ARTICLE / INVESTIGACIÓN

Cytotoxic potential activity of quercetin derivatives on MCF-7 breast cancer cell line

Husam Salman Jasim[1]*, Zainab A. Al-kubaisi[2] and Hanady S. Al-Shmgani[2]

DOI. 10.21931/RB/2023.08.01.92

[1] Chemistry Department, Ibn Al-Haitham, University of Baghdad, Baghdad, Iraq.
[2] Biology Department, Ibn Al-Haitham, University of Baghdad, Baghdad, Iraq.
Corresponding author: husam_alaythawy78@yahoo.com

Abstract: Many previous investigations have found quercetin to be a powerful antioxidant and antitumor flavonoid, but its poor bioavailability has limited its use. This current study investigated the effects of two newly synthesized Quercetin Schiff bases containing 2-amino thiadiazole-5-thiol (Q1), and its benzyl derivatives (Q2) on MCF-7 human breast cancer cells. Cell viability and apoptosis were assessed to determine the toxic effects of Q1 and Q2. Cytotoxicity valuation showed that both compounds inhibited MCF-7 cell growth, and lactate dehydrogenase (LDH) activity increased in a dose-dependent aspect compared to the control group. Comet assay results observed that Q1 and Q2 induce more serious DNA damage than the control (untreated cell); however, in all curried experiments, Q2 showed higher effects than Q1. Hence two synthesized quercetin Schiff bases can take action as a promising anticancer agent.

Key words: Quercetin derivatives, Schiff base, breast cancer, MCF-7 Cytotoxic.

Introduction

Cancer considerable one of the most significant and dangerous diseases. Breast cancer, one of the most widespread types of cancer in women, causes high mortality and morbidity. Modern studies emphasized that quercetin can prevents breast cancer by avoiding signal transduction, the exhortation of cancer cell apoptosis, and repressing proliferation, inroad, and metastases of tumor cells[1]. Generally, in the human diet, many biomolecules, such as flavonoids, can inhibit the growth of cancer cells and act as "chemo-preventers." The cancer-prophylactic effects of these biomolecules can be assigned to diverse mechanisms, containing the investment of cell-cycle arrest and/or apoptosis besides the antioxidant ability. The antioxidant properties of chemo-preventers have lately had a broad interest, ultimately the oxidative stress involved in the inception and progression of diverse pathological conditions, which includes cancer. The antioxidants are known for their capability to prohibit oxidative damage and the extensive utilization of natural food-derived antioxidants dad major consciousness as potential anti-carcinogens. Quercetin, one of these flavonoids rated as an excellent free-radical scavenger. Despite this, this activity mightily counts on the intracellular availability of reduced glutathione. Besides the antioxidant properties of quercetin as well performs a direct, proapoptotic effect in cancer cells, and prohibits the growth of diverse human cancer cell lines at diversified phases of the cell cycle. These impacts are reported in a broad assortment of cellular models and animal patterns. The altitude toxicity strives by quercetin on tumor cells skillfully corresponds with the roughly total non-attendance of any deterioration for normal, non-transformed cells[2].

Quercetin, as a significant flavonoid existing in many vegetables, fruits, and beverages[3], has another property like anti-hyperlipidemia, anti-hyperglycemia, antiviral, anti-cancer[4], anti-microbial[5], and neuroprotective[6], antioxidant[7], anti-inflammatory[8] and anti-apoptotic[9] effects that might help reduce swelling, kill cancer cells[10], arthritis[11], control blood sugar[12], quercetin derivatives are most commonly used for conditions of the blood vessels and help prevent heart disease[13]. Furthermore, the impacts of quercetin on the amelioration of the signs of Metabolic syndrome (MetS), counting sublime of glucose level, obesity, hyperlipidemia, as well blood pressure. Quercetin also participates in the management of metabolic disorders by assorted mechanisms that resemble rising adiponectin, diminishing leptin, antioxidant ability, lowering of insulin resistance, rising insulin level, and preventing calcium channel[14]. Furthermore, studies of Quercetin on cellular models display nearly comprehensive demonstration of the mechanisms which link Quercetin with oxidative cell balance also to the control of cell-cycle phases. Many studies have been raised impacts of quercetin on abnormal and normal cells. The remarkable toxicity of Quercetin for cancer cells, accompanied by the distinctive extended antiproliferative and proapoptotic influences on normal cells only at high concentrations, are decisive aspects in research of the anticancer field, Which is considered a serious objective is the recognition of drugs that selectively kill cancer cells in the absence of causes any damaging normal cells[15]. Quercetin Schiff bases also gained significant interest due to their variety of biological activities[16-18]. The thiadiazol ring also demonstrated a large spectrum of physical activities such as antioxidant[19], antibacterial, anti-influenza agents[20], anti-amnesic effect in mice[21], antiproliferative and antiangiogenic activity[22], anti-inflammatory[23] besides a large number of its derivatives obtained anticancer activity[24-26].

In this research, we demonstrated two new Quercetin derivatives as Schiff base of 1,3,4-thiadiazole 5-thiol and

Citation: Jasim H S, Al-kubaisi Z A, Al-Shmgani H S. Cytotoxic potential activity of quercetin derivatives on MCF-7 breast cancer cell line. Revis Bionatura 2023;8 (1) 92 http://dx.doi.org/10.21931/RB/2023.08.01.92
Received: 15 January 2023 / **Accepted:** 25 February 2023 / **Published:** 15 March 2023

thio benzyl derivative and studied their toxicity and anticancer activity against the MCF-7 breast cancer cell line. The research focused its attention on understanding and fighting this disease and finding an appropriate treatment with low side effects and less toxicity.

Materials and methods

Chemicals

Quercetin (3,3',4',5,7-pentahydroxyflavone, ≥ 95%) was purchased from Aldirch-Sigma (St Louis, MO, USA). All cell culture medium (RPMI-1640) and other consumable reagents were at analytical grade and obtained from Gibco (Invitrogen, MD, USA). LDH kit was purchased from Jiancheng BioEngineering (Nanjing, China). The Schiff bases of quercetin with 2-amino-1,3,4-thiadiazole-5-thiol (compound 1) was synthesized utilizing procedures described by HS Al-Shmgani *et.al*[27] and the thio etherification with bromobenzyl bromide (compound 2) was done according to procedure described by SA Saoud *et al.*[28]. These compounds were identified from their FT-IR ,^{1}H-NMR,^{13}C NMR and mass spectroscopy.

Cell culture

MCF-7 human breast cancer cell lines were obtained from the Center of Biotechnology/ Al-Nahrain University. Cells were grown in RPMI-1640 medium completed with 1% penicillin-streptomycin and 10% fetal bovine serum and maintained under sterile culture conditions.

Determination of cell viability assay

3-(4,5-dimethylthiazol-z-yl)-2,5-diphenyltetrazolium colorimetric assay (MTT) was assessed to evaluated cell variability according to Al-Kubaisi *et al.*[29]. Cells were cultivated at a concentration of 106 cell/ml in 96 cell well plates, and 100 µl of Q1and Q2 were add at different concentrations (25, 50, 100, 200 µg/ml) to each well. MTT was added after 24 h of incubation and the outcoming formazan was perfectly dissolved in 100 µL of 10% sodium dodecyl sulfate. Absorbance was read at 620 nm using microplat reader (Versa Max TM, Molecular Devices, Sunnyvale, CA). The inhibition percentage was calculated as the following:

Cell viability% = (Ab of control – Ab of treatment) / Ab of control × 100

Where Ab = optical density

Determination of lactate dehydrogenase

Lactate dehydrogenase (LDH) activity was measured to assess the cytotoxicity of Q1 and Q2 derivatives on cells following manufacture instructions. Cells were exposed to different (25,50,100, and 200 µg/ml) concentrations for 48 h. The absorbance was then read at 450 nm using (Versa Max TM, Molecular Devices, Sunnyvale, CA) microplate reader.

Determination of DNA damage

Comet assay (single-cell gel electrophoresis) was used to assess cell DNA strand damage. Briefly, cells were cultured at 106 concentration, treated with 100 µg/ml of Q1 and Q2 and incubated at 37 ºC (5% CO2) for 24 h. Low melting point agarose (in phosphate-buffered saline) was mixed with 106 cells then spread on pre-coated slides with 1% normal-melting agarose. Slides were then kept in lysis buffer for 1h at 4ºC, then placed in an electrophoresis buffer. Electrophoresis has conducted at a field of 24V and 300 mA for 30 min. After neutralization slides were stained with ethidium-bromide (2 mg/ml) and kept in the dark until observed using Leica DMR fluorescence microscopy fitted with 510 nm excitation wavelength and 590 nm emission wavelength. Images were analyzed using Komet image analysis software (version 5) from Andor Technology (Belfast, UK).

Statistical analysis

The data offered as mean ± standard error. One-way analysis of variance (ANOVA) was outright using SPSS software version 16.0. Statistical difference was accepted at P≤0.05.

Results

The chemical structure for compounds 1 and compound 2 was depicted in (Figure 1).

Cell viability

The cell viability of MCF-7 breast cancer was measured by MTT assay. Cells treated with different concentrations (25, 50, 100, 200 µg/ml) of quercetin derivative Q1 and Q2. Results on MCF-7 cells after 24 showed a significant different at the highest concentrations (100 and 200 µg/ml) compared to untreated cell (control cell). Also a considerable variance reveled between the 200 and 100 µg/ml with 25 µg/ml concentration. Effect caused dose-dependent increase manner (Figure 2). However, there was no difference between the two synthesized derivatives, indicating that the quercetin Schiff bases and the thiadiazole ring have the major effect, not the 5-thiol group.

Assessment of cytotoxicity

LDH-released enzyme widely used as a good indicator of cell membrane integrity. Results of LDH released into MCF-7 cells media after treatment with different concentrations of the two synthetic quercetin derivative for 48 h revealed significant dose increase response. Both derivatives showed a significant elevation compered to control group where the highest impact was at 200 µg/ml (Figure 3).

DNA damage assessed by Comet assay

The results from the Alkaline Single-Cell Gel Electrophoresis (SCGE) or comet assay revealed the significant damage to MCF-7 cell induced by treatment with quercetin derivative at 100 µg/ml concentration, as shown in (Figure 4A). Both compounds caused an increase in the tail DNA percentages compared to control (figure 4B). A marginally significant increase in DNA strand breaks was observed in Q2 compared to Q1.

Discussion

The anticancer activity of quercetin has been previously studied[30], but this study was carried out to determine the specific functional group and the structure responsible for that effect. Both compounds induced cell growth inhibition considering the semi-similarity in the chemical system. Inhibition of cancer cell growth index could be through suppression of cell cycle and proliferative pathways or induced apoptotic pathways. Possible mechanisms explaining

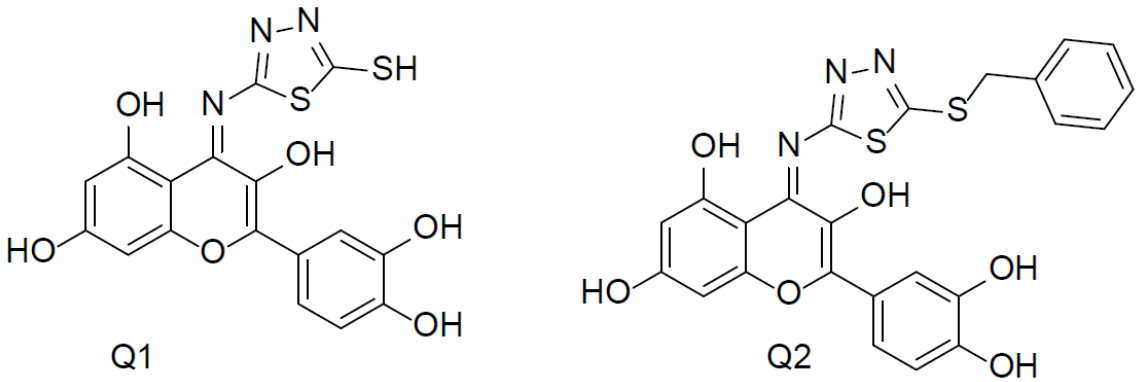


Figure 1. The molecular structure of compounds 1 and 2.

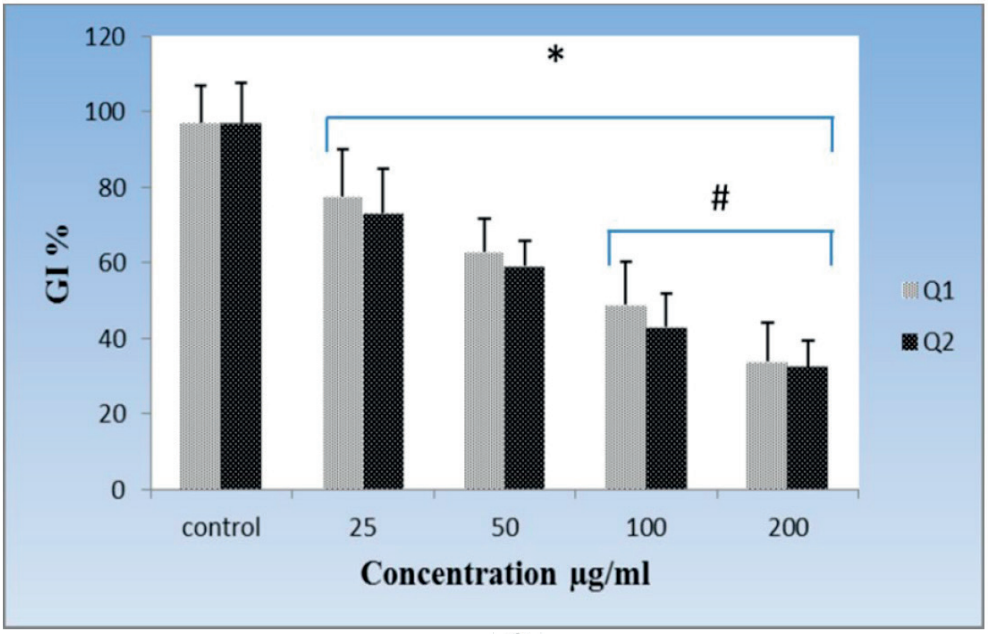


Figure 2. MCF-7 human breast cells growth inhibition in control (untreated cells) and groups treated with quercetin (Q1 and Q2) derivative compounds. * indicated a significant decrease compared to the control group. # indicated a significant decrease in 100 and 200 µg/ml compared to 25 µg/ml group. Data are checked in as mean ± SD from three independent experiments.

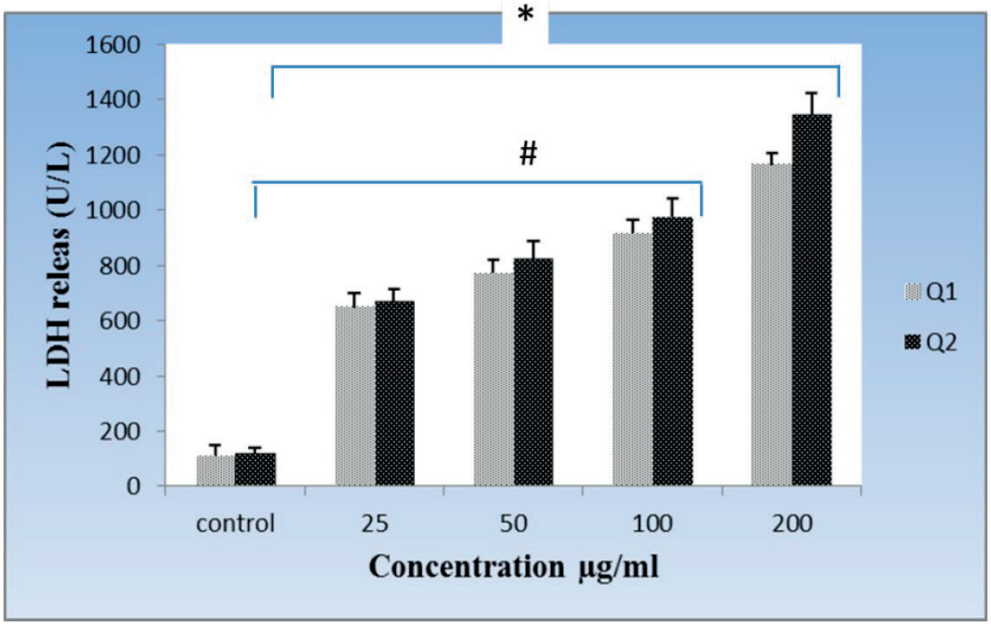
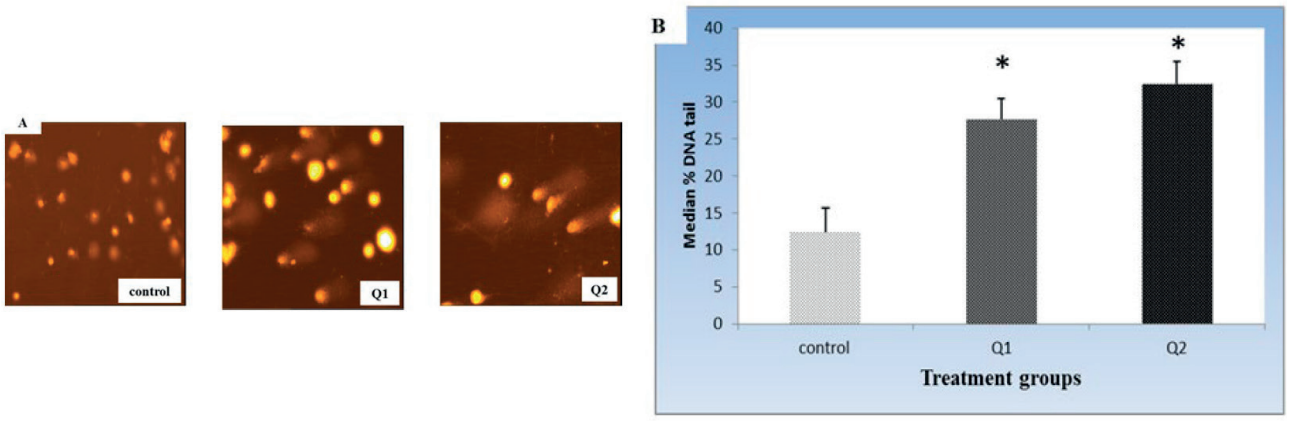


Figure 3. Lactate dehydrogenase (LDH) activity on MCF-7 human breast cells in control (untreated cells) and treated groups with quercetin (Q1 and Q2) derivative compounds. * indicated a significant decrease compared to the control group. # indicated a significant decrease in 200 µg/ml compared to other treated groups. The data are checked in as mean ± SD from three independent experiments.

its anticancer activity are either by stimulating apoptotic pathways or suppressing cell survival pathways. Their chemical structure can regulate the tendency of flavonoids to scavenge free radicals; therefore, the position and number of substitutions can impact their free radicals' inhibition activity. It is well known that compounds having an electron donating group and the lipophilic group have less cell cytotoxicity due to increasing its ability of membrane penetration and lipophilicity activity[31].

Cell death can also determine by LDH release into the medium. In this study, the phenomenon that derivative compounds structurally related to parent quercetin influen-



(a) (b)

Figure 4. (a) Comet assay results in MCF-7 human breast cancer cell after treatment with quercetin derivative (Q1 and Q2) at 100 µg/ml; (b) the results showed that both compounds had significant difference increase in the median DNA percentage compared to control (untreated cell). Data represent mean ± SD from three independent experiments.

ced solid inhibitory effects in a dose-dependent manner on MCF-7 human cancer cell line. A previous study has shown that reactive oxygen species (ROS) played a vital role in apoptosis, and increased ROS levels are associated with elevated LDH level[32]. Also, ROS production led to cell death through mitochondria pathway[33]. some previous reports indicated that quercetin and its derivatives possess strong antioxidant activity in MCF-7 cell line. Another mechanism involved in the anticancer activity of quercetin and its products is due to cell circle arrest at G2/M and G1/S[34].

One of the suggested mechanisms by which DNA damage in cells occurs is the increased production of free radicles and especially reactive oxygen species (ROS), causing oxidative stress. Oxidants induce both biochemical and morphological alterations via the oxidation of cellular components. ROS can induce DNA damage either by base modifications or strand breaks; this strand breaks due to the activation of nucleases or the formation of H_2O_2 and OH•. H_2O_2 is required for lipid peroxidation and probably the DNA damage detected by the current results. Studies have reported the preventive role of quercetin as a potent antioxidant agent through reducing ROS levels, increasing antioxidant enzyme activity and directly affecting glutathione level[35]. This study is in contrast with (36), who suggested an anti-carcinogenic activity of quercetin based on their funding that quercetin is able to protect against H_2O_2-caused DNA damage in human lymphocytes. Moreover, quercetin has been demonstrated to restrain tumor growth in human ovarian cancer by provoked apoptosis via increasing Bax and p21 expression and decreased Bcl-2 expression[37].

Conclusions

Evolution of newly synthesized quercetin derivative cytotoxicity on MCF-7 cell line by MTT, LDH and Comet assay indicated significant cytotoxic to the cell in a dose-dependent aspect. These findings highlight the potential role in breast cancer treatment, so it can be used as possible alternative therapy or supplement. Therefore, it is essential to identify the effective functional structure responsible for cytotoxicity and further to study their *in vitro* and *in vivo* effects.

Acknowledgments

We would like to thank the Center of Biotechnology/ Al-Nahrain University staff.

Bibliographic references

1. Wang R, Yang L, Li S, et al. Quercetin inhibits breast cancer stem cells via downregulation of aldehyde dehydrogenase 1A1 (ALDH1A1), chemokine receptor type 4 (CXCR4), mucin 1 (MUC1), and epithelial cell adhesion molecule (EpCAM). Medical Science Monitor: international medical journal of experimental and clinical research. 2018;24:412.
2. Gibellini L, Pinti M, Nasi M, et al. Quercetin and cancer chemoprevention. Evidence-based complementary and alternative medicine. 2011;2011
3. Jeong JH, An JY, Kwon YT, Rhee JG, Lee YJ. Effects of low dose quercetin: Cancer cell□specific inhibition of cell cycle progression. Journal of cellular biochemistry. 2009;106(1):73-82.
4. Kubina R, Iriti M, Kabała-Dzik A. Anticancer potential of selected flavonols: Fisetin, kaempferol, and quercetin on head and neck cancers. Nutrients. 2021;13(3):845.
5. Elloumi W, Mahmoudi A, Ortiz S, Boutefnouchet S, Chamkha M, Sayadi S. Wound healing potential of quercetin-3-O-rhamnoside and myricetin-3-O-rhamnoside isolated from Pistacia lentiscus distilled leaves in rats model. Biomedicine & Pharmacotherapy. 2022;146:112574.
6. Grewal AK, Singh TG, Sharma D, et al. Mechanistic insights and perspectives involved in neuroprotective action of quercetin. Biomedicine & Pharmacotherapy. 2021;140:111729.
7. Ahmad, A. Sh., F.T. Al-Rawi, Y.T. Abdul-Rahaman, TH. T. Mohammed, K.I. Mahmud. Effect of Tryptophan injection on the semen quality in Iraqi Shami buck. IOP Conference Series: Earth and Environmental Science, 2021, 761(1), 012093.
8. Lesjak M, Beara I, Simin N, et al. Antioxidant and anti-inflammatory activities of quercetin and its derivatives. Journal of Functional Foods. 2018;40:68-75.
9. AL- Shamary, W. A. .; Alkhateb, B. A. A. H. .; Abdel, E. T. . Role Of Perlite Quantity And Intervals Of Irrigation On Potatoes (Solanum Tuberosum L.) Grown In Gypsiferous Soil. Journal of Life Science and Applied Research. 2020, 1, 31-39.
10. Russo GL, Russo M, Spagnuolo C, et al. Quercetin: a pleiotropic kinase inhibitor against cancer. Advances in nutrition and cancer. 2014:185-205.
11. Haleagrahara N, Miranda-Hernandez S, Alim MA, Hayes L, Bird G, Ketheesan N. Therapeutic effect of quercetin in collagen-induced arthritis. Biomedicine & Pharmacotherapy. 2017;90:38-46.

12. Hosseini A, Razavi BM, Banach M, Hosseinzadeh H. Quercetin and metabolic syndrome: A review. Phytotherapy Research. 2021;35(10):5352-5364.
13. Chekalina N, Trybrat T, Burmak Y, Petrov Y, Manusha Y, Kazakov Y. Effect of quercetin on parameters of central hemodynamics and myocardial ischemia in patients with stable coronary heart disease. 2017;
14. Ostadmohammadi V, Milajerdi A, Ayati E, Kolahdooz F, Asemi Z. Effects of quercetin supplementation on glycemic control among patients with metabolic syndrome and related disorders: A systematic review and meta□analysis of randomized controlled trials. Phytotherapy Research. 2019;33(5):1330-1340.
15. Li S, Yuan S, Zhao Q, Wang B, Wang X, Li K. Quercetin enhances chemotherapeutic effect of doxorubicin against human breast cancer cells while reducing toxic side effects of it. Biomedicine & Pharmacotherapy. 2018;100:441-447.
16. Moodi Z, Bagherzade G, Peters J. Quercetin as a precursor for the synthesis of novel nanoscale Cu (II) complex as a catalyst for alcohol oxidation with high antibacterial activity. Bioinorganic chemistry and applications. 2021;2021
17. Shastrala K, Kalam S, Damerakonda K, et al. Synthesis, characterization, and pharmacological evaluation of some metal complexes of quercetin as P-gp inhibitors. Future Journal of Pharmaceutical Sciences. 2021;7(1):1-13.
18. Bukhari SB, Memon S, Mahroof-Tahir M, Bhanger M. Synthesis, characterization and antioxidant activity copper–quercetin complex. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2009;71(5):1901-1906.
19. Muğlu H, Akın M, Çavuş MS, Yakan H, Şaki N, Güzel E. Exploring of antioxidant and antibacterial properties of novel 1, 3, 4-thiadiazole derivatives: Facile synthesis, structural elucidation and DFT approach to antioxidant characteristics. Computational Biology and Chemistry. 2022;96:107618.
20. Dong J, Pei Q, Wang P, Ma Q, Hu W. Optimized POCl3-assisted synthesis of 2-amino-1, 3, 4-thiadiazole/1, 3, 4-oxadiazole derivatives as anti-influenza agents. Arabian Journal of Chemistry. 2022;15(4):103712.
21. da Costa Rodrigues K, de Oliveira RL, da Silva Chaves J, et al. A new arylsulfanyl-benzo-2, 1, 3-thiadiazoles derivative produces an anti-amnesic effect in mice by modulating acetylcholinesterase activity. Chemico-Biological Interactions. 2022;351:109736.
22. Faraji A, Motahari R, Hasanvand Z, et al. Quinazolin-4 (3H)-one based agents bearing thiadiazole-urea: Synthesis and evaluation of antiproliferative and antiangiogenic activity. Bioorganic Chemistry. 2021;108:104553.
23. Omar YM, Abdu-Allah HH, Abdel-Moty SG. Synthesis, biological evaluation and docking study of 1, 3, 4-thiadiazole-thiazolidinone hybrids as anti-inflammatory agents with dual inhibition of COX-2 and 15-LOX. Bioorganic Chemistry. 2018;80:461-471.
24. Bahadur A, Iqbal S, Muneer S, Alsaab HO, Awwad NS, Ibrahium HA. Synthesis, carbonic anhydrase enzyme inhibition evaluations, and anticancer studies of sulfonamide based thiadiazole derivatives. Bioorganic & Medicinal Chemistry Letters. 2022;57:128520.
25. Abas M, Bahadur A, Ashraf Z, et al. Designing novel anticancer sulfonamide based 2, 5-disubstituted-1, 3, 4-thiadiazole derivatives as potential carbonic anhydrase inhibitor. Journal of Molecular Structure. 2021;1246:131145.
26. Chidella K, Seelam N, Cherukumalli PKR, Reddy J, Sridhar G. Design and synthesis of novel 1, 2, 4-Thiadiazole linked imidazo [1, 2-b] pyridazine as anticancer agents. Chemical Data Collections. 2020;30:100554.
27. Al-Shmgani H, Shakir R, Wahed A, Elekofehinti O. Design, Synthesis, Docking, Antitumor Screening, And Absorption, Distribution, Metabolism, And Excretion Prediction Of New Hesperdin Derivative. Asian Journal of Pharmaceutical and Clinical Research. 2020;13:24-31.
28. Saoud SA, Ali KF, Shakir RM. Relationship Between the structure of Newly Synthesized derivatives of 1, 3, 4-oxadiazole Containing 2-Methylphenol and their Antioxidant and Antibacterial Activities. Oriental Journal of Chemistry. 2017;33(4):1781.
29. AL-Kubaisi ZA, Al-Shmgani H, Salman M. Evaluation of In vivo and In vitro protective effects of quercetin on lipopolysaccharide-induced inflammation and cytotoxicology. Research Journal of Pharmacy and Technology. 2020;13:3897-3902.
30. Maleki Dana P, Sadoughi F, Asemi Z, Yousefi B. Anti-cancer properties of quercetin in osteosarcoma. Cancer Cell International. 2021;21(1):1-9.
31. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. The Journal of nutritional biochemistry. 2002;13(10):572-584.
32. Liu X, Song L. Quercetin protects human liver cells from o, p'-DDT-induced toxicity by suppressing Nrf2 and NADPH oxidase-regulated ROS production. Food and Chemical Toxicology. 2022;161:112849.
33. Al-Rikabi R, Al-Shmgani H, Dewir YH, El-Hendawy S. In vivo and in vitro evaluation of the protective effects of hesperidin in lipopolysaccharide-induced inflammation and cytotoxicity of cell. Molecules. 2020;25(3):478.
34. Alhakamy NA, Fahmy UA, Eldin SMB, et al. Scorpion Venom-Functionalized Quercetin Phytosomes for Breast Cancer Management: In Vitro Response Surface Optimization and Anticancer Activity against MCF-7 Cells. Polymers. 2021;14(1):93.
35. Xu D, Hu M-J, Wang Y-Q, Cui Y-L. Antioxidant activities of quercetin and its complexes for medicinal application. Molecules. 2019;24(6):1123.
36. Wilms LC, Hollman PC, Boots AW, Kleinjans JC. Protection by quercetin and quercetin-rich fruit juice against induction of oxidative DNA damage and formation of BPDE-DNA adducts in human lymphocytes. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2005;582(1-2):155-162.
37. Gong C, Yang Z, Zhang L, Wang Y, Gong W, Liu Y. Quercetin suppresses DNA double-strand break repair and enhances the radiosensitivity of human ovarian cancer cells via p53-dependent endoplasmic reticulum stress pathway. OncoTargets and therapy. 2018;11:17.