Dasatinib/Celecoxib combination: A new hope in triple negative breast cancer treatment

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Research Article

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Abstract

Despite the tremendous efforts to implement new paradigms for breast cancer, the disease still remains a major challenge worldwide. Genetic deregulation is evident in all breast cancer subtypes and comprises a multitude of mutated genes and deregulated signaling cascades. In this sense, co-targeting Src and COX-2 signaling cascades have attracted fervent interest. This work explored the probable anti-carcinogenic effects of Dasatinib as a Src inhibitor, Celecoxib as a selective COX-2 inhibitor, and their combination in MDA-MB-231 triple-negative breast cancer cell line. Drug growth inhibition 50 (GI50) was determined using the MTT assay and the obtained results were analyzed using CompuSyn 3.0.1 software. MDA-MB-231 cells were divided into four treatment groups including a positive control, Dasatinib-treated, Celecoxib-treated, and combination-treated groups. Standard sandwich ELISA was used for the determination of the protein levels of c-Src, Bcl-2, p-AKT, FAK, PGE2, VEGF, and cyclin D1. Active caspase-3 was determined colorimetrically and the expression of *COX-2* and *c-Src* genes was quantitatively determined via quantitative real-time polymerase chain reaction. The GI50 for Dasatinib was 0.05699 μM while that for Celecoxib was 69.0976 μM. Dasatinib up-regulated c-Src gene while Celecoxib and Dasatinib/Celecoxib combination down-regulated such expression level. COX-2 gene was down-regulated by Celecoxib while it was up-regulated by both Dasatinib and Dasatinib/Celecoxib combination. On one hand, Dasatinib, Celecoxib, and their combination significantly reduced the protein levels of c-Src, Bcl-2, p-AKT, FAK, PGE2, VEGF, and cyclin D1. On the other hand, they elevated active caspase-3. To sum up, Dasatinib/Celecoxib combination increased the capability for apoptosis and suppressed proliferation, angiogenesis, migration, and invasion suggesting a strong cross-talk between Src signaling cascade and COX-2/PGE2 via the intermediate PI3K/AKT/mTOR pathway. Further in-vitro and in-vivo studies are warranted to verify the present findings.

Introduction

Breast cancer (BC) is the most frequently diagnosed cancer and the leading cause of cancer death among females according to GLOBOCAN 2018 [(1)]. As a multifaceted disease, BC comprises an array of molecular subtypes characterized by differences in molecular signatures, responses to therapies, and prognoses [(2)]. Triple-negative breast cancer (TNBC), accounting for almost 15-20% of all BCs, is the most aggressive form that lacks estrogen, progesterone, and human epidermal growth factor receptors expression [(3)].

Improved understanding of the pivotal role that signaling pathways have in the establishment and maintenance of the tumorigenic state will be crucial for the development of new chemotherapeutic agents. Small-molecule inhibitors, either as single agents or in combination therapy, provide a foundation for exploiting these pathways as probable targets in BC treatment specifically the most aggressive TNBC subtype [(4)]. Novel approaches for better disease management aimed at co-targeting more than one of these pathways. Herein, we investigated Src and cyclooxygenase-2 pathways are plausible targets involved in BC pathogenesis.

Src is a versatile target at the nexus of a multitude of signaling cascades. Src acts as an intermediate between growth factor receptor binding and downstream signaling which is vital for various cellular processes, including survival, proliferation, differentiation, invasion, and metastasis [5, 6]. Dasatinib, an inhibitor of Src/Abl family kinases, is approved by the FDA for the treatment of imatinib-resistant chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia [7, 8]. Dasatinib inhibits tumor growth in a number of solid tumors and several mechanisms underlie such suppression including G1 arrest of the cell cycle, induction of apoptosis, and inhibition of cell migration/invasion/metastasis [9].

Cyclooxygenase-2 (COX-2) is another target overexpressed in BC and is a fundamental step in BC pathogenesis acting via prostaglandin-dependent and independent mechanisms. Epidemiological studies suggest that non-steroidal anti-inflammatory drugs offer a moderate degree of benefit against BC. Nevertheless, further work is warranted to better understand how this enzyme system can be employed for therapeutic benefit. Celecoxib, as a selective COX-2 inhibitor, is believed to have potential anticancer effects in a wide variety of cancer types including colorectal, breast, and lung cancers such as suppression of cell growth, promotion of apoptotic cell death, immunoregulation, modulation of tumor microenvironment, and antiangiogenic effect. Meanwhile, COX-2-independent pathways also contribute to the anticancer effects of Celecoxib [10].

Taken together, this study was undertaken to investigate the antitumor effects of Dasatinib as a Src inhibitor, Celecoxib as a selective COX-2 inhibitor as well as their combination in MDA-MB-231 TNBC cell line.

Materials And Methods

Drugs

Dasatinib and Celecoxib (Selleckchem, TX, USA) were prepared at the concentration of 10 mM in dimethyl sulphoxide and then were stored at -20°C.

Cell lines

The current study used MDA-MB-231 cell line obtained from the American Type Culture Collection (ATCC® HTB-26™). It is an epithelial BC cell line taken from the pleural effusion of a 51-year-old Caucasian female suffering from metastatic mammary adenocarcinoma.

Cell cultures

MDA-MB-231 cells were kept as a monolayer culture in T-25 flasks in Dulbecco's Modified Eagle's Medium (Lonza Biowhitaker™, B-4800 Verviers, Belgium) supplemented with 10% (v/v) fetal bovine serum (Sigma-Aldrich Co., Germany) and 1% penicillin-streptomycin (Lonza Biowhitaker™, B-4800 Verviers, Belgium) at 37°C with 5% CO_2. Cells were passaged when they reached 80% confluence.

Growth inhibition assay

MTT assay was used to determine cell viability [11]. Briefly, MDA-MB-231 cells were seeded in 96-well plates, treated with six different concentrations of the tested drugs. The six different concentrations for Dasatinib were 0.04 µM, 0.02 µM, 0.01 µM, 0.005 µM, 0.003 µM and 0.001 µM while those for Celecoxib were 100 µM, 50 µM, 25 µM,.5 µM, 6.25 µM, and 3.125 µM. MTT (10 µl) was added after 72 hours then incubation was carried out at 37°C for 4 hours and finally the absorbance was measured at 570 nm. The GI50 was assessed for Dasatinib and Celecoxib utilizing CompuSyn 3.0.1 software.

Determination of the combination and dose reduction indices

The combination index (CI) was assessed as described earlier [12] to determine whether there is synergism, antagonism, or additive effect between Dasatinib and Celecoxib, where CI lower than 1 indicates synergism, =1 indicates additive effect and greater than 1 indicates antagonism. Additionally, the dose reduction index (DRI) was determined using the CompuSyn software as described earlier [12].

Experimental design

Three replicas of MDA-MB-231 cells received either dimethyl sulphoxide as a vehicle, Dasatinib (0.05699µM), Celecoxib (69.0976µM), or Dasatinib (0.05699µM)/Celecoxib (69.0976µM) combination. The regulatory aspects regarding the use of cell lines were followed in all the experiments.

Biochemical analyses

Protein levels of v-akt murine thymoma viral oncogene homolog 1 (p-AKT), c-Src, Focal adhesion kinase (FAK), Bcl-2, prostaglandin E2 (PGE2), cyclin-D1, and vascular endothelial growth factor (VEGF1) were determined using the following ELISA kits: Human p-AKT (Ser473) ELISA kit (RayBiotech, USA) (Cat#: PEL-AKT-S473-T), Human c-Src kinase ELISA kit (LifeSpan, Bioscience, USA) (Cat#: LS-F11230), Human FAK ELISA kit (LifeSpan, Bioscience, USA) (Cat#: MBS2515396, 96T), Bcl-2 ELISA kit (Sigma-Aldrich, USA) (Cat#: CS0520), PGE2 ELISA kit (Sigma-Aldrich, USA) (Cat#: MBS721434), cyclin-D1 ELISA kit (USCN Life Science and Technology Co.) (Cat#: E0585h) and VEGF based ELISA assay kit (Cusabio, USA) (Product Code CSB-E11718h), respectively according to the manufacturer's instructions.

Determination of capase-3 activity

Caspase 3 activity, expressed as µmol p-nitroaniline/min/ml, was assessed using Caspase-3 colorimetric kit (Sigma Aldrich, USA) (Product Code CASP-3-C) according to the manufacturer's instructions.

Gene expression analysis of *c-Src* and *Cox-2* genes using quantitative real-time polymerase chain reaction

Cox-2 and *c-Src* gene expression levels were determined using step one real-time polymerase chain reaction (PCR) system (Applied Biosystem, USA). First, total messenger RNA was isolated using the Easy-

RED™ total RNA extraction kit (Intron Biotechnology, South Korea) (Product Code 17063) according to the instructions of the manufacturer. Second, quantification and purity checking were performed using the NanoDrop 2000 spectrophotometer (Thermo Fischer Scientific, USA). Quantitative real-time PCR reactions were performed using the SensiFast™ SYBR® No-ROX one-step kit (Bioline Co., USA) (Product Code BIO-72001). Finally, the relative expression of *Cox-2* and *c-Src* genes was assessed against glyceraldehyde 3-phosphate dehydrogenase (*GAPDH)* as a housekeeping gene. The sequences of the forward and reverse primers for *c-Src* gene were: forward: 5'- GGACAGTGGCGGATTCTACATC-3' and reverse: 5'- AGCTGCTGCAGGCTGTTGA-3'; for *Cox-2,* forward, 5'CTGTTGCGGAGAAAGGAGTC-3'; reverse, 5'-TCAAACAAGCTTTTACAGGTGA-3', whereas those for *GAPDH* gene were: forward: 5'-TGCACCACCAACTGCTTAGC-3' and reverse: 5'- GGCATGGACTGTGGTCATGAG-3' [13,14,15]. To confirm the amplification of *c-Src*, *Cox-2*, and *GAPDH* genes, primer sequences were blasted against NCBI/Primer Blast. The analyses were carried out as triplicates. The relative expression of the aforementioned genes against *GAPDH* depended on the ΔΔ comparative threshold method.

Statistical analysis of the data

Data were presented as mean ± standard error of the mean. Results were analyzed using one-way analysis of variance test followed by Tukey post-hoc test. The statistical analyses were executed by Graph Pad Prism Software (version 3.0). The level of significance was fixed at $p < 0.05$.

Results

Determination of GI50 for Dasatinib and Celecoxib in MDA-MB-231 cells

The GI50 was 0.05699 μM for Dasatinib and 69.0976 μM for Celecoxib as demonstrated in (Figure 1a and 1b), respectively.

Determination Of The Combination And Dose Reduction Indices

Based on the MTT assay results shown in (Figure 2a and 2b) and the statistical analyses using Compusyn software, Dasatinib /Celecoxib combination showed a strong synergistic effect as evidenced from the combination index (CI=0.98621 μM). Likewise, the dose reduction index revealed that Celecoxib decreased the dose of Dasatinib by approximately 3.8 folds which could decrease its undesired adverse effect as a monotherapy. In addition, Dasatinib decreased the dose of Celecoxib by approximately 1.4 folds.

Effect of Dasatinib, Celecoxib and their combination on c-Src **gene expression and protein levels in MDA MB-231 cell lysates after 72 hours of treatment**

Data presented in (Figure 3a) inferred that Dasatinib up-regulated *c-Src* gene expression; however, Celecoxib and the combination down-regulated such expression compared to its expression in positive control cells. Results shown in (Figure 3b) revealed that c-Src protein levels were significantly reduced by 57%, 46%, and 76% when compared with the control group in Dasatinib-treated, Celecoxib-treated, and combination-treated cells, respectively ($p < 0.001$).

Effect of Dasatinib, Celecoxib and their combination on COX-2 gene expression level, and PGE2 protein level (Pg/mg total protein) in MDA-MB-231 cell lysates after 72 hours of treatment

As shown in (Figure 4a), Dasatinib and Dasatinib/Celecoxib combination up-regulated *COX-2* gene expression; however, Celecoxib down-regulated such expression compared to its expression in positive control cells. The results shown in (Figure 4b) revealed that PGE2 protein levels were significantly reduced by 56%, 45%, and 73% when compared with the positive control group in Dasatinib-treated, Celecoxib-treated, and combination-treated cells, respectively ($p < 0.001$). Likewise, the combination decreased PGE2 levels significantly compared with single treatments with either Dasatinib or Celecoxib ($p < 0.05$ and $p < 0.001$; respectively).

Effect of Dasatinib, Celecoxib and their combination on FAK protein level (ng/mg total protein), p-AKT protein level (Units/mg total protein), and cyclin-D1 protein level (Units/mg total protein) in MDA-MB-231 cell lysates after 72 hours of treatment

Our findings herein (Figure 5a) inferred that FAK protein levels were significantly reduced by about 59%, 50 %, and 74% in Dasatinib-treated, Celecoxib-treated, and combination-treated cells, respectively ($p < 0.001$). Moreover, Dasatinib/Celecoxib combination significantly reduced FAK protein levels when compared with single treatments with either Dasatinib or Celecoxib ($p < 0.001$). The results presented in (Figure 5b) revealed that p-AKT protein levels were significantly decreased by approximately 64%, 54%, and 77% compared with the positive control group in Dasatinib-treated, Celecoxib-treated, and combination-treated cells, respectively ($p < 0.001$). Furthermore, the combination reduced p-AKT protein levels significantly compared with single treatments with either Dasatinib or Celecoxib ($p < 0.01$ and $p < 0.001$; respectively). The findings depicted in (Figure 5c) demonstrated that cyclin D1 protein levels were significantly reduced by approximately 66%, 56%, and 76% when compared with the positive control group in Dasatinib-treated, Celecoxib-treated, and combination-treated cells, respectively ($p < 0.001$).

Effect of Dasatinib, Celecoxib and their combination on Bcl-2 protein level (Units/mg total protein), active caspase-3 (ng/mg total protein), and VEGF protein level (Pg/mg total protein) in MDA-MB 231 cell lysates after 72 hours of treatment

The data presented in (Figure 6a) showed that Dasatinib, Celecoxib, and their combination reduced Bcl-2 protein levels by 45%, 37%, and 79% compared with the positive control group ($p < 0.001$). Our results herein (Figure 6b) showed that caspase-3 protein levels were significantly increased by approximately 196%, 152%, and 538% compared with the positive control group in Dasatinib-treated, Celecoxib-treated, and combination-treated cells respectively ($p < 0.001$). Additionally, Dasatinib/Celecoxib combination

significantly elevated caspase-3 levels compared with single treatments with either Dasatinib or Celecoxib ($p < 0.001$).As presented in (Figure 6c), Dasatinib, Celecoxib, and their combination reduced VEGF protein levels significantly by about 68%, 63%, and 82% compared with the positive control group ($p < 0.001$).

Discussion

Several lines of evidence supported critical roles for Src and COX-2 signaling during breast tumorigenesis. Accordingly, there was a growing interest in studying Src and COX-2 pathways via their co-targeting by Dasatinib and Celecoxib in MDA-MB-231 TNBC cell line. To the best of our knowledge, this study is the first to assess the possible antitumor effects of Dasatinib/Celecoxib combination in MDA-MB-231 TNBC cell line.

The up-regulatory effect of Dasatinib on *c-Src* gene expression level could be a reflex mechanism resulting from the inhibition of *c-Src* on the protein level in our study. Previous studies documented an increase in the level of total Src upon treatment with c-Src inhibitors in Malignant Mesothelioma (MSTO-211H, NCI-H28, and NCI-H2052) [16, 17]. Furthermore, this is consistent with what was reported in various tumor cell lines following treatment with other Src inhibitors [18, 19].

It was documented that Dasatinib exert an effect on Src/FAK pathway [20] and this was proved by several studies in which Dasatinib inhibited growth, migration, and invasion of non-small cell lung cancer, and head and neck squamous cell carcinoma (HNSCC) cell lines [21, 22]. The molecular mechanisms suggested for Dasatinib were Src inhibition and epidermal growth factor receptor and estrogen receptor α down-regulation [23]. In colorectal cancer cell lines, overexpression of epidermal growth factor receptor was correlated with Src activation. Src enables epidermal growth factor receptor to evade degradation by inactivation of Cbl, a kinase responsible for the ubiquitination and degradation of ligand-activated receptors [24]. It was reported that Dasatinib decreased phosphorylation of c-Src in burkitt's esophageal cells [25] and MDA-MB-468 cells [26].

Our results depicted that Dasatinib significantly decreased FAK protein levels compared to the control group suggesting that it has the potential to control cell adhesion, migration, and invasion. Previous studies on different BC cell lines showed that Dasatinib strongly inhibited FAK phosphorylation at the activating site Y576 [27]. This effect was also evident in multiple studies conducted on hepatocellular carcinoma (HCC) cell lines suggesting that Dasatinib may interplay with other molecules to block FAK phosphorylation, and therefore suppresses motility and invasion [28]. Not only studies on HCC cell lines, but also studies on nasopharyngeal carcinoma cell lines showed that FAK is downstream of Src [29]. In addition, cell migration requires FAK activity, whereas FAK activation requires Src activity, suggesting a reciprocal catalytic activation mechanism of FAK and Src [30].

Consistent with mesenchymal-like tumor characteristics, MDA-MB 231 cells showed a high level of basal AKT activity. In our study, Dasatinib suppressed the phosphorylation of AKT at serine 473. The

potentiality of Dasatinib in nasopharyngeal carcinoma treatment was investigated and AKT phosphorylation was found to be reduced by Dasatinib in CNE2 cells [27].

In the present study, Dasatinib significantly reduced cyclin D1 protein levels indicating that Dasatinib has the capacity to cause cell cycle G1-S arrest. It was reported earlier that Dasatinib decreased proliferation in lung, and head and neck cancer cells [22, 31], malignant pleural mesothelioma [32], melanoma cells [33], HCT-116 colorectal cancer cells [34], nasopharyngeal carcinoma cells [35], neuroblastoma cells [36], myxoid liposarcoma [37], papillary thyroid carcinoma cells [20], breast cancer cells [26], ovarian cancer cells [38, 39], HCC cells [40], acute myeloid leukemia cells [41], and acute myeloid leukemia Kasumi-1 cells [42].

The significant increase in caspase-3 by Dasatinib in our study could be linked to the inhibition of both Src and FAK. Supporting our findings, Dasatinib promoted apoptosis in pancreatic cancer cells [44], HNSCC cells (e.g., Ca9-22, HSC3, and SCC-25 cells) [45], BC cells [46], laryngeal cancer cell line (Hep-2) [47], chronic lymphoid leukemia cells [48], neuroblastoma cells [36], nasopharyngeal carcinoma cells [35], SKOv3 and HEY ovarian cancer cells [38], and Kasumi-1 cells [42].

In the current study, Dasatinib markedly decreased VEGF protein level. Supporting our finding, a previous study conducted on chronic myeloid leukemia cells inferred that Dasatinib reduced the phosphorylation of e-Proline-Rich Homeodomain which regulates myeloid survival via direct transcriptional repression of various genes encoding VEGF signaling pathway components [49]. Another study depicted that Src family kinases affect tumor angiogenesis, where results in advanced non-small cell lung cancer patients suggested that levels of pro-angiogenic factors including VEGF are decreased by Dasatinib [50].

Our data also inferred that Dasatinib up-regulated *COX-2* gene although it was expected that Dasatinib would down-regulate *COX-2* expression. This could be the consequence of the observed reflex upregulation of src in our study which requires further investigation and evidence.

As for Celecoxib, it down-regulated *COX-2* gene expression in our study and this was confirmed by multiple studies [53, 54]. Herein, the effect of Celecoxib on PGE2 protein level was concordant with the results of another study conducted on MCF-7 BC cell line in which the PGE2 level gradually decreased in a dose-dependent manner [53]. It was suggested that Celecoxib decreased PGE2 synthesis via Wnt pathway and conversion of arachidonic acid to bioactive prostanoids [21].

Celecoxib decreased FAK protein level herein and this is consistent with what was reported earlier in HCC cells [55], non-small cell lung cancer cells [56–57], and acute myeloid leukemia cells [58]. Furthermore, Celecoxib reduced AKT protein level making our results corroborating with a previous study in which Celecoxib significantly decreased the phosphorylation of AKT in MDA-MB-231 cells but not in MDA-MB-468 cells, suggesting that the mechanism of apoptosis induction in MDA-MB-231 cells was in part

dependent upon decreased AKT phosphorylation where AKT acts as a critical signaling component in cell survival by enhancing the downstream apoptotic proteins [22].

The present data revealed a significant reduction in Cyclin D1 protein levels by Celecoxib. Consistent with the current finding, Celecoxib markedly suppressed tumor growth in a number of animal models of colon, skin, lung, bladder, and breast cancers [59]. In a spontaneous metastatic BC mouse model, Celecoxib reduced tumor growth via proliferation and angiogenesis inhibition, Bax up-regulation, and AKT and Bcl-2 down-regulation [60]. Furthermore, Celecoxib decreased cyclin D1 expression in both HN30 and HN31 HNSCC lines [61] and in mouse colon carcinoma cell line [62]. Celecoxib suppressed growth and promoted cell-cycle arrest at the G0/G1 phase in nasopharyngeal cell lines [63], BC cell lines [64], murine mammary tumor cell lines [65], human pancreatic cancer cell lines [66], and human ovarian cancer cell lines [67]. Celecoxib inhibited cell cycle progression via the G1-S transition in SKOV-3 cells *in-vivo* [68]. Celecoxib inhibited proliferation via the PGE2 pathway in BC, HCC [69], and human cholangiocarcinoma cell lines [70]. Celecoxib reduced cyclin D1 in U373 and T98G human glioblastoma cells by regulating NF-κB target genes expression and inhibited proliferation in GBM cells at least partly by suppressing NF-κB activation [71].

Our results were in agreement with several studies in which caspase-3 was increased significantly by Celecoxib in a concentration-dependent manner in both MDA-MB-231 and SK-BR-3 BC cells. This supports the notion that Celecoxib can enhance caspase 3-dependent pathways in BC cells [72]. It was found that the blockade of caspase activation is enough to suppress apoptosis [22] due to decreased AKT phosphorylation and increased Bax expression [73]. It was reported that Celecoxib prevented colon tumorigenesis by promoting apoptosis via both COX-dependent and COX-independent mechanisms [59]. It induced apoptosis in cervical cancer cells via Fas-ligand independent FADD activation in a cell type-specific manner [74] and via an apoptosome-dependent pathway, independent of death receptor pathways in lymphoma [74]. In BJMC3879 mammary adenocarcinoma cells, it enhanced apoptosis via the activation of the intrinsic mitochondrial pathway. Furthermore, Celecoxib induced apoptosis in human glioblastoma cells at least partly by suppressing NF-κB activation [71]. Celecoxib also induces a p53-independent apoptotic response which may be highly relevant in treating human neoplasms [75] and promoted apoptosis in lung cancer cells which seems to be dose-dependent [76].

Our results were in line with previous studies that reported that VEGF is reduced by Celecoxib [77, 78]. It was reported that *COX-2* overexpression in tumor cells influences angiogenesis through the production of COX-2 derived eicosanoids, which enhance endothelial cell migration and angiogenesis by elevating VEGF expression and stimulating the proliferation of endothelial cells [79, 80]. Inhibition of COX-2 activity by Celecoxib reduces all these effects and leads to inhibition of angiogenesis and reduction of tumor growth [81, 82]. A previous study found that VEGF was reduced by Celecoxib in a dose-dependent manner in MDA-MB-231 cells suggesting that COX-2/PGE2 pathway might play a pivotal role in channel formation and angiogenesis in part by enhancing proangiogenic proteins such as VEGF [22]. The

involvement of COX-2 inhibition in the antiangiogenic effect of Celecoxib was seen in a rat cornea model [83, 84]. Prostaglandins binding to its receptor might enhance VEGF expression via hypoxia-inducible factor 1 alpha explaining at least in part, the cross-talk between VEGF and COX-2/PGs pathways [85]. COX-2 independent mechanisms contributing to the antiangiogenic effects of Celecoxib was also described in rat hepatoma cells [86], human umbilical vein endothelial cells [87], ovarian SKOV-3 carcinoma xenografts [88], human colon carcinoma cells in nude mice [83], and human BC cells [89].

Taken all together, our results refer to the potential points of crosstalk between the two signaling pathways c-Src and COX-2 with their downstream molecular targets that are involved in BC. Furthermore, up to our knowledge, this is the first study that offers supporting evidence of the beneficial antitumor effects of combining Dasatinib and Celecoxib in MDA-MB-231 cells. Further preclinical and clinical investigational studies are highly recommended to explore the proposed favorable antitumor effects of combining Dasatinib and Celecoxib, not only in BC, but also in other different types of cancer.

Declarations

Ethical approval:

Not applicable

Consent to participate:

Not applicable

Consent to publish:

We declare that no part of the work referred to in here has been published before.

Author's Contributions:

N.M, M.M, M.W.H contributed to the research idea. N.M, M.M, M.W.H conceived and designed the experiments. N.M, M.M, M.W.H conducted the experiments. N.M, M.M, M.W.H, M.H contributed to data analysis and presentation. N.M wrote the manuscript. N.M, M.M, M.W.H, M.H revised the manuscript and **declared that the data were generated in-house and that no paper mill was used.**

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Availability of data and materials:

All supplementary data are available upon request

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Figures

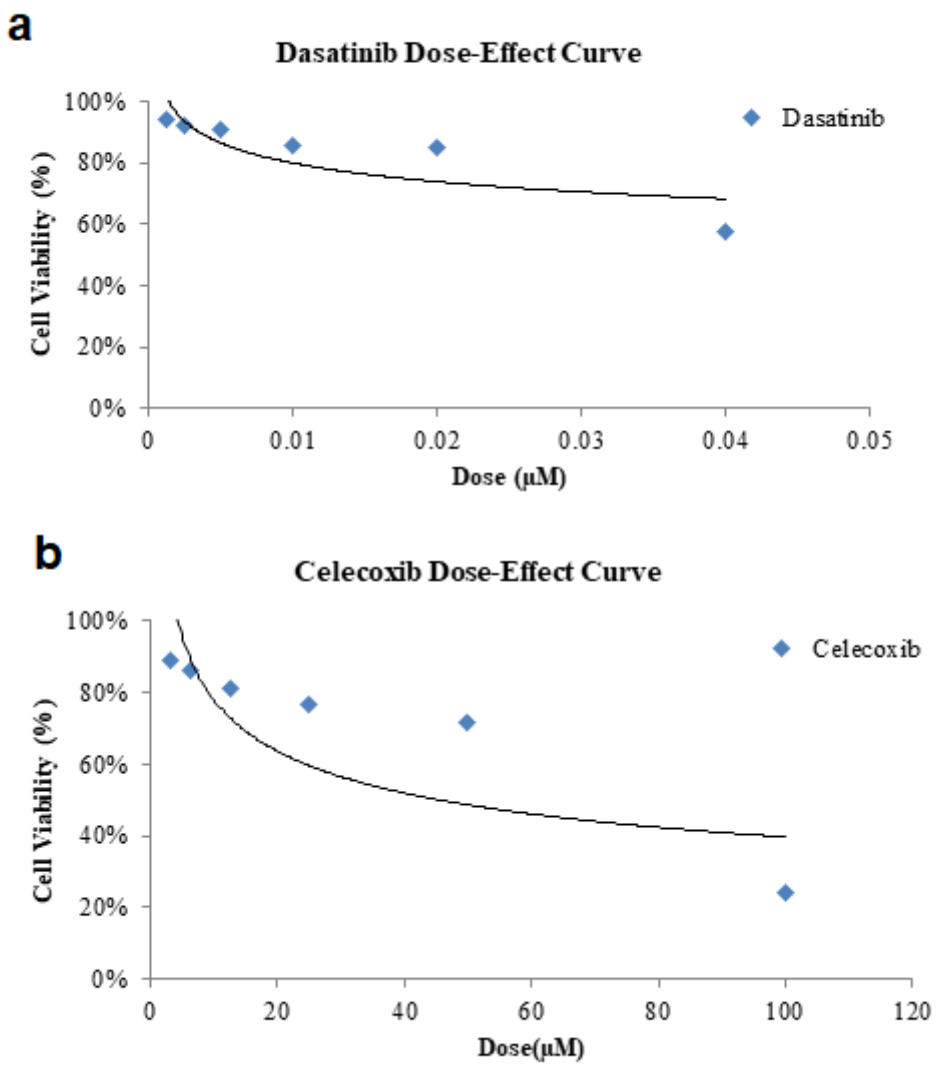

Figure 1

Viability of MDA-MB-231 cells treated with (a) Dasatinib and (b) Celecoxib. MTT was conducted to determine the cell viability. Data points represent mean ± standard error of the mean, each performed in triplicate.

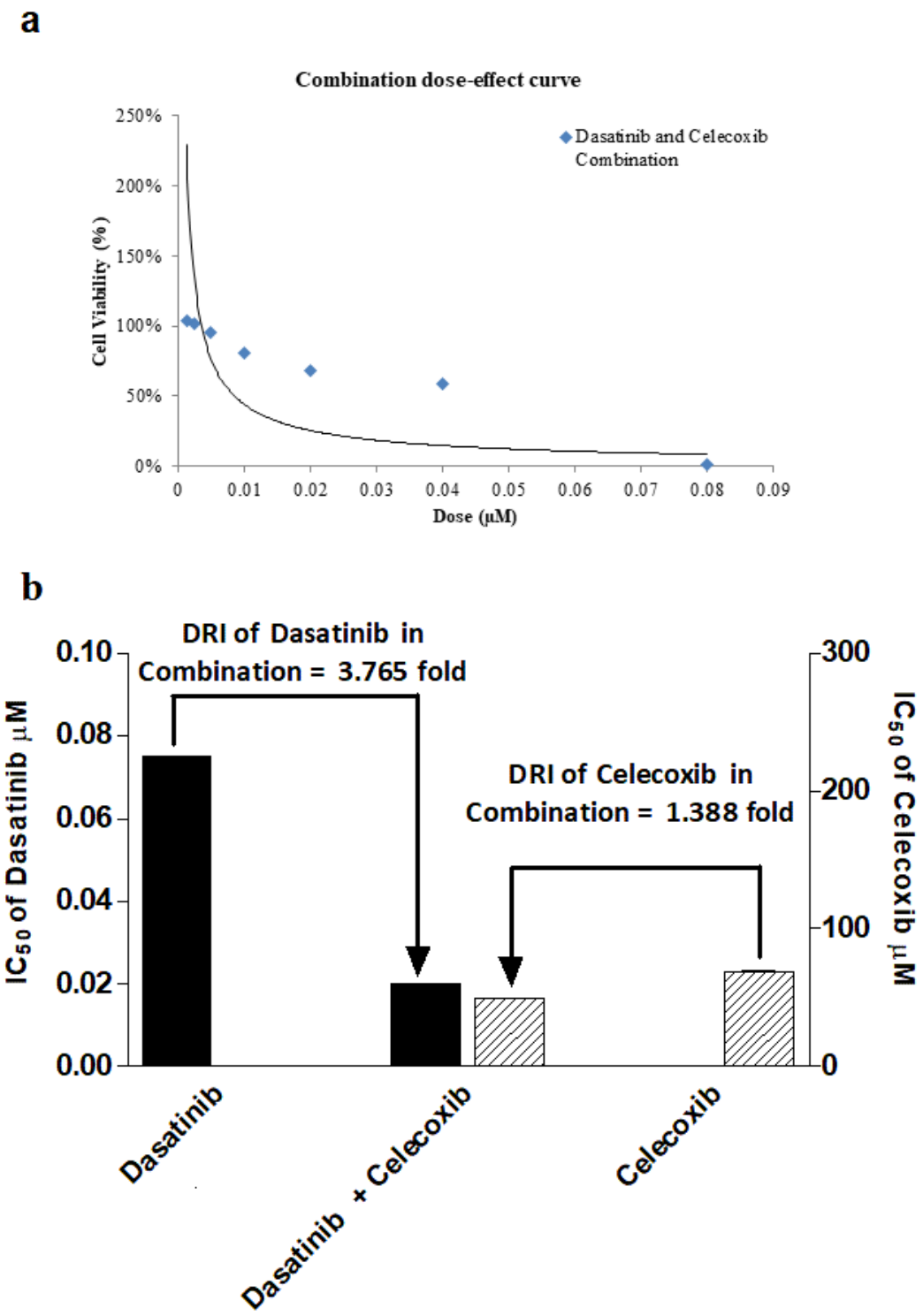

Figure 2

Viability of MDA-MB-231 cells treated with (a) Dasatinib/Celecoxib. (b) Dose reduction index for Dasatinib/Celecoxib combination. Data points represent mean ± standard error of the mean, each performed in triplicate.

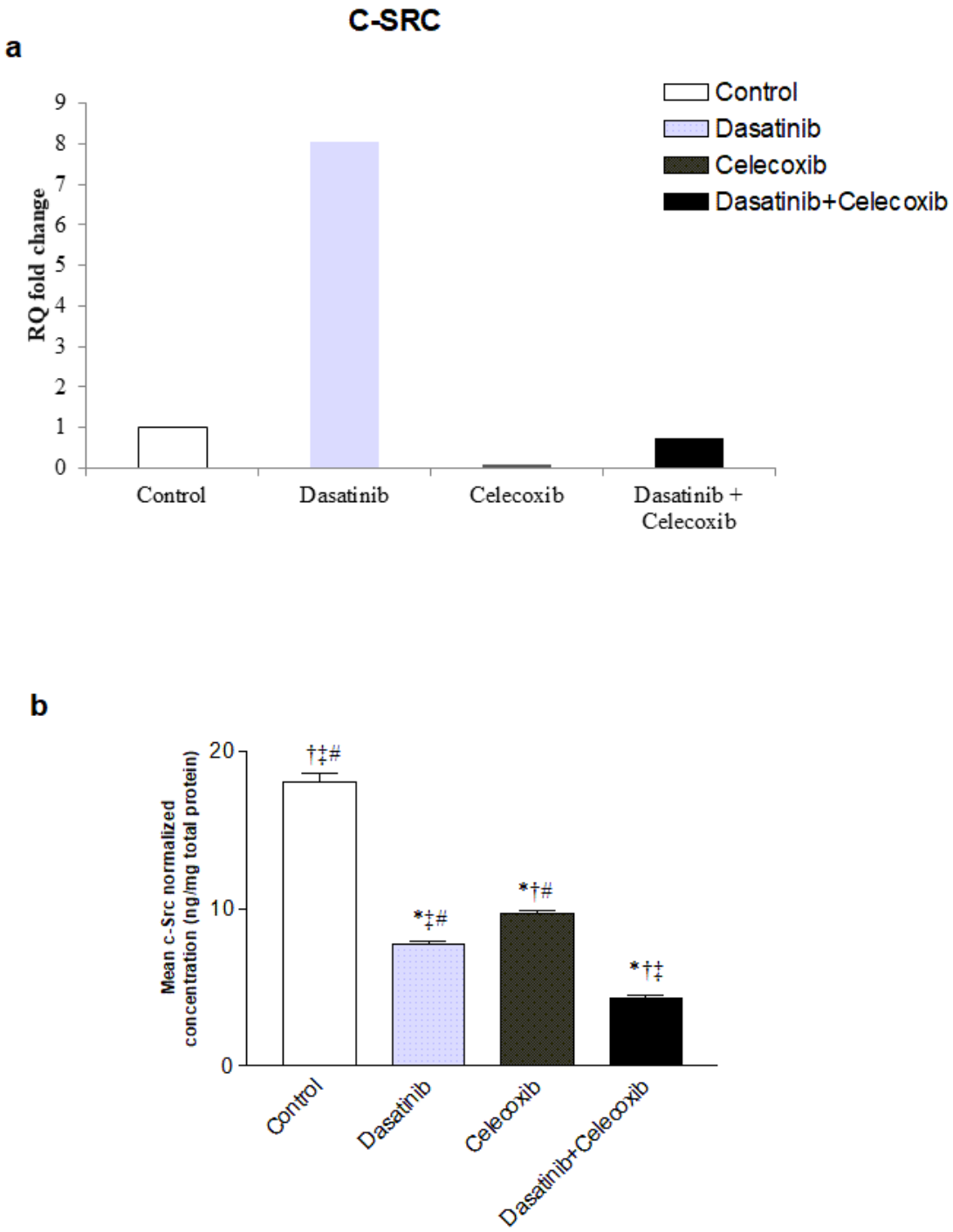

Figure 3

Effect of Dasatinib (0.05699 μM), Celecoxib (69.0976 μM) and their combination on (a) c-Src gene expression level, and (b) c-Src protein level (ng/mg total protein) in MDA-MB 231 cell lysates after 72 hours of treatment. Data presented as Mean ± standard error of the mean. * $p < 0.05$ versus Control; † $p < 0.05$ versus Dasatinib; ‡ $p < 0.05$ versus Celecoxib; # $p < 0.05$ versus Dasatinib+Celecoxib combination.

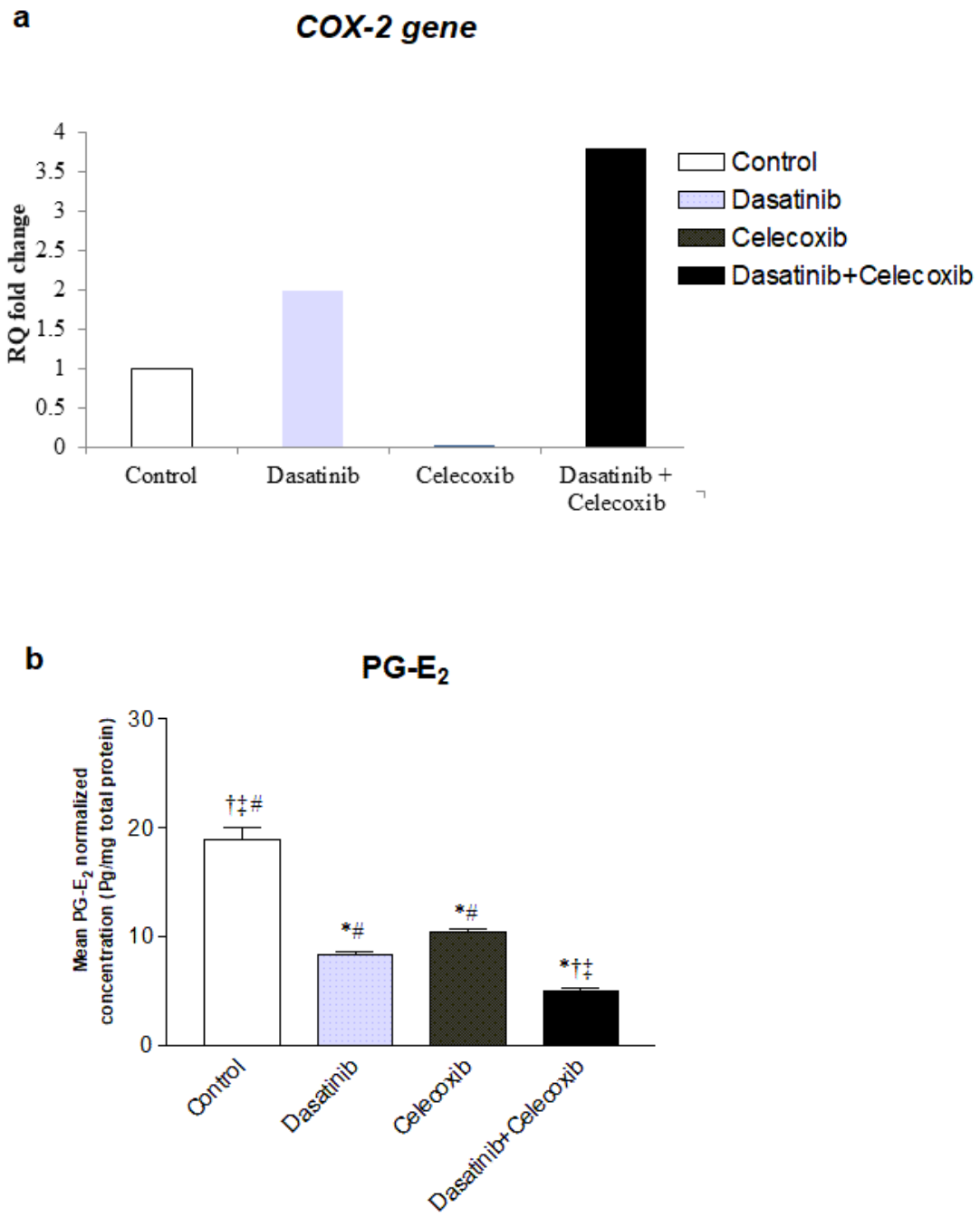

Figure 4

Effect of Dasatinib (0.05699 μM), Celecoxib (69.0976 μM) and their combination on (a) COX-2 gene expression level, and (b) PGE2 protein level (Pg/mg total protein) in MDA-MB 231 cell lysates after 72 hours of treatment. Data presented as Mean ± standard error of the mean. * $p < 0.05$ versus Control; † $p < 0.05$ versus Dasatinib; ‡ $p < 0.05$ versus Celecoxib; # $p < 0.05$ versus Dasatinib+Celecoxib combination.

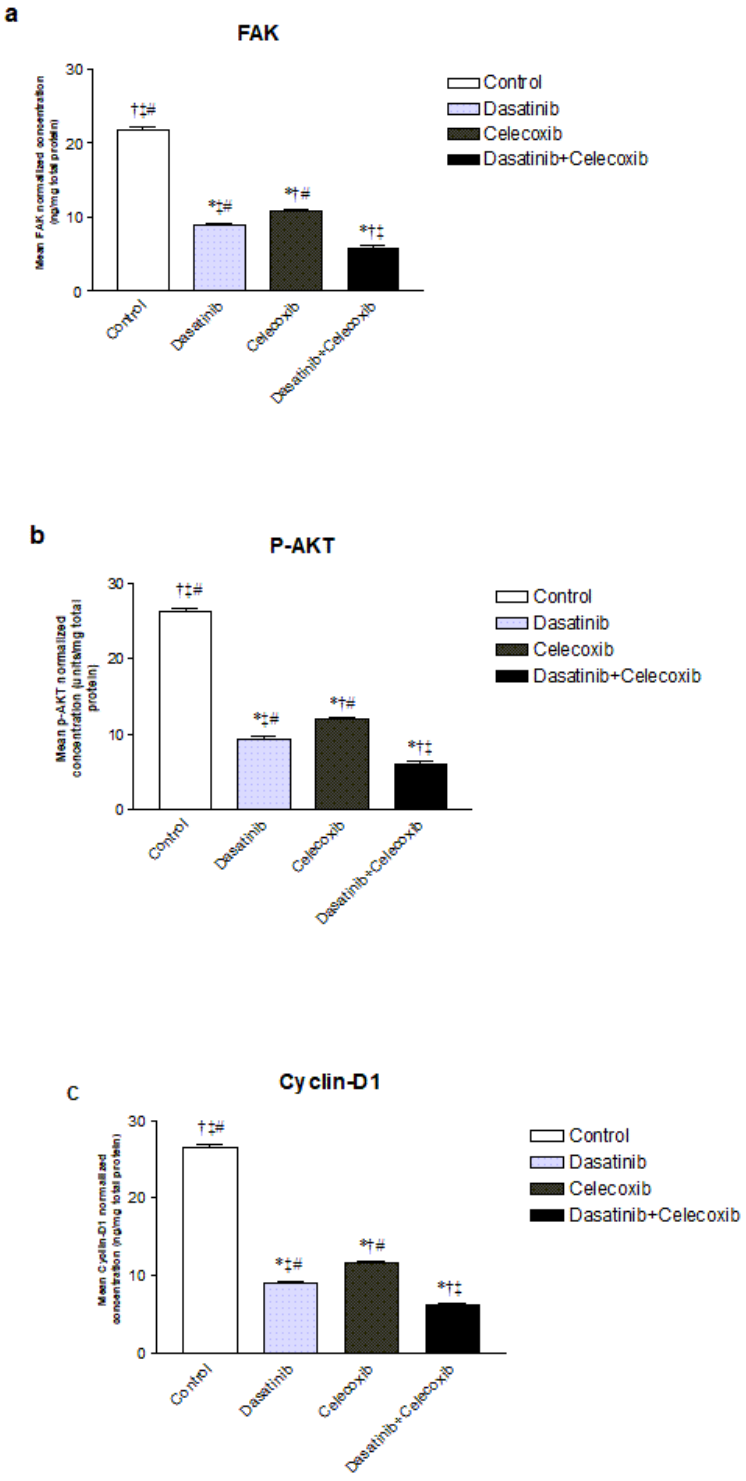

Figure 5

Effect of Dasatinib (0.05699 μM), Celecoxib (69.0976 μM) and their combination on (a) FAK, (b) p-AKT, and (c) cyclin D1 protein levels in MDA-MB 231 cell lysates after 72 hours of treatment. Data presented as Mean ± standard error of the mean. * $p < 0.05$ versus Control; † $p < 0.05$ versus Dasatinib; ‡ $p < 0.05$ versus Celecoxib; # $p < 0.05$ versus Dasatinib+Celecoxib combination.

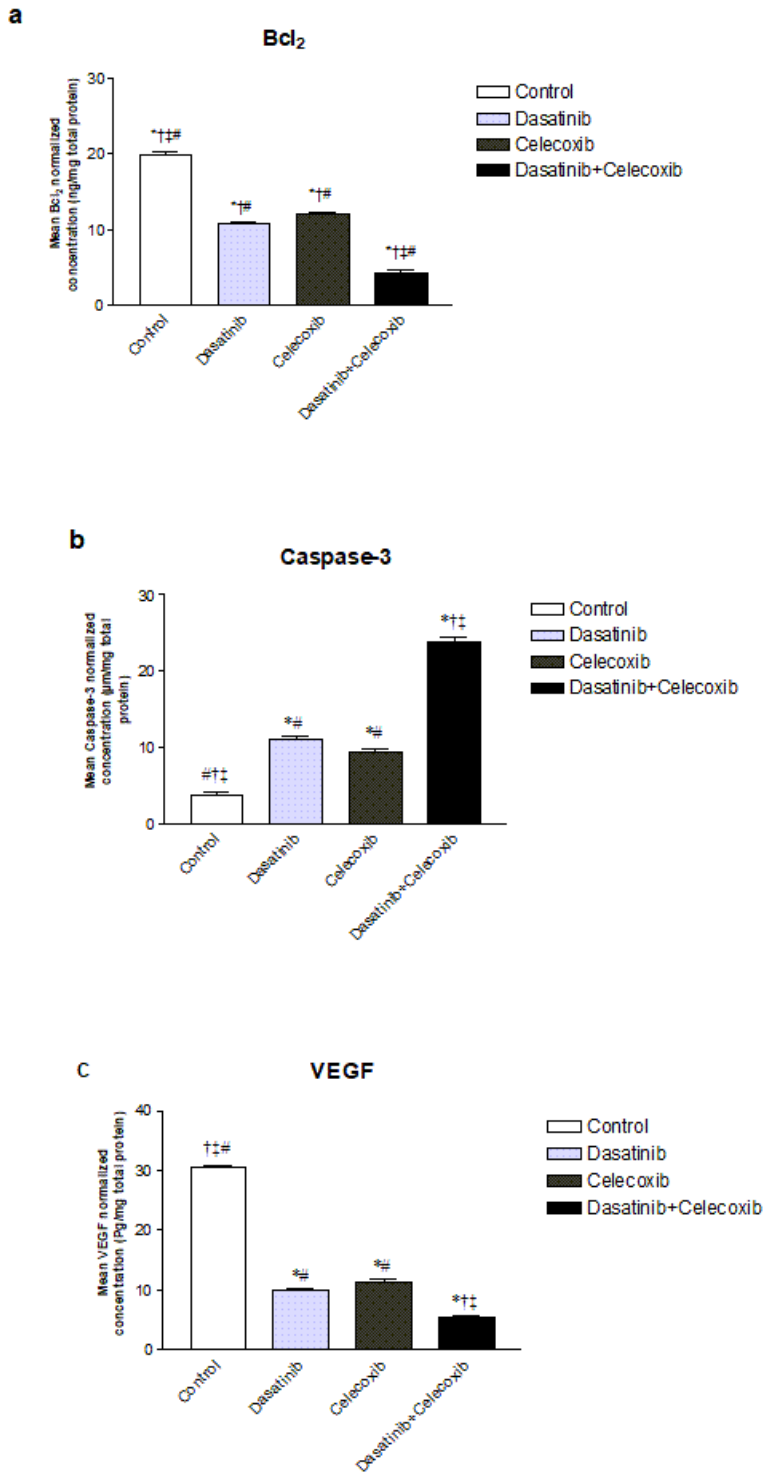

Figure 6

Effect of Dasatinib (0.05699 µM), Celecoxib (69.0976 µM) and their combination on (a) Bcl-2, (b) caspase-3, and (c) VEGF protein levels in MDA-MB 231 cell lysates after 72 hours of treatment. Data presented as Mean ± standard error of the mean. * $p < 0.05$ versus Control; † $p < 0.05$ versus Dasatinib; ‡ $p < 0.05$ versus Celecoxib; # $p < 0.05$ versus Dasatinib+Celecoxib combination.

Supplementary Files

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- 1MTTandClFig12.pzm
- 2ParametersandPCRFig345.pzm
- 3ELISAParameters.pzm