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# Evaluating the Selective Cytotoxic Effects of Mebudipine and Dibudipine: A Comparative Study in B16-F10 Melanoma and HEK293 Cell Lines

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**ABSTRACT**

**Background & Objective:** Dihydropyridine (DHP) calcium channel blockers have shown anticancer-related effects in preclinical studies, but the cytotoxic activity of the novel 1,4-DHP derivatives mebudipine and dibudipine in melanoma remains unclear. This study compared the in vitro cytotoxicities of mebudipine, dibudipine, and dacarbazine in B16-F10 melanoma cells and in the HEK293 comparative nonmelanoma line.

**Materials & Methods:** B16-F10 and HEK293 cells were treated with mebudipine, dibudipine, or dacarbazine for 24 and 48 hours. Cell viability was measured via the MTT assay. Concentration–response curves were fitted by nonlinear regression, and the $IC_{50}$ values were reported with 95% confidence intervals.

**Results:** After 24 h, B16-F10 cells showed the lowest $IC_{50}$ for mebudipine [13.51 µg/mL; 95% CI, 8.86–20.45], followed by dacarbazine [33.47 µg/mL; 95% CI, 22.24–49.57] and dibudipine [59.13 µg/mL; 95% CI, 25.62–128.90; P=0.0006]. In HEK293 cells, the preferred shared $IC_{50}$ at 24 h was 159.9 µg/mL (95% CI, 120.6–217.9; P=0.3618). After 48h, B16-F10 cells remained highly sensitive to mebudipine [9.738 µg/mL; 95% CI, 6.673–14.29] and dacarbazine [9.749 µg/mL; 95% CI, 6.627–14.42], whereas dibudipine was less potent [30.96 µg/mL; 95% CI, 15.19–57.85; P=0.0025]. In HEK293 cells at 48 h, the $IC_{50}$ values were 90.40, 206.5, and 74.66 µg/mL for mebudipine, dibudipine, and dacarbazine, respectively (P=0.0063).

**Conclusion:** Mebudipine exhibited greater cytotoxic activity against B16-F10 cells than dibudipine at both time points, with higher $IC_{50}$ values in HEK293 cells. Since HEK293 cells are transformed, these data indicate comparative cytotoxicity rather than confirmed safety in normal cells. Further mechanistic melanoma studies are warranted.

**Keywords:** *Melanoma, Cytotoxicity, Immunologic, Calcium Channel Blockers, Dihydropyridines, Dacarbazine, Cell Line, Tumor*

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## 1. Introduction

Melanoma is an aggressive skin cancer with high metastatic potential, and outcomes remain poor once distant spread occurs (1). Treatment has improved through chemotherapy, radiotherapy, targeted therapy, and immunotherapy, but durable benefits are still limited by systemic toxicity, acquired resistance, and incomplete long-term responses (2). BRAF and MEK inhibitors can produce strong early responses in selected patients; however, resistance still develops in many cases, reducing long-term disease control (3, 4). These limitations justify further evaluation of compounds that may reduce melanoma cell viability with lower toxicity toward nonmalignant cells. Calcium signaling contributes to cancer-related processes such as proliferation, apoptosis, migration, and survival (5, 6). Intracellular calcium is normally maintained at low nanomolar levels, whereas extracellular calcium remains much higher, usually in the millimolar range. This gradient is regulated by calcium transporters, calcium-binding proteins, and ion channels, including transient receptor potential channels, store-operated calcium entry channels, and voltage-gated calcium channels (7). In melanoma and other tumor models, altered calcium influx has been associated with tumor progression and treatment resistance (8). Calcium-dependent pathways, including calcium-NFAT signaling, may therefore provide a biological link between calcium

channel activity and melanoma cell behavior. However, such mechanisms were not directly tested in the present study.

Calcium channel blockers are widely used in cardiovascular medicine. Dihydropyridine derivatives reduce calcium influx in vascular smooth muscle cells and cause vasodilation (9, 10). Mebudipine and dibudipine are dihydropyridine-based compounds reported to have longer half-lives and more pharmacokinetic properties than some conventional dihydropyridines (11, 12). The cytotoxic activity of these compounds in melanoma cells has not been well examined.

This study compared the cytotoxic effects of mebudipine and dibudipine in B16-F10 murine melanoma cells after 24 and 48 hours of treatment. HEK293 cells were used as a comparative nonmelanoma cell model to estimate preferential toxicity. We hypothesized that mebudipine and dibudipine would reduce B16-F10 cell viability more strongly than they would reduce HEK293 cell viability. Any proposed effects on calcium-dependent signaling should be interpreted as mechanistic speculation, since this study assessed cell viability rather than downstream signaling events. These findings provide an initial basis for further mechanistic work in melanoma models.

## 2. Materials and Methods

### 2.1 Cell Culture Conditions and Drug Preparation

The murine melanoma cell line B16-F10 (RRID: CVCL_0159) and the human embryonic kidney-derived HEK293 cell line (RRID: CVCL_0045) were obtained from the Pasteur Institute of Iran. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, USA), 100 U/mL penicillin, and 100 µg/mL streptomycin (Gibco, USA). Cultures were maintained at 37 °C in a humidified incubator containing 5% $CO_2$. Trypsin-EDTA solution (0.25%; Gibco, USA) was used for routine cell detachment and passaging.

HEK293 cells were included as a comparative nonmelanoma cell line rather than as a normal primary cell model. This cell line is widely used in cytotoxicity screening because it is robust, reproducible, and easy to culture under standard laboratory conditions. Nevertheless, HEK293 cells are embryonic kidney-derived transformed cells and possess oncogenic features; therefore, findings in this cell line should not be interpreted as direct evidence of safety or selectivity toward healthy primary cells. Mebudipine and dibudipine were purchased from Pars Biopharmacy Research Co. (Tehran, Iran), and dacarbazine was obtained from Merck (Darmstadt, Germany). Stock solutions were prepared by dissolving 5 mg of each compound in 1.76 mL of dimethyl sulfoxide (DMSO; Merck, Germany). Working solutions were prepared by dilution in complete culture medium immediately before treatment. The tested concentration range was 0.1–200 µg/mL for mebudipine, dibudipine, and dacarbazine. The final DMSO concentration was maintained at ≤0.1% under all treatment conditions. Drug solutions were freshly prepared under conditions selected to limit compound degradation.

### 2.2 Assessment of Cell Viability via the MTT Assay

Cell viability was assessed via the MTT assay in 96-well plates. Before seeding, cell number and viability were determined by trypan blue exclusion using a hemocytometer. Cells were used when the cultures reached approximately 90% confluence, and the experiments were performed within 2 weeks of recovery from liquid nitrogen storage. B16-F10 and HEK293 cells were seeded at 7,000 cells per well in 200 µL of complete culture medium and allowed to attach overnight.

After 24 h, the culture medium was removed and replaced with 200 µL of medium containing mebudipine, dibudipine, or dacarbazine at the indicated concentrations. Untreated cells served as the maximum viability control, and cells treated with 1 mM hydrogen peroxide served as the positive cytotoxicity control. At the time of treatment, cell confluency was approximately 40–50%. Plates were incubated at 37 °C in a humidified atmosphere containing 5% $CO_2$ for 24 or 48 h. The MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was prepared as a 5 mg/mL stock solution in phosphate-buffered saline (PBS; pH 7.2) and filtered through a 0.22-µm BIOFIL® syringe filter. At the end of each treatment period, the treatment medium was removed, and 200 µL of MTT working solution was added to each well. Plates were incubated for 4 h at 37 °C in 5% $CO_2$. The supernatant was then removed, and 200 µL of DMSO plus 25 µL of Sørensen's glycine buffer (0.1 M glycine and 0.1 M NaCl; pH 10.5, adjusted with 0.1 N NaOH) was added to each well to dissolve the formazan crystals. Plates were shaken for 40 min, and the absorbance was measured at 570 nm via an ELISA plate reader (13). Cell viability was calculated via the following equation:

Cell viability (%) = (mean OD of treated wells/mean OD of control wells) × 100

The half-maximal inhibitory concentration ($IC_{50}$) values of dacarbazine, mebudipine, and dibudipine were determined at 24 and 48 h in B16-F10 and HEK293 cells. The selectivity index (SI) was calculated for each treatment duration as the $IC_{50}$ in HEK293 cells divided by the $IC_{50}$ in B16-F10 cells.

### 2.3 Replicate design and statistical analysis

The experiments were performed as three independent biological replicates. Each biological replicate included three technical wells per concentration and treatment condition. Technical wells were averaged within each biological replicate before statistical analysis to avoid treating technical replicates as independent observations. Data are presented as the means ± standard deviations (SDs). Statistical analyses were performed via GraphPad Prism software version 10.2.3 (RRID: SCR_002798). Concentration–response curves were fitted separately for each compound, cell line, and treatment duration via nonlinear regression with a sigmoidal dose–response model. $IC_{50}$ values were calculated from the fitted curves and are reported with 95% confidence intervals (CIs). Differences in the $IC_{50}$ values among mebudipine, dibudipine, and dacarbazine were evaluated within each cell line and time point via the extra sum-of-squares F test by comparing models with shared versus separate $IC_{50}$ parameters. A shared-$IC_{50}$ model was accepted when the

comparison between models was not statistically significant. A $p$-value < 0.05 was considered statistically significant.

## 3. Result

### 3.1 Cell viability after 24-hour treatment

After 24 h of exposure, mebudipine, dibudipine, and dacarbazine reduced cell viability in a concentration-dependent manner in both B16-F10 melanoma cells and HEK293 cells (Figure 1, Table 1). In B16-F10 cells (Figure 1*a*), mebudipine caused the most marked reduction in viability. The cell viability decreased from 98.32 ± 0.22% at 0.24 µg/mL to 33.11 ± 1.03% at 250 µg/mL. Dibudipine showed a weaker cytotoxic response over the same range, with viability decreasing from 98.98 ± 0.39% to 53.78 ± 1.59%. Dacarbazine had an intermediate effect, reducing viability from 98.32 ± 0.22% at 0.24 µg/mL to 43.11 ± 1.03% at 250 µg/mL. At 62.5 µg/mL, mebudipine reduced B16-F10 viability to 43.40 ± 4.50%, whereas it reduced B16-F10 viability to 76.07 ± 1.42% for dibudipine and 59.40 ± 0.86% for dacarbazine. Nonlinear regression analysis confirmed these differences. In B16-F10 cells, the $IC_{50}$ values differed significantly among the three compounds (extra sum-of-squares F test: $F$ (2,63) = 8.446, $P$ = 0.0006). The estimated $IC_{50}$ was lowest for mebudipine at 13.51 µg/mL (95% CI: 8.86–20.45), followed by dacarbazine at 33.47 µg/mL (95% CI: 22.24–49.57) and dibudipine at 59.13 µg/mL (95% CI: 25.62–128.90). Curve fits were strong, with $R^2$ values of 0.9735 for mebudipine, 0.9783 for dacarbazine, and 0.9194 for dibudipine. In HEK293 cells (Figure 1*b*), all three compounds also reduced viability after 24 h, but the reduction was less pronounced than that in B16-F10 cells. At 250 µg/mL, the viability was 43.11 ± 1.03% after mebudipine treatment, 53.78 ± 1.59% after dibudipine treatment, and 41.11 ± 1.56% after dacarbazine treatment. At intermediate concentrations, the viability of HEK293 cells was greater than that of B16-F10 cells. For example, at 62.5 µg/mL, viability remained 70.07 ± 1.42% with mebudipine, 76.07 ± 1.42% with dibudipine, and 70.73 ± 0.57% with dacarbazine. In HEK293 cells, comparison of nonlinear regression models did not reveal a statistically significant difference in the $IC_{50}$ among the three compounds (extra sum-of-squares F test: $F$ (2,63) = 1.033, $P$ = 0.3618). The shared $IC_{50}$ model was therefore preferred, yielding a common $IC_{50}$ estimate of 159.9 µg/mL (95% CI: 120.6–217.9). Under the unconstrained model, the estimated $IC_{50}$ values were 150.8 µg/mL for mebudipine (95% CI: 98.13–247.6), 240.4 µg/mL for dibudipine (95% CI: 124.3–627.6), and 135.7 µg/mL for dacarbazine (95% CI: 84.21–233.3). The corresponding $R^2$ values were 0.9782, 0.9613, and 0.9724, respectively. Consequently, the selectivity index (SI) was greater for mebudipine (approximately 11) than for dibudipine (SI ≈ 4) and dacarbazine (SI ≈ 4).

**Table 1.** Comparative $IC_{50}$ values of mebudipine, dibudipine, and dacarbazine in B16-F10 and HEK293 cells after 24 h of treatment.

| Cell line | Compound | $IC_{50}$(µg/mL) | 95% CI(µg/mL) | $R^2$ | Model comparison |
|---|---|---|---|---|---|
| B16-F10 | Mebudipine | 13.51 | 8.86–20.45 | 0.9735 | $IC_{50}$ values differed significantly among compounds; F (2,63) = 8.446, P = 0.0006 |
| B16-F10 | Dibudipine | 59.13 | 25.62–128.90 | 0.9194 | |
| B16-F10 | Dacarbazine | 33.47 | 22.24–49.57 | 0.9783 | |
| HEK293 | Mebudipine | 150.80 | 98.13–247.60 | 0.9782 | $IC_{50}$ values did not differ significantly among compounds; F(2,63) = 1.033, P = 0.3618 |
| HEK293 | Dibudipine | 240.40 | 124.30–627.60 | 0.9613 | |
| HEK293 | Dacarbazine | 135.70 | 84.21–233.30 | 0.9724 | |
| HEK293 | Shared $IC_{50}$ model | 159.90 | 120.60–217.90 | — | Preferred when curves were constrained to share one $IC_{50}$ value |

**Note: $IC_{50}$, half-maximal inhibitory concentration; CI, confidence interval. $IC_{50}$ values were estimated via nonlinear regression via sigmoidal dose–response modeling in GraphPad Prism.**

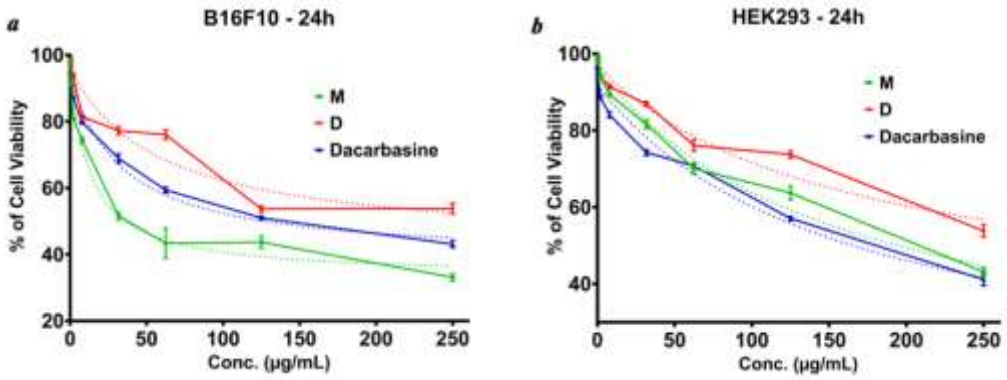


**Figure 1.** Effects of mebudipine, dibudipine, and dacarbazine on cell viability after 24 hours of treatment. B16-F10 melanoma cells (a) and HEK293 cells (b) were treated with increasing concentrations of mebudipine (M), dibudipine (D), or dacarbazine for 24 h, and cell viability was measured via the MTT assay. The data are presented as the means ± SDs from three independent biological replicates, with three technical wells per concentration in each replicate. All three compounds reduced cell viability in a concentration-dependent manner. In B16-F10 cells, mebudipine produced the greatest reduction in viability, followed by dacarbazine and dibudipine. In HEK293 cells, the reduction in viability was less pronounced, and the fitted dose–response curves revealed a weaker separation among the three compounds. The dotted lines indicate nonlinear regression-fit dose–response curves. **(Prepared by Authors, 2026)**.

### 3.2 Cell viability after 48 hours of treatment

After 48 h of treatment, mebudipine, dibudipine, and dacarbazine produced a concentration-dependent reduction in the viability of both B16-F10 melanoma cells and HEK293 cells (Figure 2, Table 2). In B16-F10 cells (Figure 2*a*), mebudipine markedly reduced cell viability from 83.98 ± 0.39% at 0.24 µg/mL to 3.45 ± 1.26% at 250 µg/mL. Dacarbazine had a similar effect at high concentrations, reducing viability from 86.73 ± 2.01% to 2.98 ± 0.75%. Dibudipine was less potent, with viability decreasing from 93.22 ± 0.58% at 0.24 µg/mL to 19.65 ± 0.11% at 250 µg/mL. This separation was clear at intermediate concentrations. At 31.75 µg/mL, the viability was 27.90 ± 1.60% with mebudipine and 25.00 ± 1.34% with dacarbazine, whereas it was 58.66 ± 0.34% with dibudipine. At 62.5 µg/mL, viability remained lower with mebudipine and dacarbazine, reaching 16.40 ± 1.85% and 22.89 ± 1.69%, respectively, compared with 37.11 ± 0.51% after dibudipine exposure. Nonlinear regression analysis of B16-F10 data revealed significant differences in the $IC_{50}$ among the three compounds. The extra sum-of-squares F test revealed significant differences in $IC_{50}$ values among the three compounds [$F(2,63) = 6.603$, $P = 0.0025$]. Mebudipine and dacarbazine had nearly identical $IC_{50}$ values: 9.738 µg/mL for mebudipine (95% CI: 6.673–14.29) and 9.749 µg/mL for dacarbazine (95% CI: 6.627–14.42). Dibudipine had a higher $IC_{50}$ of 30.96 µg/mL (95% CI: 15.19–57.85). Curve fits were strong for mebudipine and dacarbazine, with $R^2$ values of 0.9809 and 0.9774, respectively; dibudipine showed a lower, still acceptable fit, with an $R^2$ of 0.9474. In HEK293 cells (Figure 2*b*), all three compounds also reduced viability after 48 h, but the magnitude of the reduction differed among treatments. Mebudipine decreased viability from 93.65 ± 0.78% at 0.24 µg/mL to 23.11 ± 1.03% at 250 µg/mL. Dibudipine produced the weakest cytotoxic effect, with viability decreasing from 98.98 ± 0.39% to 37.11 ± 4.24%. Dacarbazine caused the greatest reduction in HEK293 cell viability, decreasing it from 97.98 ± 0.76% at 0.24 µg/mL to 13.11 ± 1.99% at 250 µg/mL. At 62.5 µg/mL, the cell viability was 46.07 ± 1.61% with mebudipine, 72.40 ± 2.64% with dibudipine, and 48.73 ± 1.19% with dacarbazine. At 125 µg/mL, viability decreased further to 33.69 ± 1.76%, 56.69 ± 1.79%, and 26.69 ± 1.79%, respectively. The fitted dose–response models in HEK293 cells also revealed significant differences in the $IC_{50}$ among the three compounds [extra sum–of–squares F test: $F(2,63) = 5.493$, $P = 0.0063$]. Dacarbazine had the lowest $IC_{50}$ at 74.66 µg/mL (95% CI: 50.91–110.5), followed by mebudipine at 90.40 µg/mL (95% CI: 62.15–134.9). Dibudipine again showed the weakest potency, with an $IC_{50}$ of 206.5 µg/mL (95% CI: 138.0–333.6). The $R^2$ values were 0.9715 for mebudipine, 0.9821 for dibudipine, and 0.9773 for dacarbazine. Calculation of the selectivity index (SI; $IC_{50}$ in HEK293/$IC_{50}$ in B16-F10) revealed that mebudipine had the highest SI (~9.3), followed by dacarbazine (~7.7) and dibudipine (~6.7).

**Table 2.** Comparative $IC_{50}$ values of mebudipine, dibudipine, and dacarbazine in B16-F10 and HEK293 cells after 48 h of treatment.

| Cell line | Compound | $IC_{50}$(µg/mL) | 95% CI (µg/mL) | $R^2$ | Model comparison |
|---|---|---|---|---|---|
| B16-F10 | Mebudipine | 9.738 | 6.673–14.29 | 0.9809 | $IC_{50}$ values differed significantly among compounds; F(2,63) = 6.603, P = 0.0025 |
| B16-F10 | Dibudipine | 30.96 | 15.19–57.85 | 0.9474 | |
| B16-F10 | Dacarbazine | 9.749 | 6.627–14.42 | 0.9774 | |
| HEK293 | Mebudipine | 90.40 | 62.15–134.90 | 0.9715 | $IC_{50}$ values differed significantly among compounds; F(2,63) = 5.493, P = 0.0063 |
| HEK293 | Dibudipine | 206.50 | 138.00–333.60 | 0.9821 | |
| HEK293 | Dacarbazine | 74.66 | 50.91–110.50 | 0.9773 | |

**Note: $IC_{50}$, half-maximal inhibitory concentration; CI, confidence interval. $IC_{50}$ values were estimated via nonlinear regression via sigmoidal dose–response modeling in GraphPad Prism.**

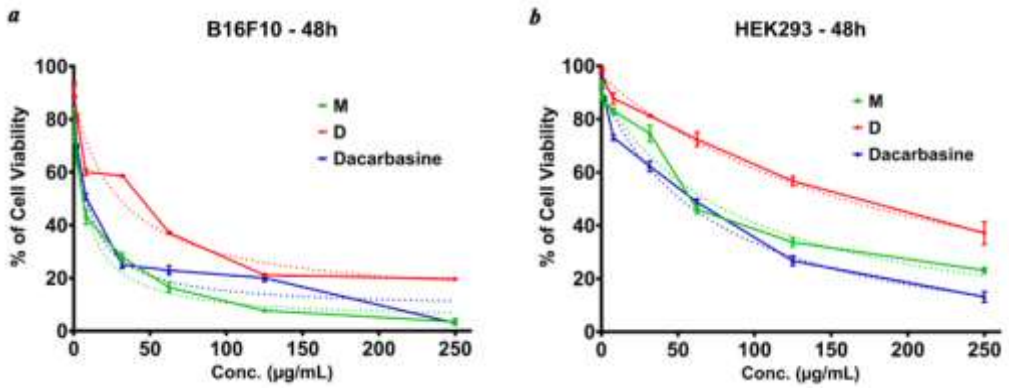


**Figure 2.** Effects of mebudipine, dibudipine, and dacarbazine on cell viability after 48 hours of treatment. B16-F10 melanoma cells (a) and HEK293 cells (b) were treated with increasing concentrations of mebudipine (M), dibudipine (D), or dacarbazine for 48 h, and cell viability was determined via the MTT assay. The data are shown as the means ± SDs from three independent biological replicates, with three technical wells per concentration in each replicate. In B16-F10 cells, mebudipine and dacarbazine produced a steep concentration-dependent reduction in viability, with near-complete loss of viable cells at the highest tested concentration. Dibudipine also reduced viability, but its effect was weaker at most concentrations. In HEK293 cells, all three compounds decreased viability after 48 h, with dacarbazine and mebudipine showing stronger cytotoxic effects than dibudipine. The dotted lines indicate nonlinear regression-fit dose–response curves. (**Prepared by Authors, 2026**).

## 4. Discussion

This study evaluated the cytotoxic effects of two 1,4-dihydropyridine calcium channel blockers, mebudipine and dibudipine, in B16-F10 murine melanoma cells, via parallel testing in HEK293 human embryonic kidney-derived comparator cells. At 24 h, mebudipine had the lowest B16-F10 $IC_{50}$ (13.51 µg/mL), compared with dacarbazine (33.47 µg/mL) and dibudipine (59.13 µg/mL; Table 1). After 48 h, the $IC_{50}$ values for mebudipine (9.738 µg/mL) and dacarbazine (9.749 µg/mL) were nearly identical, whereas dibudipine remained less active (30.96 µg/mL; Table 2). These comparisons concern short-term in vitro viability responses and do not establish therapeutic equivalence. The apparent selectivity index, calculated from the HEK293-to-B16-F10 $IC_{50}$ ratio, was highest for mebudipine at both 24 h (approximately 11.2) and 48 h (approximately 9.3). HEK293 cells are transformed and kidney-derived, so these ratios describe separation between the tested cell lines rather than safety in normal melanocytes or other healthy tissues. The higher index for mebudipine warrants testing against more relevant nonmalignant skin cells. Earlier cardiovascular studies describe the calcium channel-blocking properties of mebudipine (12). Experiments in isolated rat hepatocytes examined the metabolism of mebudipine and dibudipine (14). A review of 1,4-dihydropyridines reports broader biological activity within this chemical class (15), and separate gastric cancer work linked ORAI1/STIM1 expression with tumor-cell proliferation and invasion (16). These observations provide pharmacologic context; they do not identify the mechanism of B16-F10 viability loss. Effects of mebudipine in cardiovascular preparations (12) cannot substitute for direct calcium-flux measurements in melanoma cells.

Dibudipine has shown effects on contractility in rat atrial tissue and human internal mammary artery preparations (17). Pharmacokinetic studies in rats also reported a longer half-life than nifedipine (18). Neither finding explains why dibudipine was less active than mebudipine in the B16-F10 MTT assay; the cellular basis for this difference remains unknown.

Calcium-dependent signaling can regulate cancer-cell proliferation and survival (19). Experimental calcium-modulation studies and work on calcineurin–NFAT signaling support further investigation of these pathways (20, 21). In colorectal cancer, nifedipine reduced NFAT2 nuclear translocation and PD-L1-associated immune escape (22). The present study measured neither calcium influx nor L-type channel expression, NFAT activation, or apoptosis. A calcium-dependent mechanism for mebudipine or dibudipine in B16-F10 cells therefore remains unverified.

Resistance to systemic treatment remains a concern in melanoma (23). Dacarbazine has an established history of use in oncology, including melanoma (24, 25). Previous work examined dacarbazine responses in B16-F10 cells and the reactive intermediates responsible for its cytotoxicity (26, 27). The similar 48-h $IC_{50}$ values of mebudipine and dacarbazine do not establish comparable clinical efficacy or a shared mode of action.

Reports on small-molecule pharmacology address dihydropyridine structure–activity relationships, mineralocorticoid receptor effects of some calcium channel blockers, and P-glycoprotein ATPase inhibition by other molecules (28-31). Structure-based approaches to P-glycoprotein inhibition have also been investigated (32). These findings cannot be taken as evidence that mebudipine or dibudipine inhibits this transporter. No drug-efflux or transporter-function assay was performed.

Cancer stem-cell phenotypes require functional assessment of properties such as self-renewal and tumor initiation (33). ALDH activity and tumorsphere assays are among the methods used to characterize such populations (34, 35). Neither assay was used here, so the MTT results cannot establish targeting of melanoma cancer stem cells. The main limitations are the use of an MTT-based metabolic viability readout, one murine melanoma line, and a transformed HEK293 comparator. A lower MTT signal cannot, on its own, distinguish reduced metabolic activity from growth inhibition or cell death. Follow-up studies should include normal skin-derived cells, direct cell-death and calcium-flux assays, and clonogenic survival measurements. Animal models and pharmacokinetic assessment are needed before therapeutic activity or tolerability can be evaluated.

## 5. Conclusion

Mebudipine reduced B16-F10 melanoma cell viability at lower concentrations than dibudipine, and its 48-h $IC_{50}$ was similar to that of dacarbazine in the MTT assay. The greater $IC_{50}$ separation between B16-F10 and HEK293 cells suggests preferential activity within these two models; it does not demonstrate safety in normal tissues. Further studies should assess calcium signaling, cell-death mechanisms, and responses in primary skin-derived cells before in vivo evaluation.

## 6. Declarations

### 6.1 Acknowledgments

The authors would like to thank the Cellular and Molecular Research Center of Qom University of Medical Sciences for providing the necessary facilities to carry out the experiments.

### 6.2 Ethical Considerations

This experimental study was conducted as part of a thesis and received ethical approval from Qom University of Medical Sciences, Qom, Iran. under the ethical code "IR.MUQ.AEC.1401.032".

### 6.3 Authors' Contributions

H. A. methodology and wrote the draft. S.Z.J. performed the data analysis and wrote and reviewed the draft. Sa.K. conceptualized and reviewed the draft. Si.k. and F.H. methodology and reviewed the draft.

### 6.4 Conflict of Interest

The authors declare that they have no competing interests.

## 6.5 Fund or Financial Support

This study was financially supported by the Research Council of Qom University of Medical Sciences, Qom, Iran, in 2024.

## 6.6 Using Artificial Intelligence Tools (AI Tools)

The authors did not utilize AI Tools.

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