 1798–1813. doi: 10.1080/00397911.2021.1904991.

[19] Fawzy, N. G. et al. (2019) 'Synthesis, human topoisomerase IIα inhibitory properties and molecular modeling studies of anti-proliferative curcumin mimics', RSC Advances, 9(58), pp. 33761–33774. doi: 10.1039/c9ra05661k.

[20] Fayad, W. (2019) 'Identification of a novel anticancer compound through screening of a drug library on multicellular spheroids', 1, pp. 188–200. doi: 10.4103/epj.epj.

[21] Mosmann, T. (1983) 'Rapid Colorimetric Assay for Cellular Growth and Survival : Application to Proliferation and Cytotoxicity Assays', 65, pp. 55–63.

[22] El-Hallouty, S. M. et al. (2015) 'In vitro anticancer activity of some egyptian plant extracts against different human cancer cell lines', International Journal of PharmTech Research, 8(2), pp. 267–272.

[23] Brito, A.F. et al. (2013) 'Hepatocellular carcinoma and chemotherapy: The role of p53', Chemotherapy, 58(5), pp. 381–386. Available at: https://doi.org/10.1159/000343656.

[24] Khalil, W.K.B. et al. (2019) 'Apoptosis, reactive oxygen species and DNA damage in Familial Mediterranean Fever patients', Gene Reports, 14(November 2018), pp. 76–80. Available at: https://doi.org/10.1016/j.genrep.2018.11.010.

[25] Kamat, S. et al. (2020) 'Endophytic fungus, Chaetomium globosum, associated with marine green alga, a new source of Chrysin', Scientific Reports, 10(1), pp. 1–17. Available at: https://doi.org/10.1038/s41598-020-72497-3.

[26] Fayad, W. et al. (2011) 'Identification of Agents that Induce Apoptosis of Multicellular Tumour Spheroids: Enrichment for Mitotic Inhibitors with Hydrophobic Properties', Chemical Biology and Drug Design, 78(4), pp. 547–557. Available at: https://doi.org/10.1111/j.1747-0285.2011.01170.x.

[27] Ivascu, A. and Kubbies, M. (2006) 'Rapid generation of single-tumor spheroids for high-throughput cell function and toxicity analysis', Journal of Biomolecular Screening, 11(8), pp. 922–932. Available at: https://doi.org/10.1177/1087057106292763.

[28] Ezzat, A. et al. (2020) 'Combination treatment of MCF-7 spheroids by Pseudomonas aeruginosa HI1 levan and cisplatin', Biocatalysis and Agricultural Biotechnology, 24(February), p. 101526. Available at: https://doi.org/10.1016/j.bcab.2020.101526.

[29] Friedrich, J. et al. (2007) 'A reliable tool to determine cell viability in complex 3-D culture: The acid phosphatase assay', Journal of Biomolecular Screening, 12(7), pp. 925–937. Available at: https://doi.org/10.1177/1087057107306839.

[30]Fayad, W. et al. (2017) 'A systematic multicellular spheroids screening approach lead to the identification of antineoplastic activity in three different plant extracts from the Egyptian flora', Journal of Applied Pharmaceutical Science, 7(6), pp. 013–022. Available at: https://doi.org/10.7324/JAPS.2017.70603.