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Research Article

Evaluation of the Wound-Healing Activity and Apoptotic Induction of New Quinazolinone Derivatives

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Abstract

Background: Chemotherapeutic medication treatment for cancer is typically used in conjunction with other techniques as part of a routine regimen. It is well established that the capacity of different chemotherapeutic drugs to induce apoptosis is correlated with their anticancer efficacy. Quinazolinone-based drugs have demonstrated excellent responses from several cancer cell types. These substances have a lot of potential for use as building blocks in the creation of apoptosis inducers. ***Objective***: To assess the new quinazolinone derivatives (M1 and M2) that were recently synthesized for their potential to halt wound healing and to use the acridine orange/propidium iodide (AO/PI) double stain to assess their capacity to induce apoptosis in the chosen cancer cell lines. ***Methods***: Using the breast carcinoma cell line (MCF-7) and the lung adenocarcinoma cell line (A549), two quinazolinone derivatives (M1 and M2) were investigated for their capacity to inhibit wound healing and induce apoptosis. ***Results***: In both cell lines, the chemicals were found to be effective inducers of apoptosis and to considerably limit wound healing. ***Conclusions***: In cancer cell lines (MCF-7 and A549), compounds M1 and M2 efficiently inhibited wound repair and triggered apoptosis.

Keywords: Apoptosis, Breast cancer, Lung adenocarcinoma, Quinazolinone, Scratch test.

تقييم نشاط التئام الجروح وتحريض موت الخلايا المبرمج لبعض مشتقات الكينازولينون الجديدة

الخلاصة

الخلفية: غالبًا ما يتم علاج السرطان بأدوية العلاج الكيميائي مع طرق أخرى كجزء من النظام العلاجي القياسي. من المعروف أن النشاط المضاد للأورام للعديد من عوامل العلاج الكيميائي يرتبط بقدراتها على إحداث موت الخلايا المبرمج. أظهرت المركبات التي تعتمد على الكينازولينون أنشطة ملحوظة في خطوط الخلايا السرطانية المختلفة، وبالتالي تمثل سقالات واعدة في تصميم محفزات موت الخلايا المبرمج. **الأهداف:** تهدف الدراسة الى تقييم قدرات مشتقات الكينازولينون المُصنَّعة مؤخرًا (M1 و M2) لإيقاف التئام الجروح وكذلك قدرة هذه المركبات المُصنَّعة على تحفيز موت الخلايا المبرمج في خطوط الخلايا السرطانية المختارة عن طريق استخدام أكريدين البرتقالي/ بوديد البروبيديوم (AO / PI) صبغة مزدوجة. **الطريقة:** في هذا البحث تم اختبار اثنين من مشتقات الكينازولينون (M1 و M2) لقدرتهما على إيقاف التئام الجروح والحث على موت الخلايا المبرمج باستخدام خطين من الخلايا السرطانية ، خط خلايا سرطان الثدي (MCF-7) وخط خلايا سرطان الرئة (A549). **النتائج**: اظهرت المركبات قدرة عالية على تحفيز موت الخلايا المبرمج وثبطت بشكل معنوي التئام الجروح في كلا خطوط الخلايا السرطانية. **الأستنتاج**: في خطوط الخلايا السرطانية قيد الدراسة منعت المركبات M1 و M2 بكفاءة إصلاح الجروح وتسببت في احداث موت الخلايا المبرمج.

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INTRODUCTION

One of the most challenging medical conditions to treat is cancer, which is a serious health issue. It is distinguished by the unchecked growth and infiltration of malignant cells into adjacent tissues [1]. Due to the limited efficacy of currently known anticancer drugs to stop the growth of this terrifying disease, researchers are always looking for new targets and ways to optimize existing therapy in order to increase patient survival rates [2]. One definition of apoptosis is a naturally occurring process for cell death that is predetermined. It is especially crucial for long-lived mammals [3]. It has a high degree of regulation and seeks to eradicate any undesirable and superfluous cells. The activation of

the apoptotic pathway is caused by a number of undesirable situations, including unregulated cell proliferation [5] and DNA damage [4]. Cytochrome c, endoribonuclease G, and other mitochondrial proteins are released during apoptosis when the outer membrane of the mitochondria opens; this release is caused by an increase in the permeability of the mitochondria's transition pore. Apoptosis is brought on by the activation of caspase 3, which is the final step in the caspase 9 initiator cascade [6]. Controlling the fast proliferation of cancerous cells through the natural process of cell death is one of the key tactics used in the fight against the disease and is thought to be a very effective technique. For certain cancer types, apoptosis targeting is the most efficacious non-invasive treatment. Since cancer cells are thought to have evolved a method to evade apoptosis, numerous cytotoxic medicines have been created to target different stages of apoptosis in both the intrinsic and extrinsic routes [7,8]. Moreover, PPARγ activation and inhibition of COX2 expression have been shown to inhibit proliferation and induce apoptosis in pancreatic cancer [9]. Compounds based on 4(3H)-quinazolinone have a variety of pharmacological characteristics, including anti-inflammatory, anti-cancer effects [10], and cytotoxic potential. The inhibition of many enzymes required in cell division, such as thymidylate synthase and dihydrofolate reductase, represents the anticancer activity of quinazolinone derivatives. also, the inhibition of tyrosine kinase and histone deacetylase by 1,3,4-thiadiazole represents a promising antitumor activity for many cancer cell lines [11,12]. The current study aims to assess the potential of the newly synthesized quinazolinone derivatives (M1 and M2) (Figure 1) to trigger apoptosis in a subset of cancer cell lines and stop wound healing.

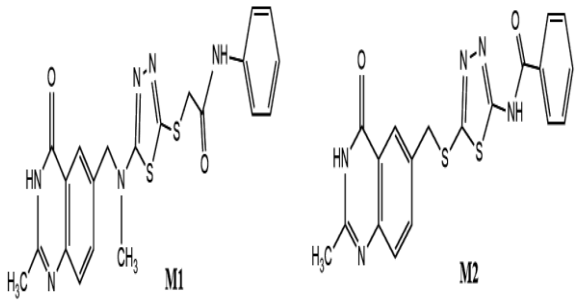

Figure 1: Chemical formula of the tested compounds.

METHODS

Materials

Human lung adenocarcinoma cell line A549 and human breast cancer cell line MCF-7 were cultured in RPMI-1640 media (Capricorn, Germany), 10% fetal bovine serum (FBS; Capricorn, Germany), Trypsin-EDTA (Capricorn, Germany), Streptomycin (Sigma), and penicillin (Sigma).

Maintenance of cell cultures

We cultured human lung adenocarcinoma cell line A549 and human breast cancer cell line MCF-7 in RPMI-1640 media supplemented with 10% fetal bovine serum, 100 g/mL streptomycin, and 100 IU/mL penicillin. After adding trypsin-EDTA, we reseeded the cells twice a week at 50% confluence before incubating them at 37 °C [13].

Scratch test for wound healing

In a 24-well plate, the selected cancer cells (MCF-7 and A549) were planted at a density of 1*105 cells per well. After bringing the cells in a monolayer to confluence, a 1 mm incision-like gap was formed by scratching them with a pipette tip. The cells were then cultivated and left to grow for 72 hours. The gap distance was computed every 24 hours using Image J software, and photos were taken every 24-72 hours. The 24 wound healing trials included four duplicates of each group (control, compound M1, and compound M2 treated groups) [14].

Apoptosis estimation

To assess apoptotic death in the cell lines, we used two different dyes: Propidium Iodide (AO/PI, Sigma) and Acridine Orange. Each well on the plate had 7,000 uniformly distributed cells. We then provided the examined chemicals (M1 and M2) at their IC50 concentrations to them for 72 hours. After that, dual staining was performed in an incubator set at 37°C. The pictures were captured using a Leica fluorescence microscope after the testing wells were precisely treated with 50µl of the AO/PI dye for 30 seconds [15].

Statistical analysis

The wound healing test determined the percentage of wound closure by examining the open wound area. The distance measured at time 0 was assumed to be 0%. Treatments were compared using an analysis of variance (ANOVA) test, with untreated cells serving as the control. Results were displayed as mean±standard deviation (SD). The AO/PI assay results for apoptosis estimation were presented as mean±SD. To compare fluorescence intensities, GraphPad Prism 6 was used, along with an unpaired t-test. A p-value of <0.05 was considered significant [16].

RESULTS

As seen in Figures 2, 3 and 5, a series of photos taken before, following 24, 48, and 72 hours after scratching were used to track the percentage of wound closure over time. In both cancer cells (MCF-7 and A549), compound M1 and compound M2 significantly impaired wound healing in the treated cells relative to the untreated control cells. After the first 72 hours of treatment, the results were more noticeable.

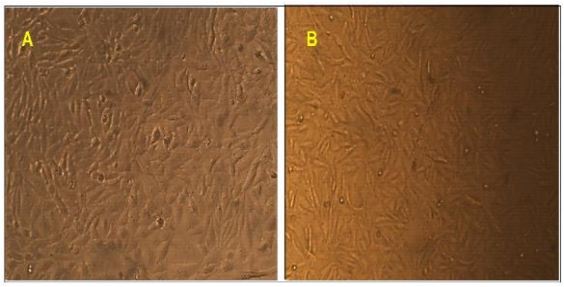

Figure 2: Monolayer of the cancer cells MCF-7 (A) and A549 (B) before scratching.

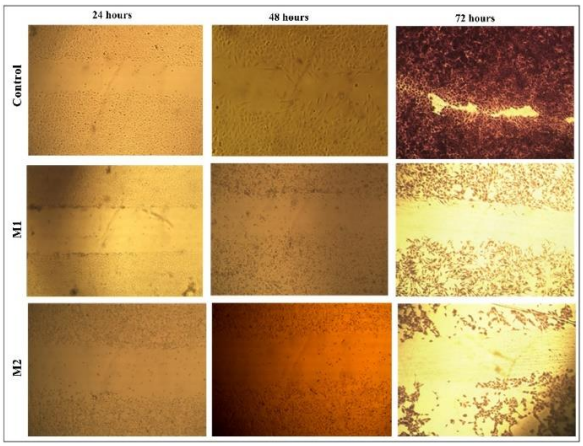

Figure 3: Time-lapse images of a representative scratch assay for the breast cancer cell line (MCF-7): control and treated cells. Groups are schemed at time 24, 48 and 72 h. for each image.

A popular dual fluorescence staining technique for identifying tumor cell apoptosis is AO/PI labeling. The statistical evaluation of the gap-closing capabilities of the investigated compounds revealed that M1 and M2 inhibit wound repair on the breast cancer cell line (MCF-7) in a significant manner.

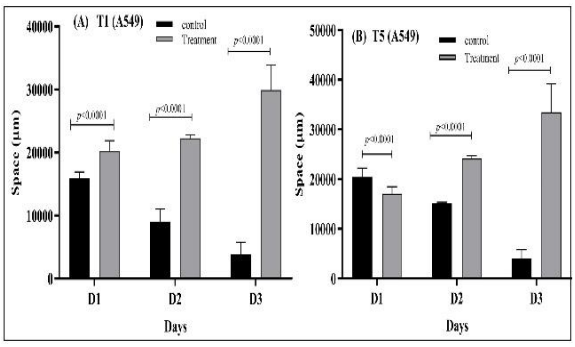

Figure 4: Measurements of gap closure representative scratch assay for the lung cancer cell line (A-549): (control and treated). Groups are schemed at time 24, 48 and 72 h. for each image. (A) Compound M1 treated cells and (B) Compound M2 treated cells). Data were expressed as means ±SD.

Nevertheless, with the passage of 24 hours, compound M2 demonstrated a more conspicuous impact on the process of wound healing. On the other hand, compound M1 inhibited gap closure on the lung cancer cell line (A-549) substantially more statistically significant than compound M2 after 24 hours of treatment (Figures 4 and 6). As seen in Figures 7 and 9, AO is distinguished by its capacity to pass through intact cell membranes and can be seen as green fluorescence, whereas apoptotic cells are stained orange or red [17].

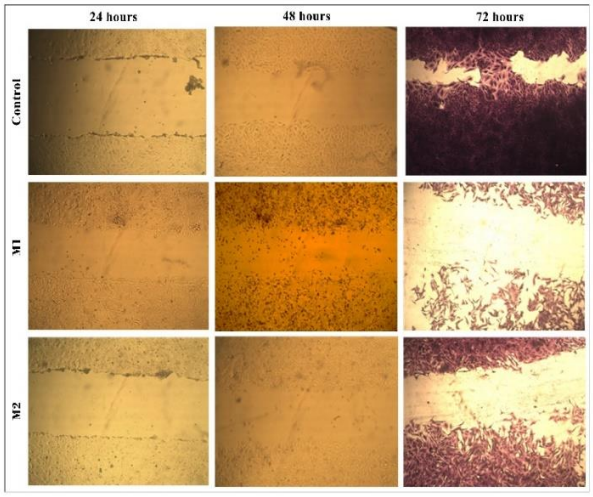

Figure 5: Time-lapse images of a representative scratch assay for the lung cancer cell line (A549): control and treated cells. Groups are schemed at time 24, 48 and 72 h. for each image.

Quantitative analysis verified the results, demonstrating in Figures 8 and 10 a notable increase in apoptotic cells in treated cancer cells relative to untreated control cells in the chosen cell lines (MCF-7 and A549).

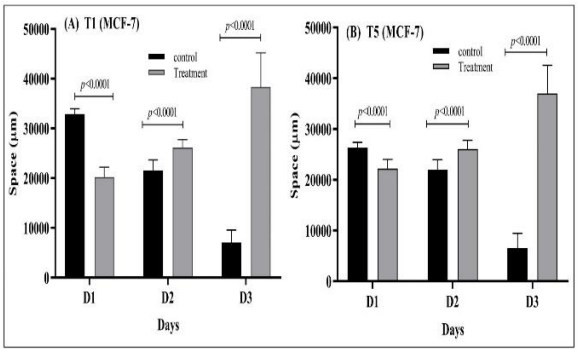

Figure 6: Measurements of gap closure representative scratch assay for the breast cancer cell line (MCF-7): (control and treated). Groups are schemed at time 24, 48 and 72 h. for each image. (A) Compound M1 treated cells and (B) Compound M2 treated cells. Data were expressed as means ±SD.

DISCUSSION

Quinazoline and its pyrimidine benzo derivatives are compounds of medical significance in the field of medicinal chemistry. These substances exhibit a diverse array of biological characteristics, such as anticancer, antifungal, antiviral, antimicrobial, and anti-inflammatory properties [18]. The quinazolinone-based compounds (M1 and M2) utilized in this investigation were previously identified as promising anticancer agents [19] due to their potent cytotoxic effects, maximal inhibition of wound closure, and promising anti-invasive and anti-adhesive properties.

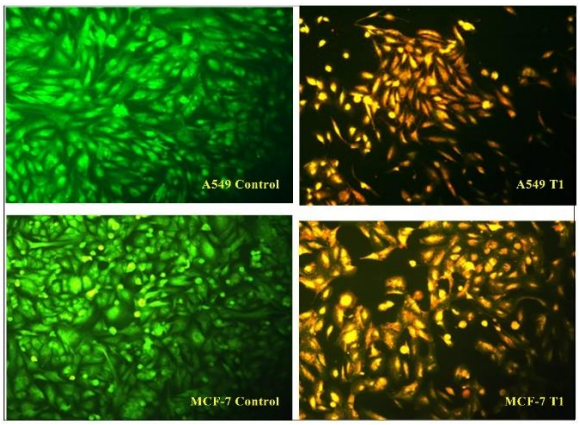

Figure 7: Analysis the ability of M1 to induce apoptosis in A549 and MCF-7 cells using AO/PI.

Using a double stain of acridinium iodide and citric acid, it was determined that the examined compounds (M1 and M2) were capable of inducing apoptosis in the selected cancer cells. Consequently, these techniques of cell morphological evaluation were implemented in order to detect indications of apoptosis [17].

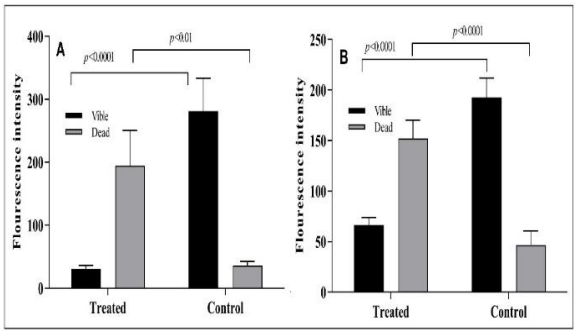

Figure 8: The fluorescence intensity of MCF-7 cells treated with tested compounds: M1 (A) and M2 (B) compared with control. Data were expressed as means±SD.

Based on a statistical examination of the gap closure capabilities of the compounds under investigation, it was determined that M1 and M2 significantly impede wound healing in the MCF-7 breast cancer cell line. However, compound M2 had a more noticeable impact on wound healing following a 24-hour treatment period.

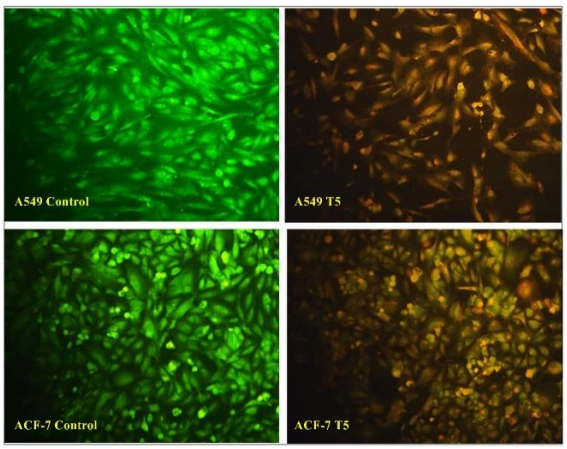

Figure 9: Analysis the ability of compound M2 to induce apoptosis in A549 and MCF-7 cells using AO/PI.

On the other hand, compound M1 significantly more statistically significant suppressed gap closure on the lung cancer cell line (A-549) than compound M2 following a 24-hour treatment period (Figures 4 and 6). Upon conducting an examination of apoptotic induction, it was observed that compound M2 induced apoptotic cell death in the breast cancer cell line (MCF-7) with a statistically significant degree of efficacy (p<0.0001), surpassing that of compound M1 (p<0.01). The same outcomes were observed on the lung cancer cell line (A-549), where compound M2 induced apoptosis more readily (p<0.0001) on the treated cells than compound M1 (p<0.01).

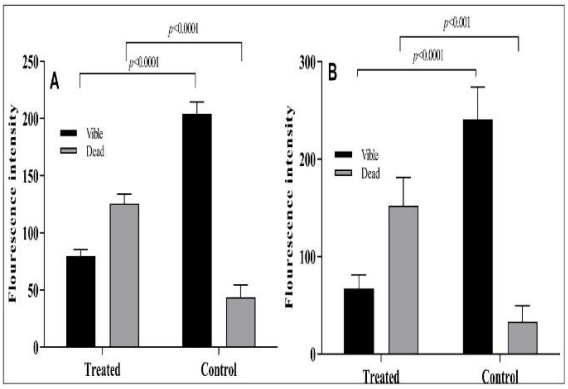

Figure 10: The fluorescence intensity of A549 cells treated with tested compounds: M1 (A) and M2 (B) compared with control. Data were expressed as means±SD.

On the basis of these findings, it can be concluded that compound M2 is more efficacious and possesses greater apoptotic capabilities against the selected cancer cell lines than compound M1. It was discovered that these compounds induced apoptosis via pathways comparable to those utilized by other quinazolinone compounds that were synthesized [20].

Conclusion

The compounds containing quinazolinone (M1 and M2) inhibited wound healing and induced apoptosis in the selected cancer cell lines (MCF-7 and A549). A comprehensive evaluation of these compounds, including their capacity to induce apoptosis and their mechanisms of action, can yield significant insights that could inform their potential application in cancer therapy.

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Conflict of interests

No conflict of interests was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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