

Cytotoxic Evaluation of Some Fused Pyridazino- and Pyrrolo-quinazolinones Derivatives on Melanoma and Prostate Cell Lines

Abstract

Background: Quinazolinone as an important class of heterocycles is attractive in medicinal research areas due to their wide range of biological effects. Cytotoxic activities of the quinazolinone derivatives in various cell lines including: HeLa, L1210 (mouse lymphocytic leukemia) and HT29 (human colon adenocarcinoma) were reported. **Materials and Methods:** In this study, a number of newly made tricyclic quinazolinone derivatives such as fused pyridazino-quinazolinones and fused pyrrolo-quinazolinones were evaluated on two cancerous cell lines, melanoma (B16F10) and prostate (PC3) using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide colorimetric assay. **Results:** The results of cytotoxicity evaluations indicated that almost all of the compounds at the concentrations of 10 and 100 μM showed significant differences in viability in comparison with negative control at 48 h exposure ($P < 0.05$). However, during 24 h exposure some of the compounds showed cytotoxicity activity. **Conclusion:** Results showed that both cell lines were sensitive to synthesized compounds and longer duration of exposure (48 h) had better results compared to that of 24 h screening.

Keywords: Cytotoxic, melanoma and prostate cancer, quinazolinone

Introduction

Of greatest problems in cancer chemotherapy is tumor cells resistance to a wide range of cytotoxic drugs. Major efforts have been carried out to synthesize new anticancer agents with improved efficacy.^[1] Some herbal components have also shown promising effects.^[2,3] Quinazolinone and its derivatives [Figure 1] are noteworthy due to their wide range of biological effects^[4,5] which include antibacterial, antifungal,^[6-14] anticancer,^[15-17] anti-inflammatory^[18-20] and antihypertensive.^[21]

Proposed anticancer mechanisms for quinazolines include: Inhibition of the DNA repair enzyme system,^[16] interaction with biological nucleophiles such as L-cysteine and sulfhydryl bearing enzymes in a Michael-type addition reaction as an alkylation agent,^[17] inhibition of epidermal growth factor receptor (EGFR) (a cellular trans-membrane tyrosine kinases that is over-expressed in a significant number of human tumors),^[16] thymidylate enzyme inhibition^[22] and inhibitory effects for tubulin polymerize.^[23,24] Raltitrexed [Figure 2] (a thymidylate enzyme inhibitor)

and gefitinib (an EGFR inhibitor) are marketed quinazolinone derivatives with anticancer activities.^[16]

In the previous works, we synthesized a series of novel quinazolinone derivatives (fused pyridazino-quinazolinones and fused pyrrolo-quinazolinones).^[25] During screening for evaluation of cytotoxicity, these molecules exhibited different cytotoxicity in the range of 10–100 μM on HeLa cell line. In this study cytotoxic effects of some of the selected compounds were measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay on melanoma and prostate cell lines after 24 and 48 h exposure.

Materials and Methods

Cell lines and cell culture

Two cancerous cell lines including prostate (PC3) and melanoma (B16F10) were purchased from national cell bank of Iran affiliated to Pasteur Institute, Tehran, Iran, and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplement with 1% antibiotics (100 units/ml penicillin and

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100 µg/ml streptomycin) plus 10% fetal calf serum till the third passage were performed before evaluating cytotoxicity effects. All the cell culture materials were purchased from Gibco, USA. Cells were grown at the temperature of 37°C and in 5% CO₂/air.

Sample preparations

The prepared stock solutions of compounds (10 mM) in dimethyl sulfoxide (DMSO) were diluted with the medium (DMEM) to reach 10, 100, 1000 µM concentrations.

In vitro cytotoxicity assay

In vitro cytotoxicity assay was initiated by separately plating (180 µl) of the melanoma and prostate cells (5×10^4 cells/ml of media) in 96-well micro plates and incubating for 24 h (37°C, air humidified 5% CO₂). After 24 h, 20 µl of each dilution of compounds was added to the 96-well micro plate containing 180 µl of the cell suspensions in order to obtain 1, 10, 100 µM concentrations. Wells containing 180 µl of the cell suspension and 20 µl of DMSO (1%) were considered as negative control while the blank wells contained only 200 µl of the DMEM medium. The micro-plates were further incubated for 24 or 48 h at the same condition. Each well was then treated with 20 µl of MTT solution for 3 h. Afterward, the media in each well was replaced with 200 µl DMSO to dissolve the blue insoluble formazan crystals. The metabolic activity in each well was determined by a rapid colorimetric assay using MTT.^[26,27] Plates were read using an enzyme-linked immunosorbent assay plate reader at 540 nm. The cell viability was determined by the following formula 1 and was compared with untreated control.

Formula 1:

$$\% \text{ survival} = \frac{\text{Mean of the well absorbance} - \text{Mean of the blank absorbance}}{\text{Mean of the negative control absorbance} - \text{Mean of the blank absorbance}}$$

Results

The cytotoxicity of compounds [Figure 3] were evaluated against melanoma and prostate cell lines at different concentrations (final concentrations 1, 10, and 100 µM) after 24 and 48 h using MTT assay [Figures 4-7]. Metabolic reduction of soluble MTT by succinic dehydrogenase enzyme of mitochondria took place when tumor cells were viable. The results are the mean of three triplicate experiments. Analysis of variance carried out by Tukey test and significance differences level was set at $P < 0.05$. The synthesized target molecules exhibited significant cytotoxicity in the range of 10–100 µM on melanoma and prostate cell lines after 48 h.

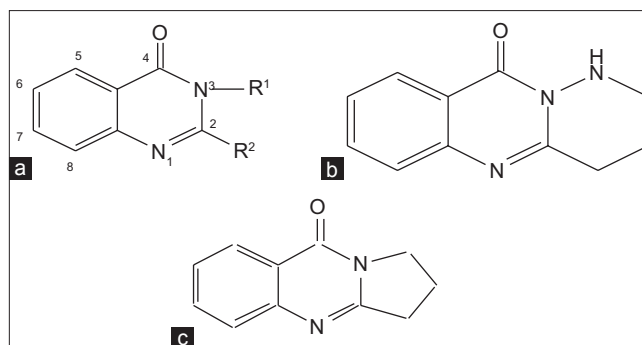


Figure 1: Quinazolinone basic structure (a) fused-pyridazino-quinazolinones (b) and fused-pyrrolo-quinazolinones (c) derivatives

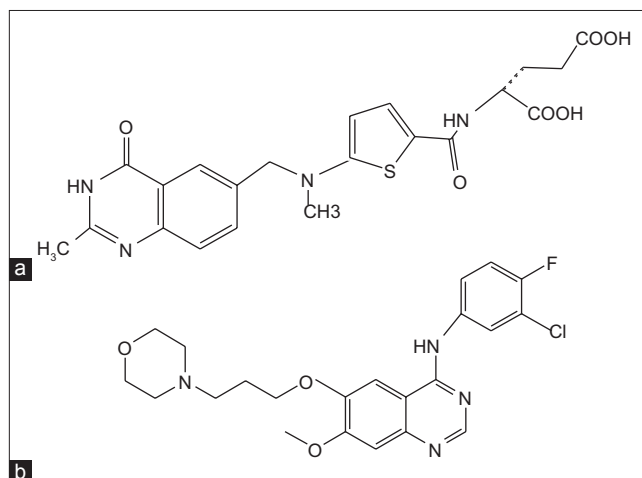


Figure 2: Raltitrexed (a) and gefitinib (b)

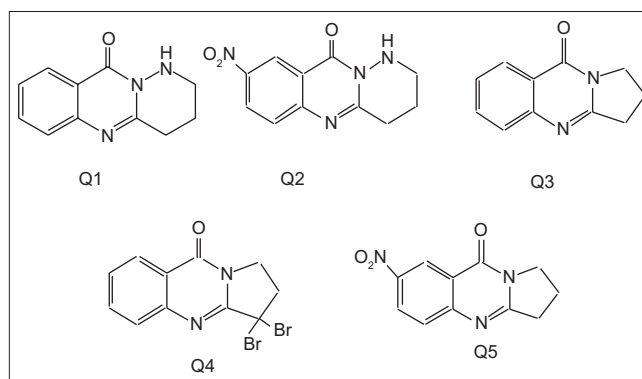


Figure 3: Fused-pyridazino-quinazolinones (Q1 and Q2) and fused-pyrrolo-quinazolinones (Q3, Q4, Q5) derivatives

Discussion

Quinazolinone derivatives have therapeutic benefit as anticancer agents for activity in early and advanced tumors.^[15] Amine or substituted amine on 4th position and either halogens or electron-rich substituents on 6th position of quinazolinone can improve activity against cancer cell lines.^[15]

In the previous work, novel quinazolinone derivatives (fused pyridazine-quinazolinones and fused pyrrolo-quinazolinones

and other derivatives) were synthesized and screened against Hella cell line by our group.^[28] Some of these compounds showed significant cytotoxic activity on HeLa cell line in the range of 10–100 μ M and obtained results revealed that the nitro substituted compounds were more cytotoxic than their bromo-containing counterparts also compounds Q3 and Q4 exhibited acceptable cytotoxicity approximately 50% at 10 μ M concentration on this cell line. It could be concluded that the existence of a substituent NO₂ group on 6th position of the phenyl ring could improve the cytotoxic effects of tested compounds. In this study, a selection of quinazolinone derivatives was screened against melanoma and prostate cell lines, using the MTT colorimetric assay.

Following 48 h exposure of compounds to melanoma cell line, significant differences in viability ($P < 0.05$) were resulted compared to the negative control at 1, 10 and 100 μ M concentrations [Figure 5]. At 24 h exposure, only Q3 and Q4 showed significant activities at all concentrations. For other derivatives (Q1, Q5, Q2) higher concentrations (10 and 100 μ M) were necessary [Figure 4].

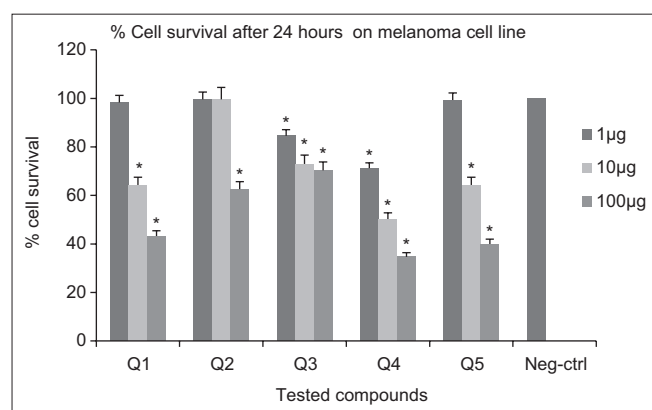


Figure 4: Cytotoxic effects of compounds on melanoma cell line following exposure to different concentrations of compounds (after 24 h). Data are presented as mean \pm standard deviation, * $P < 0.05$, $n = 3$, Neg-ctrl: Negative control

Significant differences in viability ($P < 0.05$) at 1, 10, 100 μ M concentrations were observed after 24 h exposure of Q4 to prostate cell line. Nitro-derivatives of fused pyrrolo-quinazolinone Q5 and fused pyridazine-quinazolinone Q2 showed significant differences in viability ($P < 0.05$) at 10, 100 μ M concentrations [Figure 6]. A 48 h continuous drug exposure on prostate cell line exhibited a significant difference in viability ($P < 0.05$) at 1, 10, 100 μ M concentrations for all tested compounds except Q3 [Figure 7].

Conclusion

Results showed that both cell lines were sensitive to synthesized compounds, and longer duration of exposure (48 h) had better results compared to that of 24 h screening. Although this compound showed a promising result on these two cell lines, however, its beneficial effects in human cancers and the mechanism of action is not clear and should be established.

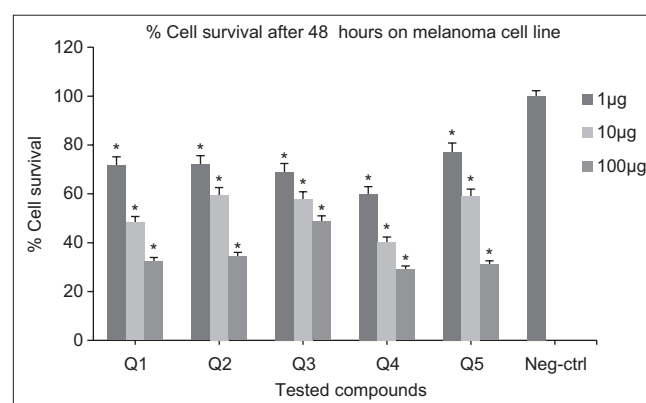


Figure 5: Cytotoxic effects of compounds on melanoma cell line following exposure to different concentrations of compounds (after 48 h). Data are presented as mean \pm standard deviation, * $P < 0.05$, $n = 3$, Neg-ctrl: Negative control

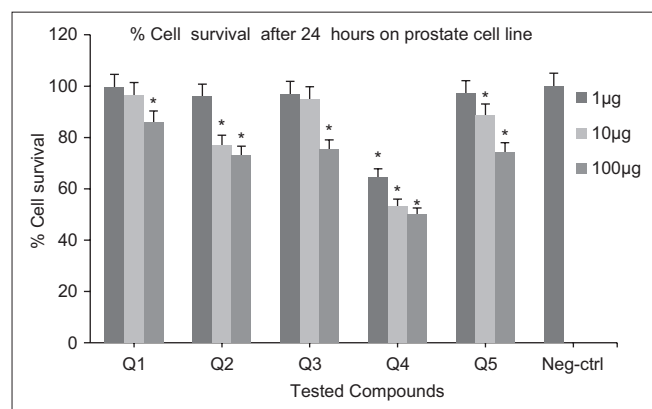


Figure 6: Cytotoxic effects of compounds on prostate cell line following exposure to different concentrations of compounds (after 24 h). Data are presented as mean \pm standard deviation, * $P < 0.05$, $n = 3$, Neg-ctrl: Negative control

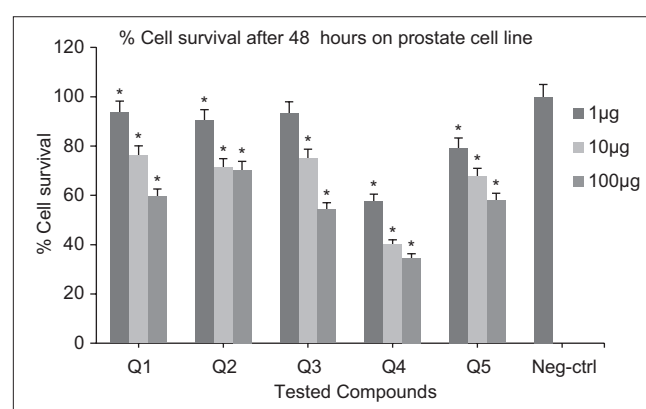


Figure 7: Cytotoxic effects of compounds on prostate cell line following exposure to different concentrations of compounds (after 48 h). Data are presented as mean \pm standard deviation, * $P < 0.05$, $n = 3$, Neg-ctrl: Negative control

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Nil.

Conflicts of interest

There are no conflicts of interest.

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