current issues in molecular biology

MDPI

Article

Anti-Migratory and Anti-Invasive Effects of 1,3,8-Triazaspiro[4.5]decane-2,4-dione in PC-3 Prostate Cancer Cells: Association with FAK/Src Signalling

Javier de Jesus Vasconcelos-Ulloa [1,2], Victor García-González [1,2], Ignacio A. Rivero [3], Eloy Andrés Pérez-Yépez [4], Angel Pulido-Capiz [1,2], Mario Israel Oregel-Cortez [1,2], Raul Díaz-Molina [1,2,5,*] and Octavio Galindo-Hernández [1,2,*]

1 Faculty of Medicine and Nutrition, Autonomous University of Baja California, Mexicali 21000, Baja California, Mexico; javier.vasconcelos@uabc.edu.mx (J.d.J.V.-U.); vgarcia62@uabc.edu.mx (V.G.-G.); pulido.angel@uabc.edu.mx (A.P.-C.); mario.israel.oregel.cortez@uabc.edu.mx (M.I.O.-C.)
2 Multidisciplinary Laboratory of Metabolic Studies and Cancer, Autonomous University of Baja California, Mexicali 21000, Baja California, Mexico
3 Graduate and Research Center in Chemistry, Technological Institute of Tijuana, Tijuana 22510, Baja California, Mexico; irivero@tectijuana.mx
4 Genomics Laboratory, National Cancer Institute, Mexico City 14080, Mexico; eperezy@conahcyt.mx
5 Department of Education and Research, Health Services of the Mexican Social Security Institute for Welfare (IMSS-BIENESTAR), Mexicali 21000, Baja California, Mexico
* Correspondence: rauldiaz@uabc.edu.mx (R.D.-M.); octavio.galindo@uabc.edu.mx (O.G.-H.)

Abstract

Cancer is known as a microevolutionary disease associated with genetic and epigenetic modifications, which is considered a global problem for which there is no fully effective treatment. Prostate cancer (PCa) is the second most commonly diagnosed cancer in men, with 1,414,259 new global cases (14.1%) in 2020. The aim of the present study was to evaluate the activity of a molecule of the triazaspirane family in PC-3 prostate cancer cells, through possible negative regulation of the FAK/Src signal transduction pathway. The effects of 1,3,8-triazaspiro[4.5]decane-2,4-dione (TSDD) on cell migration, invasion, and FAK/Src-related signalling were evaluated in PC-3 prostate cancer cells. In vitro experiments demonstrated reduced migration and invasion following TSDD treatment, accompanied by changes in MMP-9-associated gelatinolytic activity and the relative phosphorylation levels of FAK and Src. Although these findings suggest a possible association between the observed anti-migratory and anti-invasive effects and FAK/Src-related signalling.

Keywords: prostate cancer; PC-3 cells; triazaspirane; cell migration; invasion; FAK; Src

Check for updates

Academic Editor: Peter C. Hart

Received: 5 September 2026
Revised: 9 November 2026
Accepted: 17 November 2026
Published: 22 November 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

Cancer is considered a microevolutionary disease associated with genetic and epigenetic modifications [1], which is characterised by increased cell proliferation, evasion of growth suppressors, resistance to cell death, generation of immortalised cells, induction of angiogenesis, and invasion and metastasis [2].

Cancer is currently considered a major issue due to the complexity of establishing effective treatments. In 2020, according to the GLOBOCAN report, 19,292,789 new cases of cancer and 9,958,133 cancer-associated deaths were registered. Prostate cancer (PCa) ranks as the second most commonly diagnosed cancer in men, with 1,414,259 new cases (14.1%) reported in 2020 [3]. Currently, there are several treatment options for cancer, with surgery, chemotherapy and radiotherapy being the most widely used; however, these established

treatments compromise patient quality of life and are currently not completely effective [4]. Thus, it is important to identify alternatives, using new compounds that can be applied as coadjuvant drugs and improving the effectiveness of established treatments. Currently, the effectiveness of azaspirane-type molecules is being considered; these compounds have been associated with various beneficial effects, including immunoregulatory activity, which has been reported in in vivo models through the control of the inflammatory response, with important benefits being observed in murine models of rheumatoid arthritis, thus establishing that they exert an anti-inflammatory action and improve the preservation of bone integrity [5]. Thus far, there is little evidence of the anti-carcinogenic capacity of these molecules; however, the current research sets an interesting precedent for further research into these types of compounds.

N,N-diethyl-8,8-dipropyl-2-azaspiro[4,5]decane-2-propanamine (atiprimod) exhibits anti-proliferative and anti-angiogenic properties in myeloma cells through activation of pro-apoptotic proteins such as caspase-3 and -8, inhibition of Akt and STAT3 phosphorylation, and a decrease in vascular endothelial growth factor and interleukin-6 production [6,7]. This, together with a decrease in protein expression levels of JAK2 and phosphorylated p-JAK2 and thus negative regulation of the JAK-STAT pathway, which is overactive in tumour cells, may impact the mechanisms of tumour progression [8].

Focal adhesion kinase (FAK) and Src are key components of signalling networks involved in the regulation of cell adhesion, migration, and invasion, making them biologically relevant proteins in the study of prostate cancer progression. Considering the involvement of the FAK/Src signalling axis in these cellular processes, the present study aimed to evaluate the effects of 1,3,8-triazaspiro[4.5]decane-2,4-dione (TSDD) on migration and invasion in PC-3 prostate cancer cells, together with changes in MMP-9-associated gelatinolytic activity and the phosphorylation status of FAK and Src. These analyses were conducted to explore the potential association between the effects of TSDD on cell migration and invasion and alterations in FAK/Src-related signalling.

2. Materials and Methods

2.1. Cell Culture

The present study was an explanatory, experimental, quantitative and in vitro project. The PC-3 PCa cell line (CRL-1435) was used in the current study, which was acquired from the American Type Culture Collection (ATCC, Manassas, VA, USA). PC-3 tumour cell cultures were grown in 100 mm diameter boxes with Dulbecco's Modified Eagle Medium (DMEM) supplemented with 3.7 g/l sodium bicarbonate, 5% foetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) and antibiotics, in a humidified atmosphere containing 5% CO_2 at 37 °C. The cell line was periodically evaluated with the Venor™ GeM Mycoplasma Detection Kit, PCR-based (MilliporeSigma, Burlington, MA, USA), confirming negative contamination with *Mycoplasma* spp.

2.2. Preparation of the TSDD Compound

The TSDD compound was synthesised at the Graduate and Research Centre, Technological Institute of Tijuana (Tijuana, Baja California, Mexico), under methodology published by Rivero et al. [9]. The molecule was obtained in a pure state and a stock of 1 mM concentration was generated. The stock solution of TSDD was prepared using sterile distilled water as a solvent based on the favourable solubility of the compound in aqueous media and its compatibility with the cell culture systems employed. Finally, the stock solution was taken for the stimulation of PC-3 tumour cells according to the conditions of each experiment. The compound was purified and validated through spectroscopic and analytical methods, as previously described by Rivero et al. [9] and Santiago-Ruiz et al. [10].

2.3. Wound Closure Migration Test

To evaluate cell migration, a wound closure assay was conducted using PC-3 PCa cells. Cells were grown to 100% confluence and subjected to serum starvation by incubation in DMEM without FBS for 18 h, followed by treatment with mitomycin C for 2 h to inhibit cell proliferation. Linear wounds were generated using a sterile 200 µL pipette tip, and detached cells were removed by washing twice with 1× PBS. Subsequently, the cells were exposed to the TSDD compound for 24 h. In this assay, three concentrations of TSDD (10, 20 and 50 µM) were employed to assess the potential dose-dependent effects of TSDD on migratory capacity. While the 10 µM concentration was selected for subsequent experiments based on its pronounced inhibitory effect, all three concentrations were tested to ensure comprehensive evaluation. After the incubation period, the cells were fixed with cold methanol, stained with Coomassie Brilliant Blue, and washed with 1× PBS, and images were captured using a Vista Vision-VWR inverted microscope equipped with a digital camera (VWR International, Radnor, PA, USA).

2.4. Boyden Chamber Invasion Assay

The cell invasion assay was performed using a Boyden chamber (pore size, 8 µm diameter). Tumour cell cultures were subjected to FBS starvation for 18 h. In the Boyden chamber inserts, 15 µL Matrigel (BD Biosciences, Bedford, MA, USA) was added with 45 µL DMEM, and the insert was incubated for 30 min to ensure solidification of the DMEM-Matrigel. A cell passage was also carried out, from which 100 µL cells were taken from each insert and placed on the solidified DMEM-Matrigel. The different stimulus conditions (negative control (DMEM), positive control (DMEM + FBS), and treatments with TSDD at 10 µM), with a total volume of 600 µL, were placed on the working plate and incubated at 37 °C for 24 h. Subsequently, the medium was removed from each condition. The cells were fixed with cold methanol for 10 min, and a cotton swab was used to remove the excess cells that did not migrate from the upper chamber. Coomassie Brilliant Blue was then added for 10 min to stain the cells fixed at the bottom of the insert, followed by three washes with double distilled water. The analysis was concluded by visualisation of the Boyden chamber membrane, with images captured using an inverted microscope coupled to a camera.

2.5. Immunodetection

TSDD (10 µM) was used to stimulate confluent cell cultures, which were then lysed, and the amount of protein was quantified for each condition using the micro-Bradford method. The lysates were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis on 10% gels, after which they were transferred to polyvinylidene fluoride (PVDF) membranes. The PVDF membranes were then blocked with 5% milk for 2 h, followed by the addition of primary antibodies (1:500) at 4 °C overnight. The following antibodies were used for the Western blot technique: anti-p-FAK (Tyr397) (cat. no. sc-81493), anti-FAK (total) (cat. no. sc-271126), anti-p-Src (Tyr419) (cat. no. sc-81521), anti-Src (total) (cat. no. sc-19), anti-E-cadherin (cat. no. sc-8426), anti-N-cadherin (cat. no. sc-59987), and anti-β-actin (cat. no. sc-47778). All antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Subsequently, each membrane was washed three times (10 min each) with 0.1% PBS-Tween, followed by incubation with a horseradish peroxidase (HRP)-conjugated, species-specific secondary antibody (cat. no. sc-358914, dilution 1:5000) (Santa Cruz, CA, USA) at 37 °C for 2 h with agitation, and a further three washes (10 min each) with 0.1% PBS-Tween. The autoradiographs were scanned and the bands were semi-quantified using the ImageJ 1.52a software (National Institutes of Health, Bethesda, MD, USA).

2.6. Zymography

PC-3 cell cultures were stimulated with TSDD (10 µM), and the media were obtained, which were concentrated under the following conditions: Centrifuged at 2500 rpm for 2 h using Centricon filters (MilliporeSigma, Burlington, MA, USA). Subsequently, electrophoresis was performed, and samples and gels were prepared under the following conditions: Equal volumes of concentrated conditioned media were mixed with sample buffer (2.5% SDS, 4 µg/mL phenol red and 1% sucrose) without reducing agents, and loaded onto 8% acrylamide gels copolymerised with gelatine at 1 mg/mL. The obtained gels were washed twice with 2.5% Triton X-100 and incubated in activation buffer (50 mM Tris-HCl, 5 mM $CaCl_2$, pH 7.4) for 24 h at 37 °C, after which the gels were fixed and stained with Coomassie Brilliant Blue G-250 reagent in 30% methanol and 10% acetic acid. Proteolytic activity was identified by the presence of bands against the stained background of unconsumed substrate. Gels were scanned and bands were semi-quantified using ImageJ 1.52a software.

2.7. Statistical Analysis

The results obtained are presented as the mean ± standard deviation using GraphPad Prism Version 8.0.2 (GraphPad, Software, Boston, MA, USA). Data were analysed using a one-way ANOVA with a Dunnett's test. $p < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. Impact of TSDD on Cell Migration

The TSDD molecule showed inhibitory effects on the migration of PC-3 PCa cells. Figure 1A shows the inhibitory effect of the various concentrations of TSDD (10, 20 and 50 µM) over a time course of 24 h, with the 10 µM concentration showing the highest statistical significance with respect to the other conditions. In Figure 1B, the graph was obtained from at least three independent experiments and was analysed by one-way ANOVA.

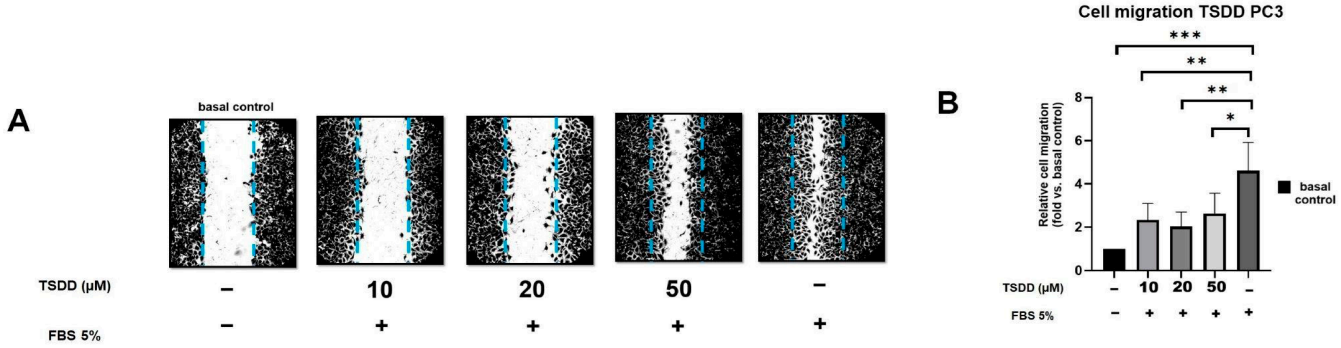


Figure 1. Effects of TSDD on cell migration. (**A**) A 24 h time course. Conditions are represented from left to right: Control, DMEM; TSDD 10 µM, 10 µM TSDD + DMEM + FBS 5%; TSDD 20 µM, 20 µM TSDD + DMEM + FBS 5%; TSDD 50 µM, 50 µM TSDD + DMEM + FBS 5%; FBS, DMEM + FBS 5%. (**B**) The graph represents the means of each condition ± SD of at least three independent experiments. Data were normalised to the basal control (DMEM without FBS), which was assigned a relative value of 1.0. Statistical comparisons were performed against the FBS-stimulated control (DMEM supplemented with 5% FBS). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, were determined using one-way ANOVA with Dunnett's test. Blue dashed lines indicate the boundaries of the wound area in the cell migration assay. CTRL, control; DMEM, Dulbecco's Modified Eagle Medium; FBS, foetal bovine serum; SD, standard deviation.

3.2. Effect of TSDD on Cell Invasion

In the cell invasion assay, the TSDD molecule was revealed to have a determinant effect in suppressing cell invasion after a 24 h stimulation of the cells. The positive control, 5% FBS in DMEM, promoted cell invasion; however, in the presence of TSDD an inhibitory effect was observed using the 10 µM concentration (Figure 2A). This concentration was selected based on the results of cell migration studies. Figure 2B shows that there was a statistically significant difference between treatment with TSDD (10 µM) and the positive control (FBS), suggesting the effectiveness of TSDD in suppressing cell invasion.

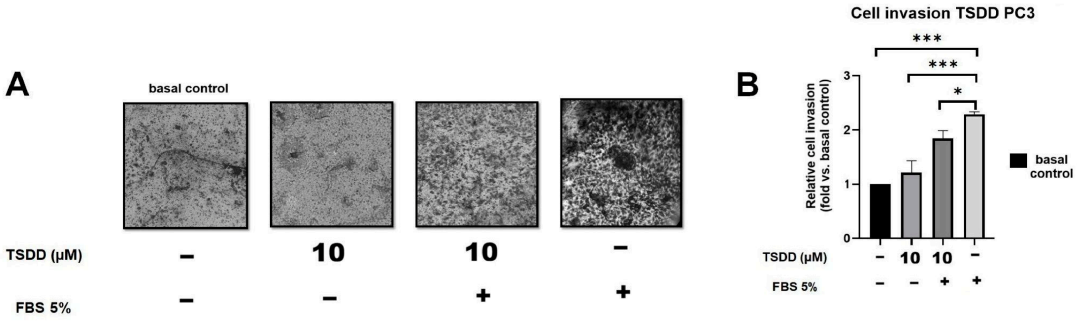


Figure 2. The cell invasion assay. (**A**) A 24 h time course. Conditions are plotted from left to right: CTRL, DMEM; TSDD DMEM, 10 µM 1,3,8-triazaspiro[4.5]decane-2,4-dione + DMEM; TSDD DMEM/FBS, 10 µM 1,3,8-triazaspiro[4.5]decane-2,4-dione + DMEM + FBS 5%; FBS, DMEM + FBS 5%. (**B**) The graph represents the means of each condition ± SD of at least three independent experiments. Conditions are represented from left to right: CTRL, DMEM; TSDD DMEM, 10 µM 1,3,8-triazaspiro[4.5]decane-2,4-dione + DMEM; TSDD DMEM/FBS, 10 µM 1,3,8-triazaspiro[4.5]decane-2,4-dione + DMEM + FBS 5%; FBS, DMEM + FBS 5%. Data were normalised to the basal control (DMEM without FBS), which was assigned a relative value of 1.0. Statistical comparisons were performed against the FBS-stimulated control (DMEM supplemented with 5% FBS). * $p < 0.05$, *** $p < 0.001$, were determined using one-way ANOVA with Dunnett's test. CTRL, control; DMEM, Dulbecco's Modified Eagle Medium; FBS, foetal bovine serum; SD, standard deviation.

3.3. Determination of MMP-9 Secretion

The zymogram in Figure 3A shows how the addition of TSDD (10 µM) resulted in a decrease in MMP-9 secretion compared with the control condition (DMEM). Notably, it is important to mention that in response to FBS treatment, an ideal contrast was not obtained, which may be due to the bias generated by the proteins found in FBS. After statistical analysis of the results of at least three independent experiments, Figure 3B shows a statistically significant difference denoting a suppression of MMP secretion as a consequence of triazaspirane activity.

3.4. Modulation of p-FAK and p-Src Levels by TSDD

Western blot analysis was performed to evaluate changes in the phosphorylation-related signals of p-FAK and p-Src in PC-3 cells following treatment with TSDD (10 µM) for 5, 15, and 30 min (Figure 4A). Densitometric analysis of three independent experiments revealed that FBS stimulation increased the relative p-FAK signal compared with the basal control. In contrast, TSDD-treated cells exhibited significantly lower p-FAK levels at all evaluated time points compared with the FBS-stimulated control, with the greatest reduction observed at 30 min (Figure 4B). Similarly, FBS stimulation increased the relative p-Src signal, whereas TSDD treatment resulted in significantly lower p-Src levels at 5, 15, and 30 min compared with the FBS-stimulated control (Figure 4C). Overall, these findings suggest that TSDD treatment is associated with reduced phosphorylation-related signals of FAK and Src under the experimental conditions evaluated.

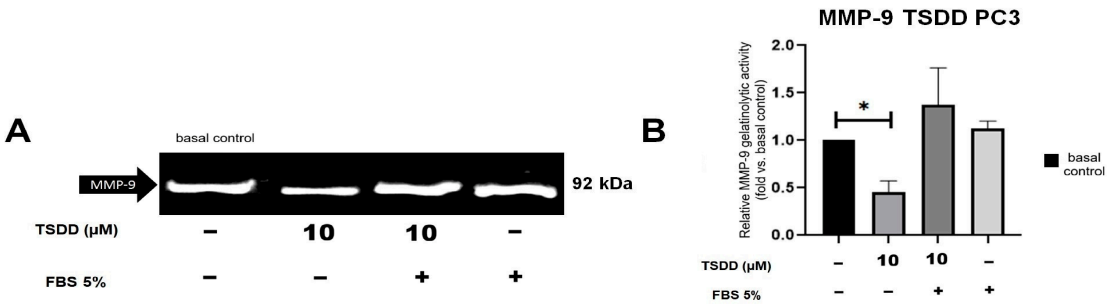


Figure 3. The determination of MMP-9 secretion. (**A**) A 24 h time course. Conditions are represented from left to right: CTRL, DMEM; TSDD DMEM, 10 μM 1,3,8 triazaspiro[4.5]decane-2,4-dione + DMEM; TSDD DMEM/FBS, 10 μM 1,3,8-triazaspiro[4.5]decane-2,4-dione + DMEM + FBS 5%; FBS, DMEM + FBS 5%. (**B**) The graph represents the means of each condition ± SD of at least three independent experiments. Conditions are represented from left to right: CTRL, DMEM; TSDD DMEM, 10 μM 1,3,8-triazaspiro[4.5]decane-2,4-dione + DMEM; TSDD DMEM/FBS, 10 μM 1,3,8-triazaspiro[4.5]decane-2,4-dione + DMEM + FBS 5%; FBS, DMEM + FBS 5%. Data were normalised to the basal control (DMEM without FBS), which was assigned a relative value of 1.0 and was also used as the reference group for statistical comparisons. * $p < 0.05$, analysed by one-way ANOVA with Dunnett's test. CTRL, control; DMEM, Dulbecco's Modified Eagle Medium; FBS, foetal bovine serum; SD, standard deviation.

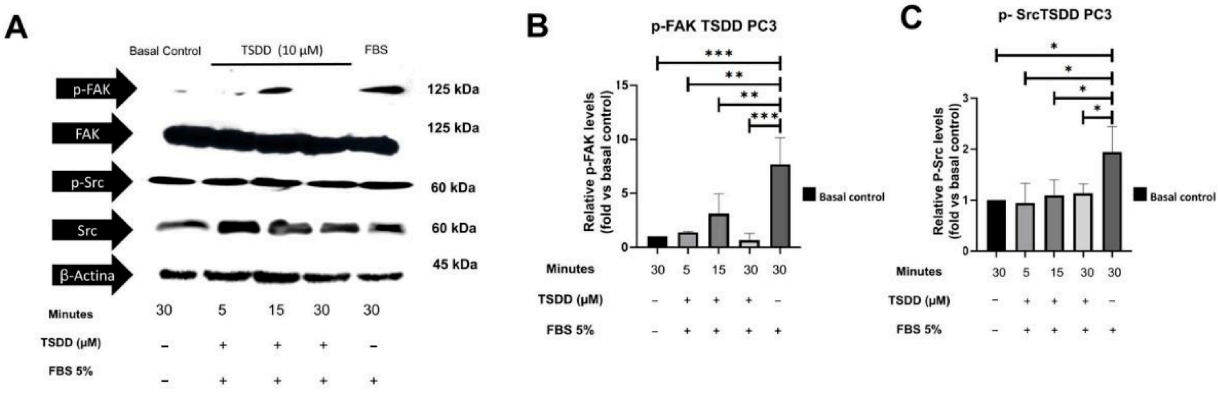


Figure 4. p-FAK and p-Src expression determination. (**A**) A time course of 5, 15 and 30 min and the detection of p-FAK and p-Src. Conditions are depicted from left to right: CTRL, DMEM; TSDD (5 min), 10 μM 1,3,8-triazaspiro[4.5]decane-2,4-dione + DMEM + FBS 5%; TSDD (15 min), 10 μM 1,3,8-triazaspiro[4.5]decane-2,4-dione + DMEM + FBS 5%; TSDD (30 min), 10 μM 1,3,8-triazaspiro[4.5]decane-2,4-dione + DMEM + FBS 5%; FBS, DMEM + FBS 5%. (**B**) The graph represents the mean relative expression levels of p-FAK ± SD of at least three independent experiments. Conditions are represented from left to right: CTRL, DMEM; TSDD (5 min), 10 μM 1,3,8-triazaspiro[4.5]decane-2,4-dione + DMEM + FBS 5%; TSDD (15 min), 10 μM 1,3,8-triazaspiro[4.5]decane-2,4-dione + DMEM + FBS 5% (FBS); TSDD (30 min), 10 μM 1,3,8-triazaspiro[4.5]decane-2,4-dione + DMEM + FBS 5%; FBS, DMEM + FBS 5%. (**C**) The graph represents the mean relative expression levels of p-Src ± SD of at least three independent experiments. Conditions are represented from left to right: CTRL, DMEM; TSDD (5 min), 10 μM 1,3,8-triazaspiro[4.5]decane-2,4-dione + DMEM + FBS 5%; TSDD (15 min), 10 μM 1,3,8-triazaspiro[4.5]decane-2,4-dione + DMEM + FBS 5%; TSDD (30 min), 10 μM 1,3,8-triazaspiro[4.5]decane-2,4-dione + DMEM + FBS 5%; FBS, DMEM + FBS 5%. Data were normalised to the basal control (DMEM without FBS), which was assigned a relative value of 1.0. Statistical comparisons were performed against the FBS-stimulated control (DMEM supplemented with 5% FBS). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Data were analysed by one-way ANOVA followed by Dunnett's test. CTRL, control; DMEM, Dulbecco's Modified Eagle Medium; FBS, foetal bovine serum; SD, standard deviation.

3.5. Determination of N-cadherin and E-cadherin Levels

Western blot analysis was performed to evaluate N-cadherin and E-cadherin protein levels in PC-3 cells following treatment with TSDD (10 µM) for 24 h (Figure 5A). N-cadherin levels showed numerical variations among the experimental conditions, with higher relative expression in TSDD-treated cells compared with the basal control. However, no statistically significant differences were observed between the TSDD-treated and FBS-stimulated groups (Figure 5B). Similarly, E-cadherin levels showed no statistically significant differences among the experimental conditions, including the basal control, FBS-stimulated control and TSDD-treated group (Figure 5C). Overall, these findings indicate that TSDD did not significantly alter the relative abundance of the detected E-cadherin and N-cadherin protein bands under the experimental conditions evaluated.

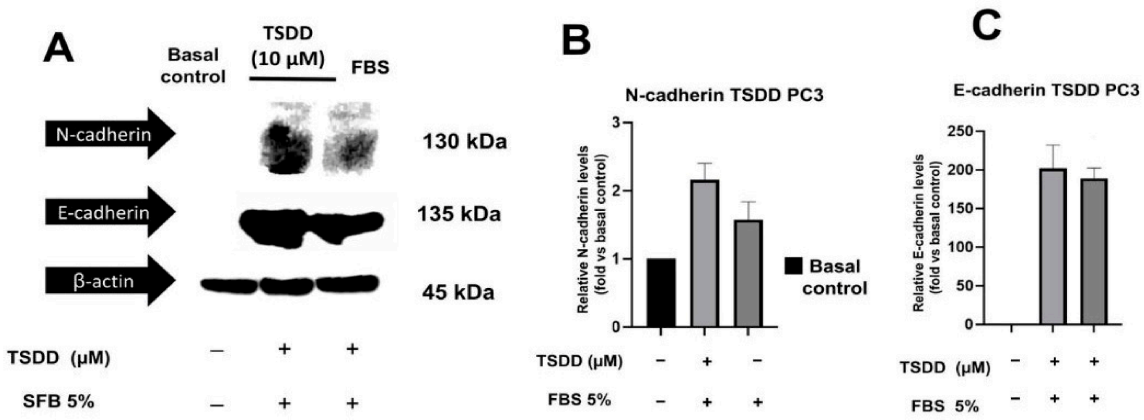


Figure 5. The determination of E-cadherin and N-cadherin expression. (**A**) A 24 h time course and detection of E-cadherin and N-cadherin. Conditions are represented from left to right: CTRL, DMEM; TSDD, 10 µM 1,3,8-triazaspiro[4.5]decane-2,4-dione + DMEM + FBS 5%; FBS, DMEM + FBS 5%. (**B**) The graph represents the mean relative expression levels of N-cadherin ± SD of at least three independent experiments. Conditions are represented from left to right: CTRL, DMEM; TSDD, 10 µM 1,3,8-triazaspiro[4.5]decane-2,4-dione + DMEM + FBS 5%; FBS, DMEM + FBS 5%. (**C**) The graph represents the mean relative expression levels of E-cadherin ± SD of at least three independent experiments. Conditions are represented from left to right: CTRL, DMEM; TSDD, 10 µM 1,3,8-triazaspiro[4.5]decane-2,4-dione + DMEM + FBS 5%; FBS, DMEM + FBS 5%. Data were analysed by one-way ANOVA. CTRL, control; DMEM, Dulbecco's Modified Eagle Medium; FBS, foetal bovine serum; SD, standard deviation.

4. Discussion

PCa is considered one of the most commonly diagnosed types of cancer, with an estimated 1.4 million new cases by the year 2020. It has been established that the areas with the highest incidence of PCa are Northern and Western Europe, the Caribbean, North and South America, Southern Africa, Australia and New Zealand [11]. In Mexican men, PCa is one of the main health problems, and it is the highest cause of cancer cases in men [29.9% of total cases (26,742 cases)] [3]. One of the factors associated with the high mortality rate of this type of cancer is the late diagnosis of patients, with the disease often being identified in advanced stages. Notably, PCa is described as clinically heterogeneous, with few tumours considered to have an aggressive phenotype because most are classified as indolent. Furthermore, another important complication of PCa is the resistance to androgen deprivation therapy, which leads to the development of castration-resistant PCa, which is strongly associated with the development of metastasis, the main cause of PCa-associated death [12].

Given the limited variety of effective treatment alternatives and the large number of problems faced by patients, such as urinary dysfunction, erectile dysfunction and sexual difficulties, as well as the fact that surgery does not have a good prognosis for treated patients [13,14], novel compounds that have positive effects to improve the quality of life of patients with PCa have been sought. Among the variety of compounds assessed are azaspiranes; these compounds have been tested in different types of cancer, such as multiple myeloma and lymphoma, where favourable results have been found in both in vitro and in vivo studies, demonstrating their application and potential area of study for future research [6].

Therefore, the present study proposed to evaluate the antitumour potential of a triazaspirane-type compound, 1,3,8-triazaspiro[4.5]decane-2,4-dione (TSDD), through assays of cellular processes associated with metastasis, such as cell migration and invasion. In addition, its effects on MMP-9-associated gelatinolytic activity and phosphorylation-related signals of the FAK/Src pathway were assessed. Hydantoin derivatives are known to be used in the treatment of epilepsy and cardiac arrhythmia; however, they have also been found to be useful in treating tumour cells. These compounds are quite prevalent in nature and offer diversity in their chemical structure, in particular, their antiproliferative effects have been observed in K562 leukaemia cells [15]. In modern medicinal chemistry, spiro compounds have been attributed to a wide range of physiological activities due to their considerable similarity to a large number of natural compounds, with spirooxindoles and spirohydantoins being some highly effective examples [16]. It is important to mention that spiro compounds are formed by a system of two orthogonally placed rings due to the sp3 hybridisation of the central spiro carbon, which allows them to adjust and introduce various heterocylic motifs, a fact that gives them a structure similar to various biomolecules such as proteins, thus favouring their interaction with biological systems [17]. Heterocyclic compounds present an interesting variety of forms that have been taken as a basis for the formation of drugs due to their similarity to natural compounds, vitamins and biomolecules [18].

In this context, atiprimod represents an example of an azaspirane-type compound that has been investigated for its potential antitumour effects, including interactions with signalling mechanisms associated with tumour progression [7,8]. These findings support continued investigation of structurally related compounds as potential modulators of cellular processes involved in cancer progression.

In the context of cancer progression, the FAK/Src signalling axis plays a central role in regulating key processes such as cell adhesion, migration, invasion and survival. This pathway is frequently dysregulated in malignant cells, making it an attractive therapeutic target for the development of anti-metastatic agents [19]. FAK activation involves autophosphorylation at Y397, which facilitates the recruitment of Src through its SH2 domain and promotes subsequent phosphorylation events, including those at Y576/Y577, required for full FAK activation [20]. Given the involvement of these proteins in signalling networks associated with tumour progression, the present study evaluated whether the effects of TSDD on PC-3 cell migration and invasion were accompanied by changes in the phosphorylation-related signals of FAK and Src. This approach allowed the exploration of a possible association between the biological effects of TSDD and FAK/Src-related signalling, without assuming direct interaction with or inhibition of either kinase.

The FAK/Src signalling axis is also involved in the regulation of cytoskeletal rearrangement, focal adhesion dynamics and the formation of invasive cellular structures, processes that contribute to tumour cell migration and invasion. Furthermore, FAK/Src-related signalling has been associated with mechanisms involved in epithelial–mesenchymal transition (EMT), including changes in cell adhesion and cellular plasticity. These processes may

contribute to the acquisition of an invasive phenotype and metastatic progression. However, although these mechanisms provide a biological context for interpreting the effects of TSDD, the present experimental findings do not establish that the compound directly regulates these downstream processes or induces changes in EMT. The involvement of FAK/Src signalling in the regulation of invasive cellular behaviour has been extensively described, highlighting its relevance in tumour progression [21].

During the analysis of cell migration, it was revealed that the triazaspirane-type molecule could significantly inhibit this process. This finding is supported by previous research, such as that carried out by Sulaiman et al. [22], who used an azaspirane-type molecule called CIMO. In addition, Pan et al. [23] indicated that it is important to establish that an inhibitor of the FAK protein has a significant inhibitory activity on cell migration. In the current study, a dose-response evaluation was performed using TSDD concentrations ranging from 1 to 50 µM. Among the tested concentrations, 10 µM produced statistically significant effects on cell migration and was therefore selected for subsequent experiments. For clarity, only concentrations showing statistically significant effects are presented in Figure 1. Similarly, in cell invasion assays, an essential component in assessing the development in cellular activity of PCa, it was observed that this result acts as an indicator of various phenomena linked to tumour progression. In the present study, inhibitory effects were detected when using the TSDD molecule at a concentration of 10 µM compared with the FBS control. This result is in agreement with the findings reported by Sulaiman et al. [22], who observed similar effects using the aforementioned CIMO molecule.

Another crucial element in the analysis of tumour progression is related to overactivation of the FAK/Src pathway leading to increased activation and secretion of MMPs. The upregulation and secretion of MMPs is linked to cell proliferation and invasion as well as an increase in tumour size. In a previous study, particular emphasis was placed on type 9 MMPs, known as gelatinases, which serve a decisive role in the development of invasion due to their ability to degrade the basement membrane; this effect is attributed to their affinity for collagen types IV, V, VII, X and XIV; gelatin; elastin; galectin-3; and fibronectin [24]. The present study exhibited a marked regulatory effect of TSDD on MMP secretion, showing significantly lower levels in the cells treated with 10 µM TSDD. This result may be important in the management of the regression of the EMT phenomenon, as it has been described that EMT is mediated by MMP activity, leading to loss of cell polarity and adhesion [25]. In a previous study by Jiang et al. [26] using sinomenine hydrochloride, a bioactive compound extracted from *Sinomenium acutum*, it was revealed that from a concentration of 0.0625 to 0.25 mM this compound could regulate the activity of MMP-2 and MMP-9, which was shown to be related to inhibition of the NF-κB pathway. This demonstrates an important relationship with the present results, as NF-κB activity is linked to upregulation of MMPs. This could indicate that TSDD possesses a possible regulatory effect on this signalling pathway, providing new perspectives for future evaluations with the molecule presented in the present study.

After confirming the inhibition of biological activity of PC-3 cancer cells, protein expression assays were performed to understand the possible mechanism underlying this inhibitory effect. The main focus was directed towards the expression of the phosphorylated forms of proteins linked to the FAK/Src pathway, as this represents the active form after receiving its activation stimulus. The data obtained in the present study revealed a suppressive effect of 10 µM TSDD on the active form of FAK, and after a longer exposure time there was a greater decrease in the expression of p-FAK protein. A previous study provided evidence of inhibition of migration and invasion in breast cancer cells through the use of shikonin; after its application, this compound was shown to exert a significant inhibitory effect on some of the mechanisms associated with tumour progression, and an important

relationship was revealed with the suppression of the expression of proteins related to signalling pathways such as STAT3, FAK and Src [27]. Based on the findings described above, the potential involvement of other signalling pathways, such as JAK-STAT3, emerges as a relevant target for further investigation of triazaspirane-type compounds, particularly regarding their role as regulatory molecules in tumour progression. In addition to the aforementioned findings, Src is recognised as a protein that undergoes a notable increase in its catalytic activity in various types of invasive cancer. The inhibition of its activity is therefore considered crucial to reduce the migratory and invasive capacity of cancer cells. Notably, the drug AZD0530, a Src kinase inhibitor, has been associated with the reversal of the invasive phenotype in breast cancer cells and has been implemented in phase II clinical studies. Since Src serves a role in migration and motility and at the same time regulates cell survival and proliferation pathways, it is considered an essential target for regulation in tumour cells [28]. In the results of the present study, it was observed that after exposure to TSDD (10 μM) for different durations, there was a significant suppressive effect on Src phosphorylation levels.

An important factor to take into consideration in the evaluation of tumour progression is the modification of cell–cell contacts; these contacts are mediated by calcium-dependent transmembrane glycoproteins called cadherins, which are essential in cell structuring and homeostasis. The study of two cadherins in particular has become relevant: N-cadherin and E-cadherin. N-cadherin is recognised as a factor linked to tumour progression and is considered a biomarker of EMT when increased, whereas E-cadherin is a marker of epithelial tissue, and its decrease is related to the generation of metastasis [29,30].

In the present study, TSDD treatment (10 μM) was associated with reduced migration and invasion of PC-3 prostate cancer cells, accompanied by a decrease in MMP-9-associated gelatinolytic activity and changes in the relative immunoreactive signals of phosphorylated FAK and Src. Although a numerical increase in E-cadherin expression was observed, this difference was not statistically significant, and no significant changes were detected in N-cadherin expression. Therefore, the available results do not support a definitive effect of TSDD on epithelial–mesenchymal transition. Given the established involvement of FAK/Src signalling in cell migration and invasion, the observed phosphorylation-related changes suggest a possible association between this signalling axis and the cellular response to TSDD. Nevertheless, these findings do not demonstrate direct inhibition of FAK or Src kinase activity, nor do they establish that modulation of this pathway is responsible for the observed reduction in migration and invasion. Further mechanistic studies are required to determine the molecular targets of TSDD and clarify the signalling events underlying its biological effects.

5. Conclusions

The present study provides evidence that 1,3,8-triazaspiro[4.5]decane-2,4-dione (TSDD) reduces the migratory and invasive behaviour of PC-3 prostate cancer cells under the experimental conditions evaluated. These findings were accompanied by changes in MMP-9-associated gelatinolytic activity and lower relative immunoreactive signals of phosphorylated FAK and Src, suggesting a possible association between the cellular effects of TSDD and signalling mechanisms involved in cell motility and invasion.

Several limitations should be considered when interpreting these findings. The study was conducted using a single prostate cancer cell line, and certain Western blot bands exhibited high signal intensity and limited definition.

Future investigations should focus on validating the biological effects of TSDD in additional prostate cancer models and employing complementary molecular and biochemical approaches to determine whether FAK/Src signalling contributes directly to its cellular

effects. Overall, these findings provide a preliminary basis for further investigation of TSDD and related triazaspirane compounds as modulators of cellular processes associated with prostate cancer progression.

Author Contributions: J.d.J.V.-U., I.A.R., O.G.-H. and R.D.-M. conceived and designed the analysis. J.d.J.V.-U., A.P.-C. and M.I.O.-C. contributed data or analysis tools. J.d.J.V.-U. performed the analysis. J.d.J.V.-U., O.G.-H. and R.D.-M. wrote the paper. V.G.-G., E.A.P.-Y., O.G.-H. and R.D.-M. performed writing—reviewing and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the 7th Special Call to Support Regional Needs 2022 (award no. 106/2/C/157/7) from the Autonomous University of Baja California (UABC).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data generated in the present study may be requested from the corresponding author.

Acknowledgments: The first author would like to express their gratitude to the National Council of Humanities, Sciences and Technologies (CONAHCYT) of Mexico for the support received through the granting of a scholarship for doctoral studies. The authors would also like to thank the Autonomous University of Baja California (UABC) for the financial support provided to carry out this project.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

Akt	Protein kinase B
DMEM	Dulbecco's Modified Eagle Medium
EMT	Epithelial-Mesenchymal Transition
FAK	Focal Adhesion Kinase
FBS	Foetal bovine serum
GLOBOCAN	Global Cancer Observatory
HRP	Horseradish peroxidase
JAK	Janus kinase
JAK2	Janus kinase 2
MMP-9	Matrix metalloproteinase-9
PBS	Phosphate-Buffered Saline
PCa	Prostate cancer
PVDF	Polyvinylidene fluoride
SD	Standard deviation
Src	Tyrosine-protein kinase Src
STAT	Signal Transducer and Activator of Transcription
STAT-3	Signal Transducer and Activator of Transcription 3
TSDD	1,3,8-triazaspiro[4.5]decane-2,4-dione

References

1. Orsolic, I.; Carrier, A.; Esteller, M. Genetic and epigenetic defects of the RNA modification machinery in cancer. *Trends Genet.* **2023**, *39*, 74–88. [CrossRef] [PubMed]
2. Moses, C.; Garcia-Bloj, B.; Harvey, A.R.; Blancafort, P. Hallmarks of cancer: The CRISPR generation. *Eur. J. Cancer* **2018**, *93*, 10–18. [CrossRef] [PubMed]
3. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.* **2021**, *71*, 209–249. [CrossRef] [PubMed]
4. Nevedomskaya, E.; Baumgart, S.J.; Haendler, B. Recent Advances in Prostate Cancer Treatment and Drug Discovery. *Int. J. Mol. Sci.* **2018**, *19*, 1359. [CrossRef] [PubMed]

5. Griswold, D.E.; Martin, L.D.; Badger, A.M.; Breton, J.; Chabot-Fletcher, M. Evaluation of the cutaneous anti-inflammatory activity of azaspiranes. *Inflamm. Res.* **1998**, *47*, 56–61. [CrossRef] [PubMed]
6. Hamasaki, M.; Hideshima, T.; Tassone, P.; Neri, P.; Ishitsuka, K.; Yasui, H.; Shiraishi, N.; Raje, N.; Kumar, S.; Picker, D.H.; et al. Azaspirane (N-N-diethyl-8,8-dipropyl-2-azaspiro [4.5] decane-2-propanamine) inhibits human multiple myeloma cell growth in the bone marrow milieu in vitro and in vivo. *Blood* **2005**, *105*, 4470–4476. [CrossRef] [PubMed]
7. Amit-Vazina, M.; Shishodia, S.; Harris, D.; Van, Q.; Wang, M.; Weber, D.; Alexanian, R.; Talpaz, M.; Aggarwal, B.B.; Estrov, Z. Atiprimod blocks STAT3 phosphorylation and induces apoptosis in multiple myeloma cells. *Br. J. Cancer* **2005**, *93*, 70–80. [CrossRef] [PubMed]
8. Faderl, S.; Ferrajoli, A.; Harris, D.; Van, Q.; Kantarjian, H.M.; Estrov, Z. Atiprimod blocks phosphorylation of JAK-STAT and inhibits proliferation of acute myeloid leukemia (AML) cells. *Leuk. Res.* **2007**, *31*, 91–95. [CrossRef] [PubMed]
9. Rivero, I.A.; Reynoso, E.A.; Ochoa-Teran, A. Microwave-assisted synthesis of cycloalkanespirohydantoins and piperidylspirohydantoins as precursors of restricted α-amino acids. *Arkivoc* **2011**, *2011*, 260–271. [CrossRef]
10. Santiago-Ruiz, S.; Polverini, E.; Manjarrez, J.; Espinoza, K.A.; Reynoso, E.; Rivero, I.A. Virtual screening of putative anticonvulsant hydantoin-derived drugs and biological evaluation. *Ann. Med. Chem. Res.* **2016**, *2*, 1016–1023.
11. Bergengren, O.; Pekala, K.R.; Matsoukas, K.; Fainberg, J.; Mungovan, S.F.; Bratt, O.; Bray, F.; Brawley, O.; Luckenbaugh, A.N.; Mucci, L.; et al. 2022 Update on Prostate Cancer Epidemiology and Risk Factors-A Systematic Review. *Eur. Urol.* **2023**, *84*, 191–206. [CrossRef] [PubMed]
12. Kensler, K.H.; Rebbeck, T.R. Cancer Progress and Priorities: Prostate Cancer. *Cancer Epidemiol. Biomark. Prev.* **2020**, *29*, 267–277. [CrossRef] [PubMed]
13. Popiołek, A.; Brzoszczyk, B.; Jarzemski, P.; Piskunowicz, M.; Jarzemski, M.; Borkowska, A.; Bieliński, M. Quality of Life of Prostate Cancer Patients Undergoing Prostatectomy and Affective Temperament. *Cancer Manag. Res.* **2022**, *14*, 1743–1755. [CrossRef] [PubMed]
14. Houédé, N.; Rébillard, X.; Bouvet, S.; Kabani, S.; Fabbro-Peray, P.; Trétarre, B.; Ménégaux, F. Impact on quality of life 3 years after diagnosis of prostate cancer patients below 75 at diagnosis: An observational case-control study. *BMC Cancer* **2020**, *20*, 757. [CrossRef] [PubMed]
15. Zagórska, A.; Czopek, A.; Jaromin, A.; Mielczarek-Puta, M.; Struga, M.; Stary, D.; Bajda, M. Design, Synthesis, and In Vitro Antiproliferative Activity of Hydantoin and Purine Derivatives with the 4-Acetylphenylpiperazinylalkyl Moiety. *Materials* **2021**, *14*, 4156. [CrossRef] [PubMed]
16. Ivanenkov, Y.A.; Kukushkin, M.E.; Beloglazkina, A.A.; Shafikov, R.R.; Barashkin, A.A.; Ayginin, A.A.; Serebryakova, M.S.; Majouga, A.G.; Skvortsov, D.A.; Tafeenko, V.A.; et al. Synthesis and Biological Evaluation of Novel Dispiro-Indolinones with Anticancer Activity. *Molecules* **2023**, *28*, 1325. [CrossRef] [PubMed]
17. Ramdani, L.H.; Talhi, O.; Taibi, N.; Delort, L.; Decombat, C.; Silva, A.; Bachari, K.; Vasson, M.-P.; Caldefie-Chezet, F. Effects of Spiro-bisheterocycles on Proliferation and Apoptosis in Human Breast Cancer Cell Lines. *Anticancer Res.* **2016**, *36*, 6399–6408. [CrossRef] [PubMed]
18. Bourzikat, O.; El Abbouchi, A.; Ghammaz, H.; El Brahmi, N.; El Fahime, E.; Paris, A.; Daniellou, R.; Suzenet, F.; Guillaumet, G.; El Kazzouli, S. Synthesis, Anticancer Activities and Molecular Docking Studies of a Novel Class of 2-Phenyl-5,6,7,8-tetrahydroimidazo [1,2-b]pyridazine Derivatives Bearing Sulfonamides. *Molecules* **2022**, *27*, 5238. [CrossRef] [PubMed]
19. Valente, A.C.M.M.; de Farias, G.H.L.; Bernardo, A.C.R.; de Souza Alves, C.C.; de Moura Pereira, M.B.; Tognon-Ribeiro, R. Focal adhesion kinase inhibition decreases cell viability and induces apoptosis of JAK2 V617F positive cells. *Braz. J. Pharm. Sci.* **2023**, *59*, e23075. [CrossRef]
20. Roberts, W.G.; Ung, E.; Whalen, P.; Cooper, B.; Hulford, C.; Autry, C.; Richter, D.; Emerson, E.; Lin, J.; Kath, J.; et al. Antitumor activity and pharmacology of a selective focal adhesion kinase inhibitor, PF-562,271. *Cancer Res.* **2008**, *68*, 1935–1944. [CrossRef] [PubMed]
21. Poh, A.R.; Ernst, M. Functional roles of SRC signaling in pancreatic cancer: Recent insights provide novel therapeutic opportunities. *Oncogene* **2023**, *42*, 1786–1801. [CrossRef] [PubMed]
22. Sulaiman, N.B.S.; Mohan, C.D.; Basappa, S.; Pandey, V.; Rangappa, S.; Bharathkumar, H.; Kumar, A.P.; Lobie, P.E.; Rangappa, K.S. An azaspirane derivative suppresses growth and induces apoptosis of ER-positive and ER-negative breast cancer cells through the modulation of JAK2/STAT3 signaling pathway. *Int. J. Oncol.* **2016**, *49*, 1221–1229. [CrossRef] [PubMed]
23. Pan, Q.; Wang, Q.; Zhao, T.; Zhao, X.; Liang, Y.; Shi, M.; Chen, C.; Lin, F. FAK inhibitor PF-562271 inhibits the migration and proliferation of high-grade serous ovarian cancer cells through FAK and FAK mediated cell cycle arrest. *Med. Oncol.* **2023**, *40*, 215. [CrossRef] [PubMed]
24. Almutairi, S.; Kalloush, H.M.; Manoon, N.A.; Bardaweel, S.K. Matrix Metalloproteinases Inhibitors in Cancer Treatment: An Updated Review (2013–2023). *Molecules* **2023**, *28*, 5567. [CrossRef] [PubMed]
25. Zhang, J.; Hu, Z.; Horta, C.A.; Yang, J. Regulation of epithelial-mesenchymal transition by tumor microenvironmental signals and its implication in cancer therapeutics. *Semin. Cancer Biol.* **2023**, *88*, 46–66. [CrossRef] [PubMed]

26. Jiang, Y.; Jiao, Y.; Liu, Y.; Zhang, M.; Wang, Z.; Li, Y.; Li, T.; Zhao, X.; Wang, D. Sinomenine Hydrochloride Inhibits the Metastasis of Human Glioblastoma Cells by Suppressing the Expression of Matrix Metalloproteinase-2/-9 and Reversing the Endogenous and Exogenous Epithelial-Mesenchymal Transition. *Int. J. Mol. Sci.* **2018**, *19*, 844. [CrossRef] [PubMed]
27. Thakur, R.; Trivedi, R.; Rastogi, N.; Singh, M.; Mishra, D.P. Inhibition of STAT3, FAK and Src mediated signaling reduces cancer stem cell load, tumorigenic potential and metastasis in breast cancer. *Sci. Rep.* **2015**, *5*, 10194. [CrossRef] [PubMed]
28. Oswald, A.J.; Symeonides, S.N.; Wheatley, D.; Chan, S.; Brunt, A.M.; McAdam, K.; Schmid, P.; Waters, S.; Poole, C.; Twelves, C.; et al. Aromatase inhibition plus/minus Src inhibitor saracatinib (AZD0530) in advanced breast cancer therapy (ARISTACAT): A randomised phase II study. *Breast Cancer Res. Treat.* **2023**, *199*, 35–46. [CrossRef] [PubMed]
29. Kaszak, I.; Witkowska-Piłaszewicz, O.; Niewiadomska, Z.; Dworecka-Kaszak, B.; Ngosa Toka, F.; Jurka, P. Role of Cadherins in Cancer-A Review. *Int. J. Mol. Sci.* **2020**, *21*, 7624. [CrossRef] [PubMed]
30. Lin, W.H.; Cooper, L.M.; Anastasiadis, P.Z. Cadherins and catenins in cancer: Connecting cancer pathways and tumor microenvironment. *Front. Cell Dev. Biol.* **2023**, *11*, 1137013. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.