Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20494 – 20509
Received 05 August 2021; Accepted 15 September 2021.

Study of the effects of NCI667916 and NCI201725 on the invasion and migration of U87 cell line

Manar Zraikat[1*],Walhan Alshaer[2], Tasneem Alshelleh[1], Ahmed M Sada Alhanjori[1], Diala Walid Abu-Hassan[3], Ghada O Tuffaha[4],Mutasem Taha[4*]

Department of Pharmacology, Faculty of medicine, University of Jordan, Amman, Jordan[1]

Cell Therapy Center, The University of Jordan, PO Box: 5825, Amman, Jordan[2]

Department of Physiology and biochemistry, Faculty of medicine, University of Jordan, Amman, Jordan[3]

Department of Pharmaceutical Sciences, Faculty pharmacy, University of Jordan, Amman, Jordan[4]

* Manar Zraikat m.zraikat@ju.edu.jo

Department of Pharmacology, Faculty of Medicine, University of Jordan, Amman, Jordan

*Mutasem Tahamutasem@ju.edu.jo

Department of Pharmaceutical Sciences, Faculty pharmacy, University of Jordan, Amman, Jordan

Abstract

Aim: This work aims to study the effect of twoNCI compounds,NCI667916 andNCI201725 on the invasion and migration of glioblastoma U87 cells using 3Dspheroid invasion model and 2D scratch migration model.

The method involves the use of two famous tumor dissemination assays including the 3D invasion spherical model and the 2D scratch model using the U87 glioblastoma cells

The results showed inhibitory effects of both compounds compared to the control on the invasion and migration of the U87 cells in both the 3D invasion model and the 2D migration model.

The compounds NCI667916 and NCI201725 have shown positive inhibitory effects on the invasion of U87 spheres in 3D collagen invasion assay and in the 2D scratch migration assay.

Key words:

Cancer, Invasion, migration, Glioblastoma, invasion assay, migration assay.

Introduction

The tumour dissemination process involves metastasis, such as migration, invasion, adhesion, and angiogenesis. Metastasis is a process includes the detachment of cells from the primary tumour and movement of the cells on the basement membrane (migration), then penetration through the basement membrane by degradation of the ECM proteins (invasion). [1, 2]. The importance of the tumour dissemination process

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20494 – 20509
Received 05 August 2021; Accepted 15 September 2021.

in the development of tumors made this process a very important target for therapy[3].

This work aims to investigate the effect of two compounds on the tumor dissemination process. The two compounds are NCI667916 and NCI201725 (from the National Cancer Institute list of compound). The effects of these compounds wereinvestigated on the dissemination of Glioblastoma using two representing models for two major dissemination processes including the invasion and migration process. The cell line chosen for this purpose was the Glioblastoma U87 cellsknown to have high cell surface expression of $\alpha_v\beta_3$ integrin [4, 5].

$\alpha_v\beta_3$ integrinisknown to be significantly over-expressed on the surface of some solid tumour cells tumors such as melanoma, glioblastoma, prostate and breast cancers, and plays an important role in inducing metastasis, migration, invasion, angiogenesis in these cancers [6].$\alpha_v\beta_3$ integrin is considered to be important due to its high expression on the surface of many solid tumors in addition to its important role in inducing metastasis in glioblastoma[7, 8].
There are currently no FDA-approved antagonists for α_v integrins although many inhibitors are currently being researched for cancer therapy and other diseases like osteoporosis [9, 10]. For example, LM609 is a monoclonal antibody that targets $\alpha_v\beta_3$ integrin [11]thus blocking angiogenesis associated with TNFα and bFGF. The main problem associated with LM609 is the high immunogenic response that limited its use and so encouraged further research to be carried out, resulting in vitaxin[12]. Vitaxin is a humanized mouse monoclonal antibody derived from LM609 and is able to recognize $\alpha_v\beta_3$. Vitaxin blocks angiogenesisinduced by TNFα and bFGF. However Vitaxin did not progress beyond Phase I trials as was found to be ineffective [13]. Etaracizumab (known as Abegrin) is a humanized engineered monoclonal antibody against $\alpha_v\beta_3$ integrin and has completed Phase I and II clinical trials in prostate cancer and melanoma[14-16].
On the other hand, MK-0429 (Figure 1) represents a family of small molecules that targets $\alpha_v$integrins and has completed a Phase I/II study as potential treatment of solid tumors[17]. MK-0429 is being investigated to be used for the treatment of prostate cancer [18], ophthalmological problems, and osteoporosis [17]. Similarly, Cilengitide (Figure 1,a cyclic peptide antagonist of $\alpha_v\beta_3$ (IC_{50} = 2.3nM) and $\alpha_v\beta_5$ (IC_{50} = 37nM) integrins[19], was investigated for glioblastoma[20], melanoma [21], breast cancer [22] and head and neck cancer [23]. Low concentrations of Cilengitide*in vivo* resulted in stimulating tumor growth and angiogenesis indicating complex role of integrins in tumorigenesis[24]. Although phase I and phase II clinical trials indicated promising activity of Cilengitideagainst glioblastoma as monotherapy and in combination with other compounds [25]-[26], phase III clinical studies showed the addition of Cilengitide to glioblastoma standard therapy failed to meet the primary endpoint of increasing overall survival leading to the decision that Cilengitide will not be developed as an anticancer drug in the future[27, 28].

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20494 – 20509
Received 05 August 2021; Accepted 15 September 2021.

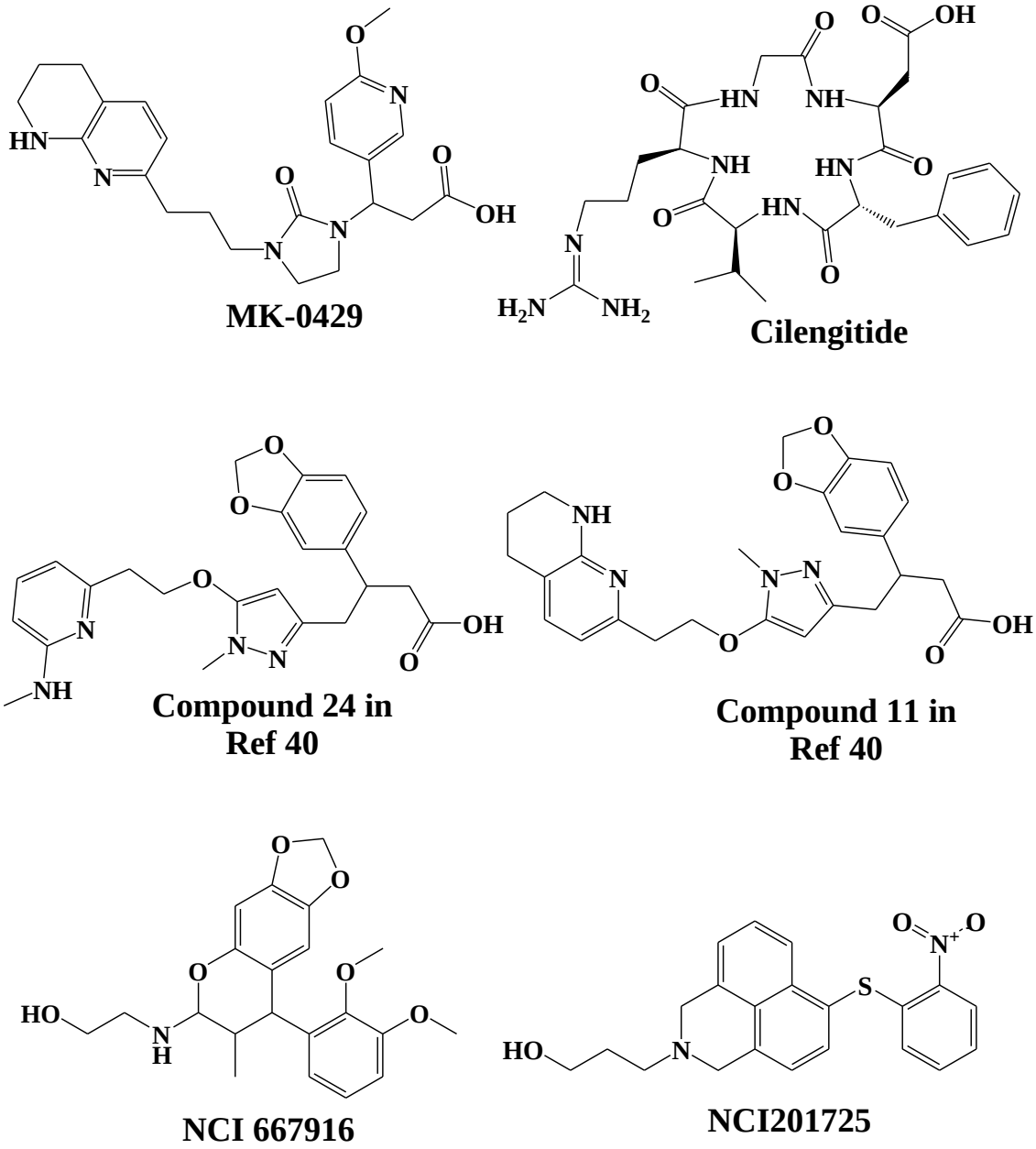

Figure 1. Chemical Structures of known $\alpha_v\beta_3$ integrin inhibitors including tested compoundsNCI667916 and NCI201725.

Interest in the development in new clinically viable $\alpha_v\beta_3$ integrinblockers prompted us to implement a similarity search protocol to screen the National Cancer Institute database for new inhibitors. MK-0429 and two drug-like potent $\alpha_v\beta_3$ integrin inhibitors (compounds 24 and 11 in reference 40) were used as molecular templates while the molecular similarity was assessed using MDL-public keys as molecular fingerprints and Tanimoto indexas similarity criteria [41]. Out of the top-ranking molecules, NCI667916 (similarity score = 0.71) and NCI201725 (similarity score =0.52) showed significant activity in subsequent *in vitro* assays.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20494 – 20509
Received 05 August 2021; Accepted 15 September 2021.

The anti-invasive effects of these compoundswere determined by testing specific concentrations and determining their effect on the invasion of U87 cells from spheres in 3D collagen invasion assay and 2D scratch assay.

The concentrations of these compounds were selected based on MTT tests done to determine the IC_{50} of those compounds.The concentrations selectedfor the invasion assay were less than the valuesof the IC_{50} of the compounds. The anti-migration properties of the compounds were determined based on using the same concentrations used in the invasion assay. The effect studied was the inhibitory effect of the certain concentrations of the compounds on the migration of U87 cells in scratch assay.

Material and methods

The cell line and reagents:

The U87-MG Glioma cell line was purchased from the European Collection of Cell Cultures ((ECACC), Salisbury, Wiltshire, England). Standard conditions of 37°C and 5% CO2 humidified atmosphere were used to maintain the cells. In the 3 D invasion assay, the Collagen usedwas catalogue number C4243.The reagents used in the MTT assay werepurchased from Sigma-Aldrich (Poole, UK).

Methods

MTT Assay

The U87 cells were treated with different concentrations of the tow compounds over 7 days before doing the MTT assay. The cells were incubatedat 37°C, 5% CO2 for 4 hours with previously prepared MTT solution. The optical density of the plates was read at 570 nm after removal of the MTT solution. The assays were done in triplicates.

Collagen Invasion Assay:

The collagen invasion assay was prepared as previously shown in[29]. Briefly,The U87 spheroids were prepared from hanging drops and seeded between two layers of Collagen I in 8-chamber cover glass (Nunc, Lab-TeK, Thermo scientific). The collagen was over layered by 200 µL of RPMI and incubated at 37°C, 5% CO2 for 7 days. Image-Jprogram was used to analyze pictures taken for the spheroidsover 7 days. The t-test was used to analyses of the data.

Scratch Assay

The scratch assay was done in plates containing 70 -80 % confluent cells. First, the scrachswere done and the cells were washed with media. Second, the different concentrations of the compounds were added to 2 ml of full media for each well and added to the scratched wells. Finally the pictures were taken in different times including after 4 hours, 24 hours and 48 hour.The representative pictures of the scratch were analyzed by ImageJ program.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20494 – 20509
Received 05 August 2021; Accepted 15 September 2021.

Results and discussion

Compounds NCI667916 and NCI201725 were evaluated against U87 cells since theyoverexpress $\alpha_v\beta_3$ integrin. The two compounds were selected based on fingerprint similarity with known $\alpha_v\beta_3$ integrin inhibitors (see figure 1).

Two assays were selected to investigate the effects of the two compounds on the invasion and migration of the U87 cells. The first assay is the three-dimensional (3D) spheroid invasion assay. This assay is considered to mimic the *in vivo* invading capacity of tumors[30]. The 3D structure of the spheres mimic the 3D structure of the tumors*in vivo*[31]. furthermore the presence of collagen layers mimic the *in vivo*tumor microenvironment [32, 33]. The layers of collagen in the 3D assay also work as barriers between the drug and the target spheres making the assay more challenging and more able to reflect the real situations [32].

To evaluate tumor migration potential, we implemented the two-dimensional (2D) scratch assay [34, 35]. This assay describes the2D migration of tumor cells[36, 37]. The two compounds were tested by the two methods using identical concentrations for the purpose of comparison.

Initially, the two compoundswere evaluated by MTT assay that was repeated three times for each. Both NCI667916and NCI201725 yieldedIC$_{50}$ values of 7.0 µM. The concentrations used in the subsequent three-dimensional (3D) and two-dimensional (2D) assays were less than this value, i.e., at 0.5 µM and 5.0 µM.The measurements were repeated in triplicates.

Both compounds showed clear inhibitory effect on the invasion of the U87 spheres at the two tested concentrations.The inhibitory effects of both concentrations of the compound NCI667916 were comparable (figures 2 and 3). Furthermore, the compound NCI201725inhibited the invasion of U87 spheroids at both concentrations in a comparable way(figures4 and 5).

Both compounds inhibited the ability of U87 cells to migrate. The inhibitory effect of NCI667916 at the two concentrations wasgradual and proportional to the concentrations used(figures 6 and 7). However,the same two concentrations of NCI201725 yielded comparable effects (figures8 and9).

Clearly, the ability of both compounds to inhibit migration of U87 cells is more pronounced compared to their inhibition on invading spheres. This difference is supposed to be due to the presence of collagen in the 3D invasion assay making this assay more reflective to the realistic situation.

Future extra experimental work is recommended including investigating the effect of these compounds on extra invasion assays in addition to investigate the safety of these compounds before progressing towards further clinical studies.

Conclusion

In conclusion both the compounds NCI667916 and NCI201725 have shown positive inhibitory effects on the invasion of U87 spheres in 3D collagen invasion assay and in the 2D scratch migration assay and future investigating studies on those compounds is recommended.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20494 – 20509
Received 05 August 2021; Accepted 15 September 2021.

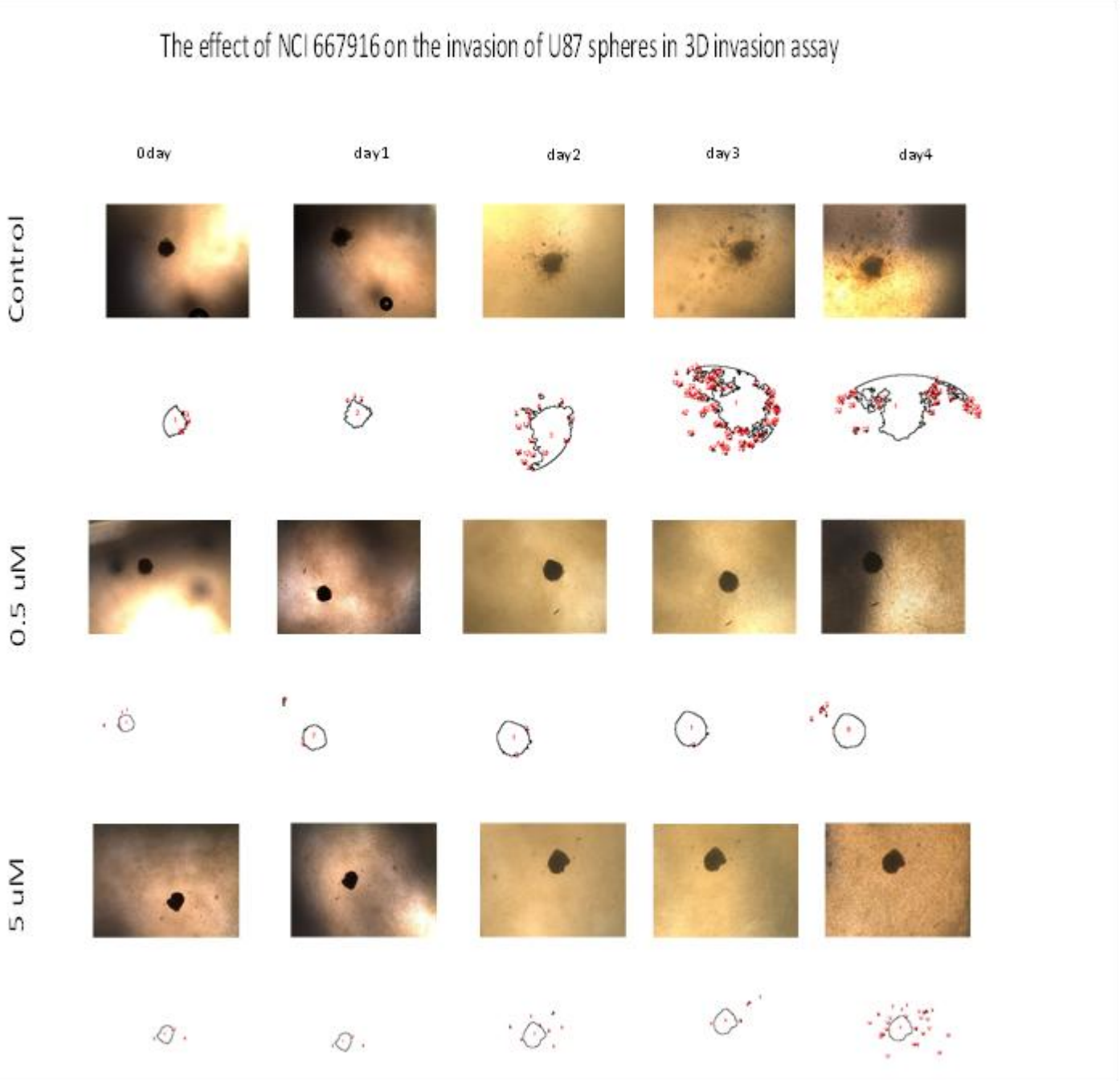

Figure2. the effect of the compound NCI667916 on the invasion of U87 spheres.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20494 – 20509
Received 05 August 2021; Accepted 15 September 2021.

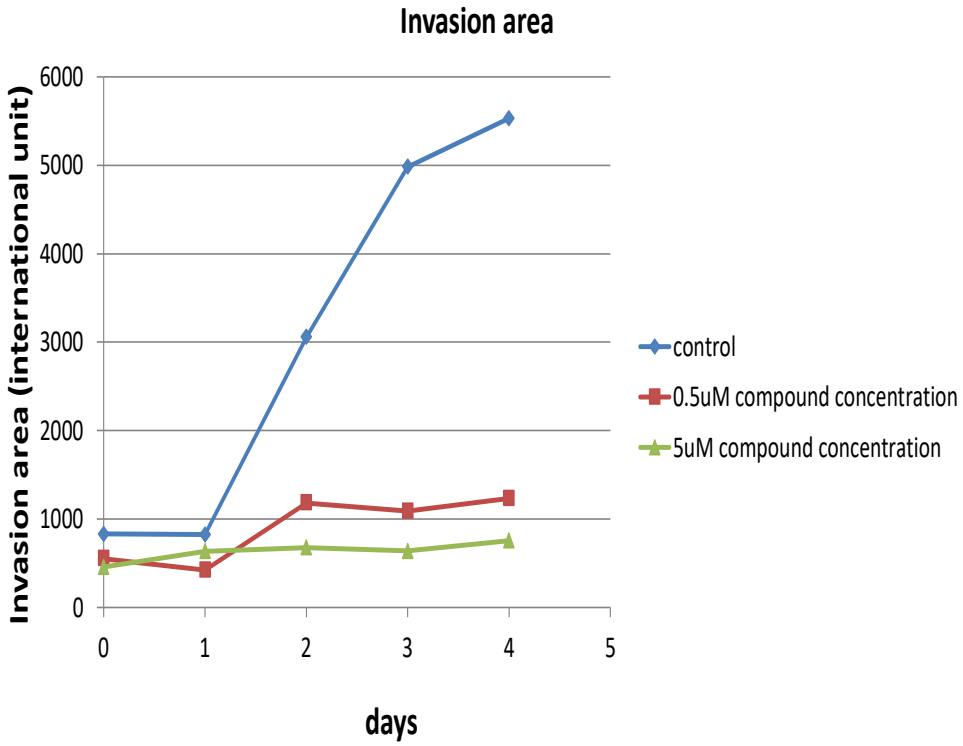

Figure 3. diagram shows the effect of the compound NCI667916 on the invasion of U87 spheres in the 3D invasion assay. Mathematical representation of the 3 D invasion assay results

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20494 – 20509
Received 05 August 2021; Accepted 15 September 2021.

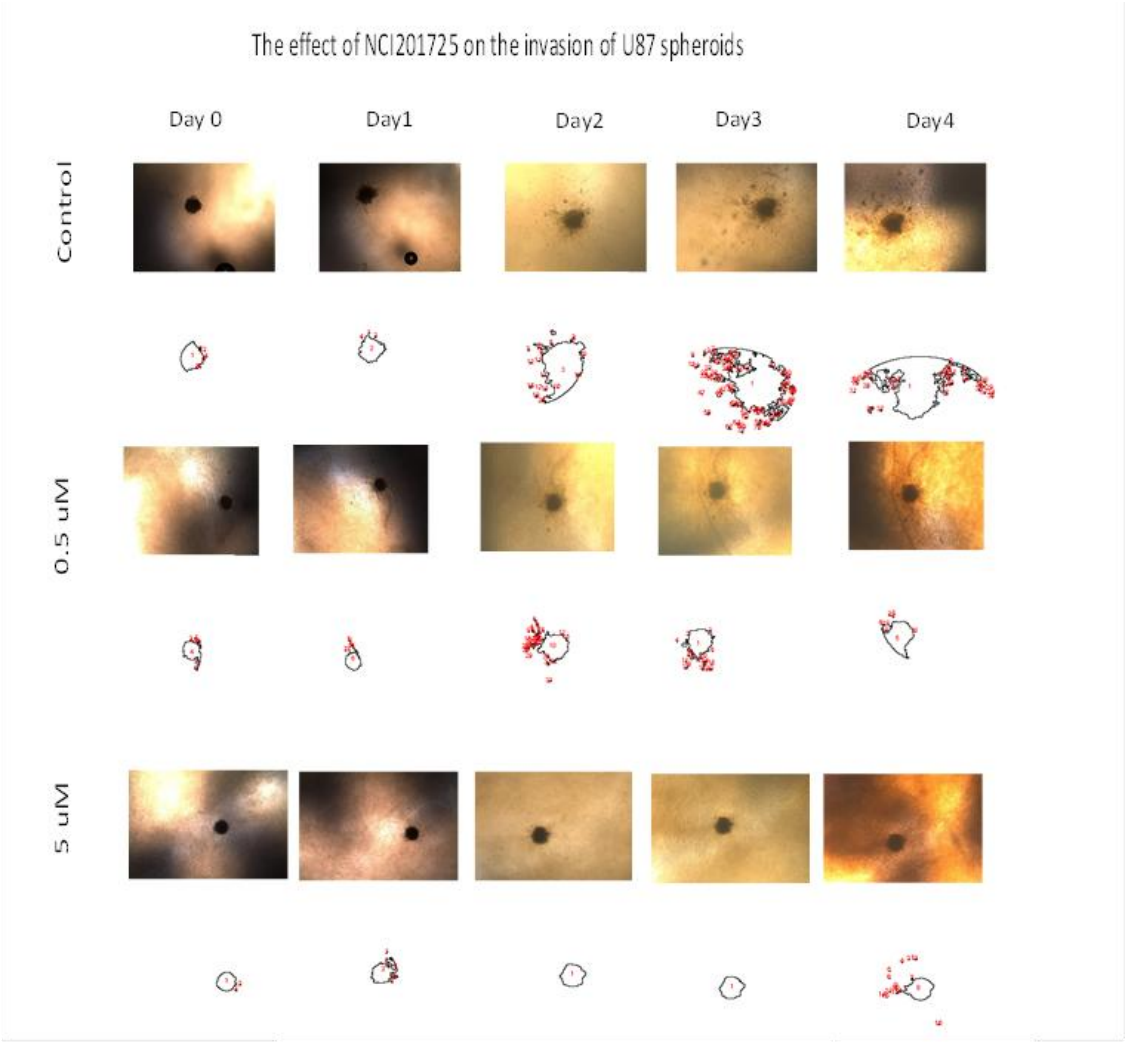

Figure 4. the effect of the compound NCI201725 on the invasion of U87 spheroids in collagen.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20494 – 20509
Received 05 August 2021; Accepted 15 September 2021.

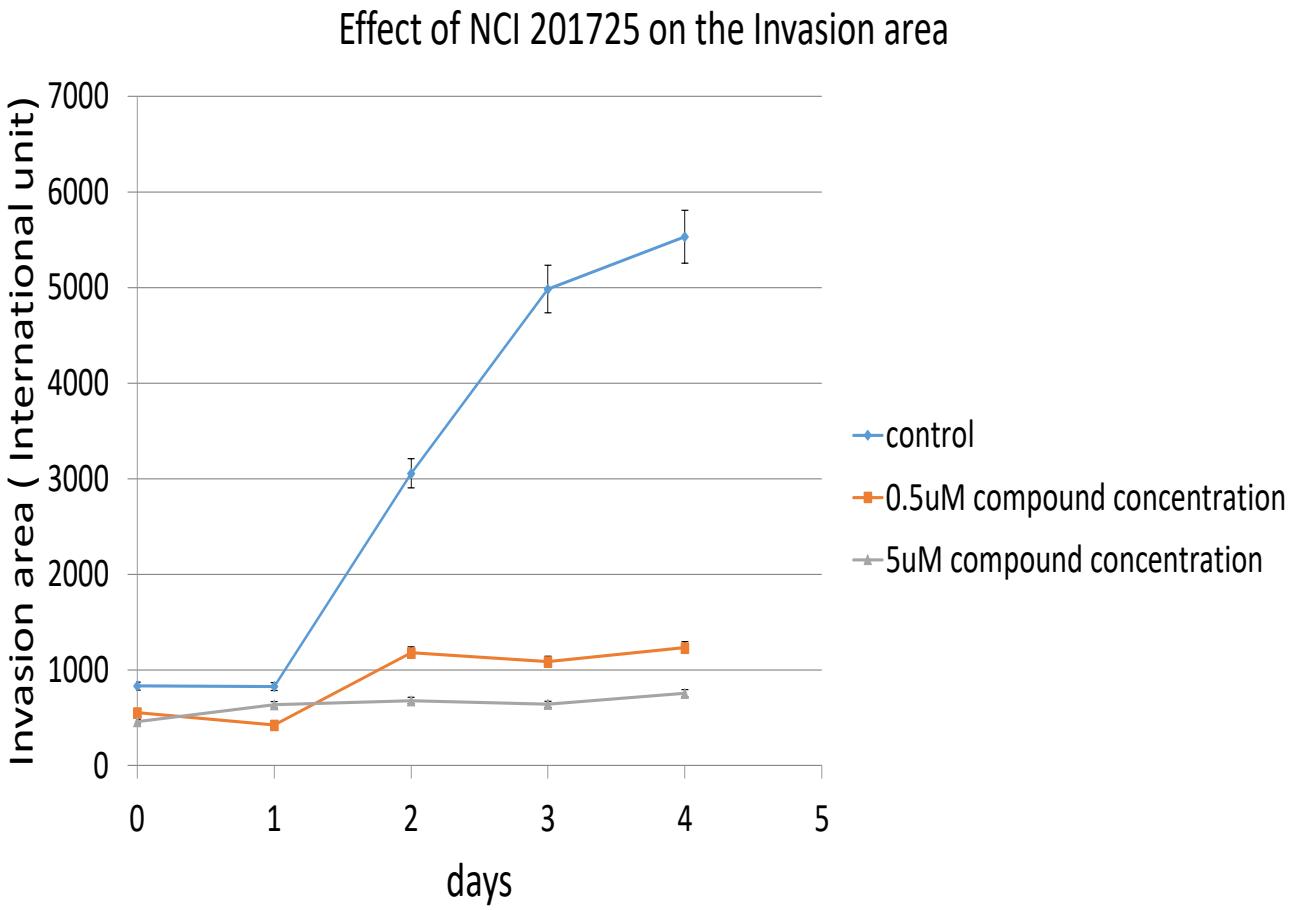

Figure5. Diagram shows the effect of the compoundNCI201725 on the invasion of U87 spheroids in collagen. Mathematical representation of the 3 D invasion assay results

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20494 – 20509
Received 05 August 2021; Accepted 15 September 2021.

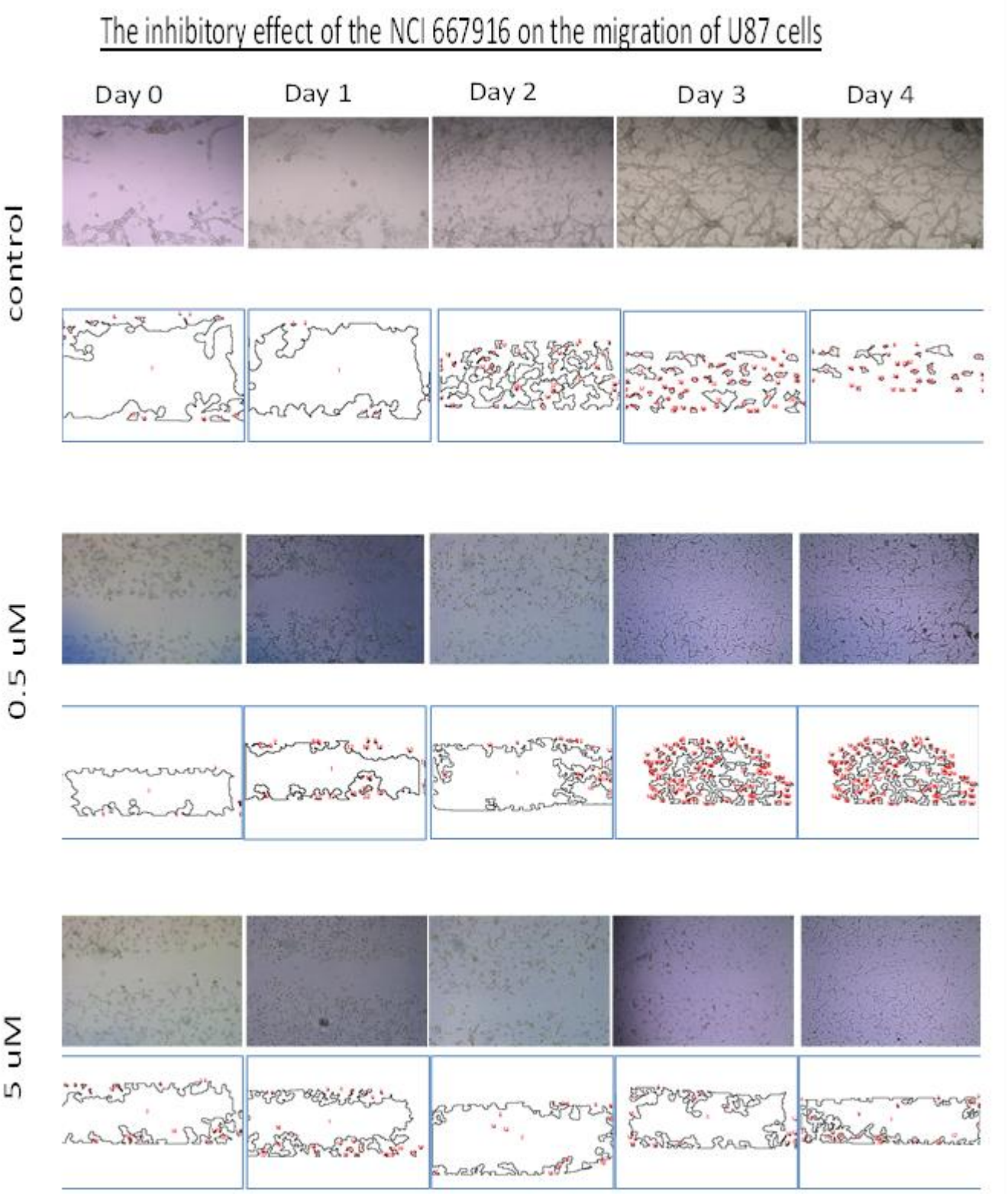

Figure 6. the anti-migrationeffect of the NCI 667916 on the U87 cell in 2D scratch assay.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20494 – 20509
Received 05 August 2021; Accepted 15 September 2021.

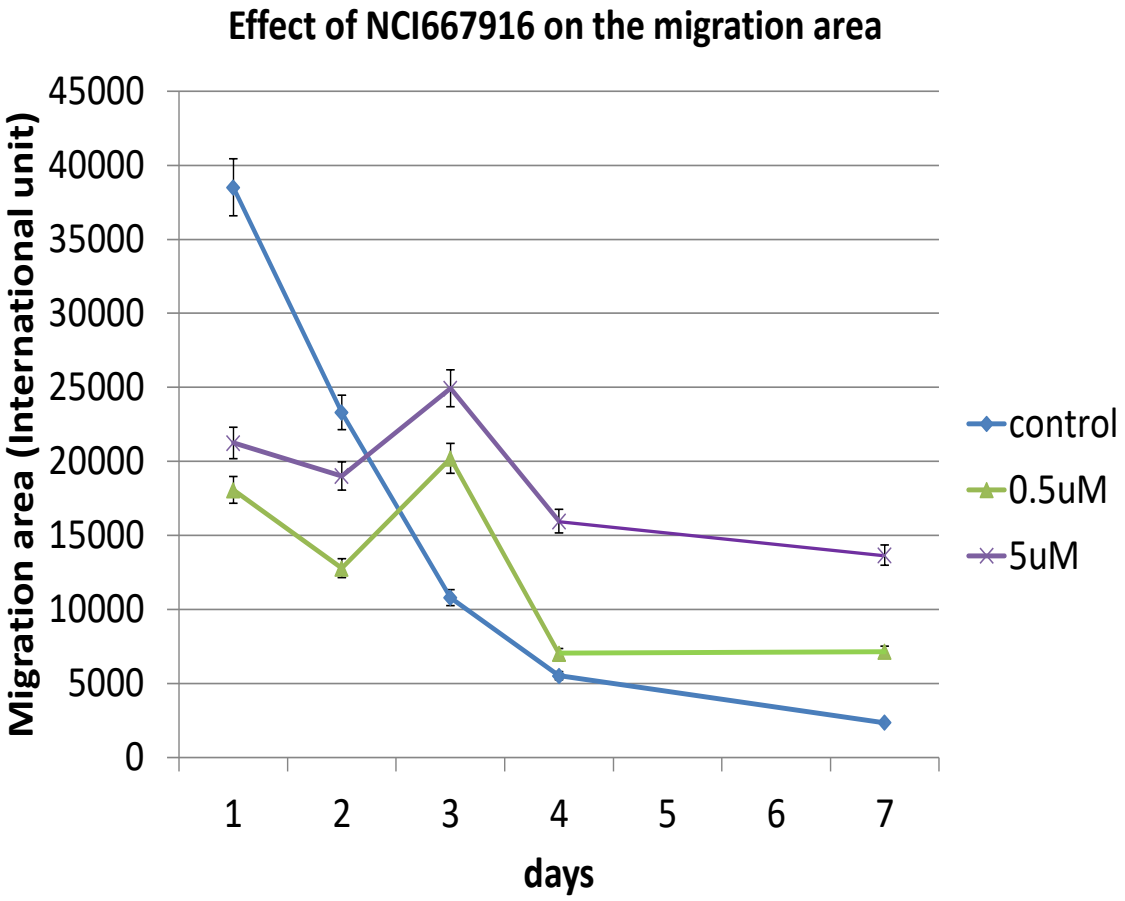

Figure 7. a diagram shows the anti- migration effect of the compound NCI 667916 on the 2D scratch assay.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20494 – 20509
Received 05 August 2021; Accepted 15 September 2021.

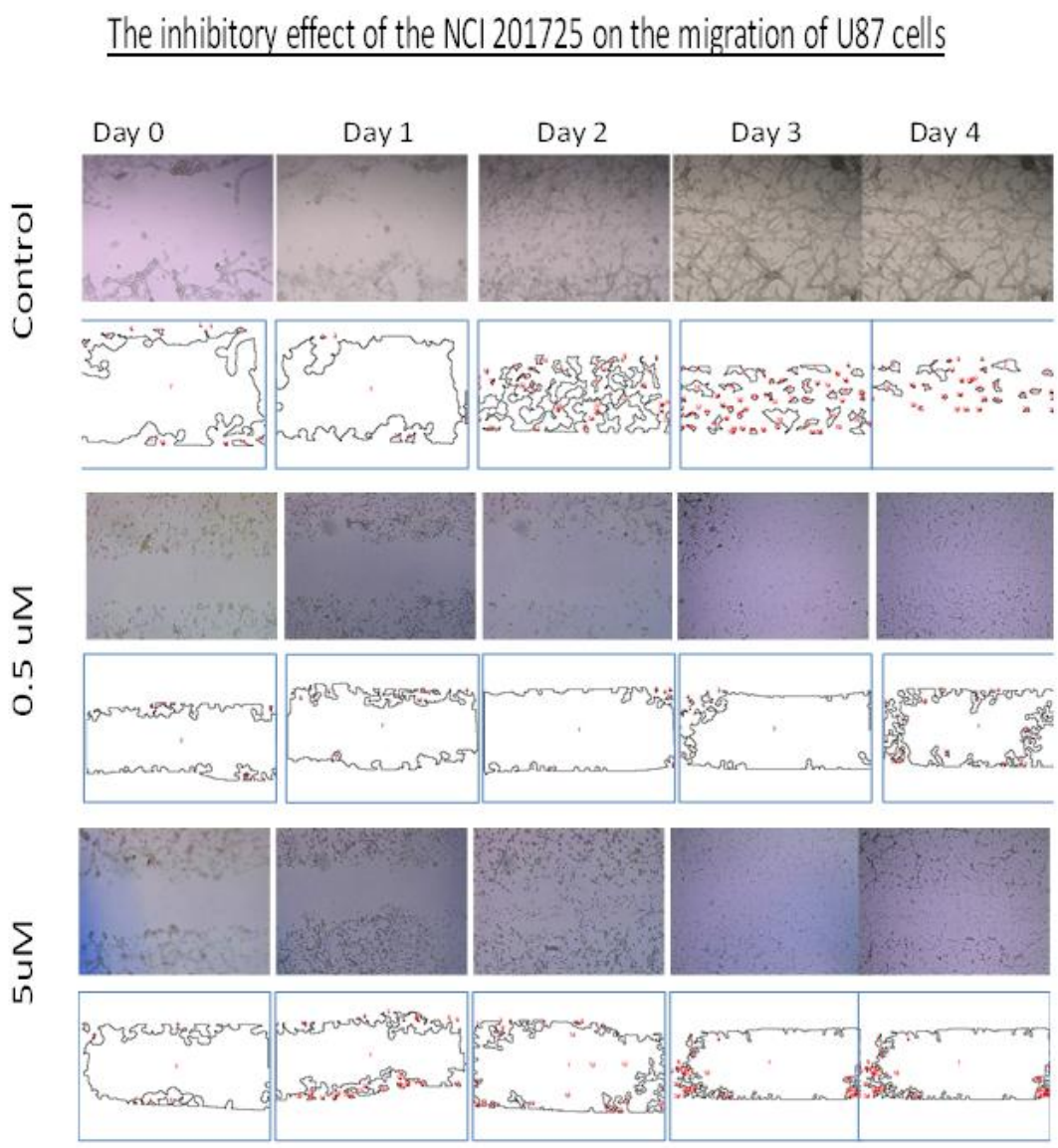

Figure 8. the anti-migration effect of the compound NCI201725 on the U87 cell le in 2D scratch assay.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20494 – 20509
Received 05 August 2021; Accepted 15 September 2021.

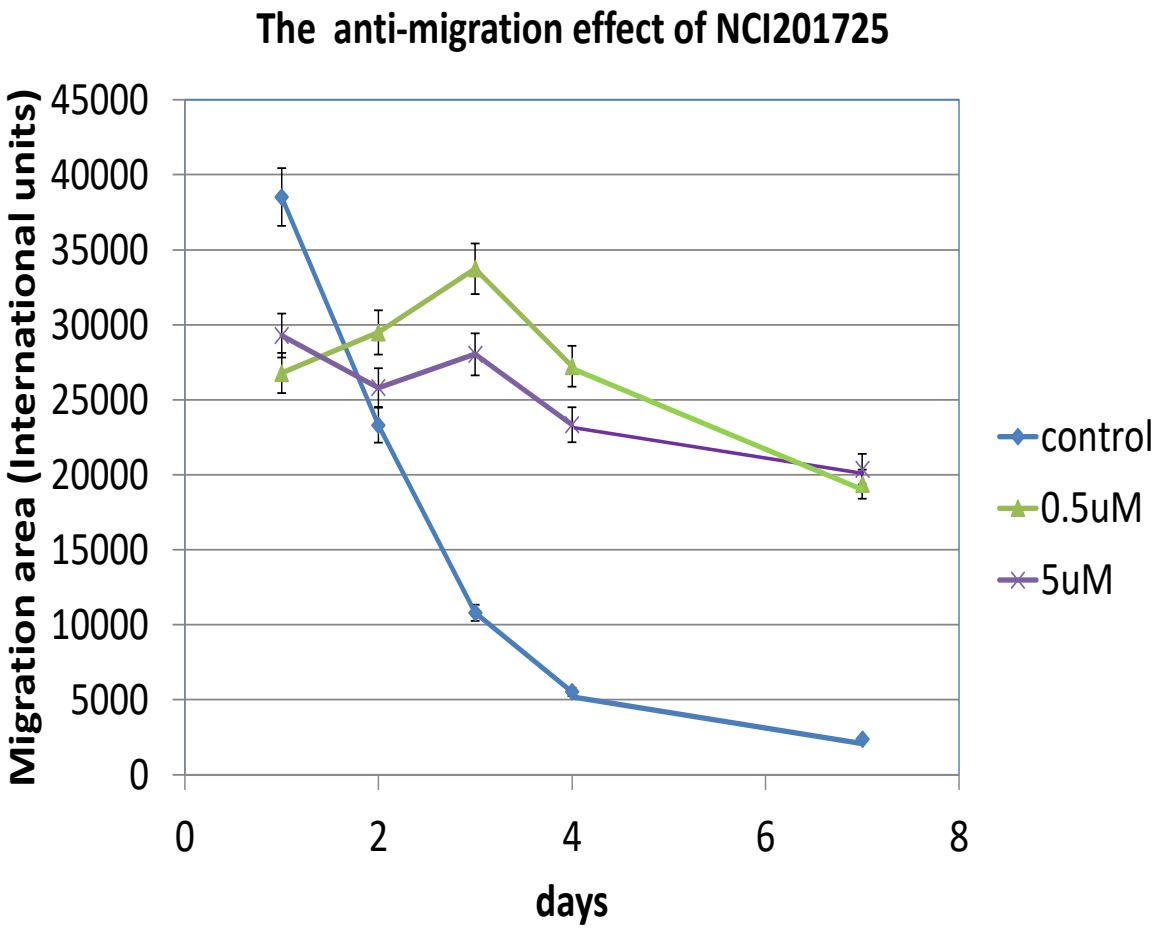

Figure9. diagram shows the anti-migration effect of the compound NCI201725 in 2D scratch assay.

List of abbreviations

- three dimension (3 D)
- two-dimension (2 D)
- fifty percent inhibitory concentration (IC50)
- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)
- European Collection of Cell Cultures ((ECACC),

Declarations

- **Ethics approval and consent to participate**

Not applicable

- **Consent for publication**

Not applicable

- **Availability of data and materials**

All data generated or analysed during this study are included in this published article [and its supplementary information files.

- **Competing interests**

The authors declare that they have no competing interests" in this section.

- **Funding**

This work is funded by the dean of scientific research in the University of Jordan.

References

.1 Egeblad, M., E.S. Nakasone, and Z. Werb, Tumors as organs: complex tissues that interface with the entire organism. Developmental cell, 2010. **18**(6): p. 884-901.

.2 Barkan, D., J.E. Green, and A.F. Chambers, Extracellular matrix: a gatekeeper in the transition from dormancy to metastatic growth. European Journal of Cancer, 2010. **46**(7): p. 1181-1188.

.3 van der Kaaij, R.T., et al., Treatment of peritoneal dissemination in stomach cancer patients with cytoreductive surgery and hyperthermic intraperitonealchemotherapy (HIPEC): rationale and design of the PERISCOPE study. 2017. **6**(7): p. e136.

.4 Desgrosellier, J.S. and D.A. Cheresh, Integrins in cancer: biological implications and therapeutic opportunities. Nature Reviews Cancer. **10**(1): p. 9-22.

.5 Monferran, S., et al. αvβ3 andαvβ5 integrins control glioma cell response to ionising radiation through ILK and RhoB. International Journal of Cancer 2008 [cited 123 2]; 357-364.[

.6 Cheresh, D., Tumor progression induced by integrin±ζv ²3ζmediated anchorage-dependent and-independent signaling (79.3). The FASEB Journal. **28**(1 Supplement): p. 79.3.

.7 Francia, G., et al., Mouse models of advanced spontaneous metastasis for experimental therapeutics. Nature Reviews Cancer, 2011. **11**(2): p. 135-141.

.8 Hanahan, D .and R.A. Weinberg, Hallmarks of cancer: the next generation. Cell, 2011. **144**(5): p. 646-674.

.9 Millard, M., S. Odde, and N. Neamati, Integrin targeted therapeutics. Theranostics, 2011. **1**: p. 154.

.10 Sun, C.-C., X.-J. Qu, and Z.-H. Gao, Integrins: players in cancer progression and targets in cancer therapy. Anti-cancer drugs. **25**(10): p. 1107-1121.

.11 Kaul, D.K., et al., Monoclonal antibodies to αVβ3 (7E3 and LM609) inhibit sickle red blood cell–endothelium interactions induced by platelet-activating factor Presented in part at the INSERM Symposium, Paris, France, September 2, 1995 (ref 24), and at the 40th Annual Meeting of the American Society of Hematology, Miami, FL, December 7, 1998. Blood, 2000. **95**(2): p. 368-374.

.12 Cheresh, D.A., Human endothelial cells synthesize and express an Arg-Gly-Asp-directed adhesion receptor involved in attachment to fibrinogen and von Willebrand factor. Proceedings of the National Academy of Sciences, 1987. **84**(18): p. 6471-6475.

.13 Rader, C., D.A. Cheresh, and C.F. Barbas Iii, A phage display approach for rapid antibody humanization: designed combinatorial V gene libraries.

Proceedings of the National Academy of Sciences, 1998. **95**(15): p. 8910-8915.

.14 Hersey, P., et al., A randomized phase 2 study of etaracizumab, amonoclonal antibody against integrin±ـv±¯,²3ـdacarbazine in patients with stage IV metastatic melanoma. Cancer. **116**(6): p. 1526-1534.

.15 Cai, W., et al., In vitro and in vivo characterization of 64Cu-labeled AbegrinTM, a humanized monoclonal antibody against integrin±ـv.²3ـCancer research, 2006. **66**(19): p. 9673-9681.

.16 Heckmann, D. and H. Kessler, Design and chemical synthesis of integrin ligands. Methods in enzymology, 2007. **426**: p. 463-503.

.17 Jinushi, M., et al., ATM-Mediated DNA Damage SignalsMediate Immune Escape through Integrin-αvβ3–Dependent Mechanisms. Cancer research, 2012. **72**(1): p. 56-65.

.18 Rosenthal, M.A., et al., Evaluation of the safety, pharmacokinetics and treatment effects of an ²3ـ½ـ±ـintegrin inhibitor on bone turnover anddisease activity in men with hormoneâ‚¬refractory prostate cancer and bone metastases. Asiaâ‚¬Pacific Journal of Clinical Oncology. **6**(1): p. 42-48.

.19 Ruoslahti, E. and M.D. Pierschbacher, Arg-Gly-Asp: a versatile cell recognition signal. Cell, 1986. **44** :(4)p. 517-518.

.20 Nisato, R.E., et al., αvβ3 and αvβ5 integrin antagonists inhibit angiogenesis in vitro. Angiogenesis, 2003. **6**(2): p. 105-119.

.21 Taga, T., et al., αv-Integrin antagonist EMD 121974 induces apoptosis in brain tumor cells growing on vitronectin and tenascin. International journal of cancer, 2002. **98**(5): p. 690-697.

.22 Burke, P.A., et al., Cilengitide targeting of αvβ3 integrin receptor synergizes with radioimmunotherapy to increase efficacy and apoptosis in breast cancer xenografts. Cancer research, 2002. **62**(15): p. 4263-4272.

.23 Raguse, J.-D., et al., Cilengitide (EMD 121974) arrests the growth of a heavily pretreated highly vascularised head and neck tumour. Oral oncology, 2004. **40**(2): p. 228-230.

.24 Reynolds, A.R., et al., Stimulation of tumor growth and angiogenesis by low concentrations of RGD-mimetic integrin inhibitors. Nature medicine, 2009. **15**(4): p. 392-400.

.25 Nabors, L.B., et al., Phase I and correlative biology study of cilengitide in patients with recurrent malignant glioma. Journal of Clinical Oncology, 2007. **25**(13): p. 1651-1657.

.26 Mas-Moruno, C., F. Rechenmacher, and H. Kessler, Cilengitide: the first anti-angiogenic small molecule drug candidate. Design, synthesis and clinical evaluation. Anti-cancer agents in medicinal chemistry, 2010. **10**(10): p. 753.

.27 Reardon, D.A., et al., Cilengitide: an RGD pentapeptide αvβ3 and αvβ5 integrin inhibitor in development for glioblastoma and other malignancies. Future Oncology, 2011. **7**(3): p. 339-354.

.28 Stupp, R., et al., Phase I/IIa study of cilengitide and temozolomide with concomitant radiotherapy followed by cilengitide and temozolomide maintenance therapy in patients with newly diagnosed glioblastoma. Journal of clinical oncology. **28**(16): p. 2712-2718.

.29 Stupp, R., et al., Cilengitide combined with standard treatment for patients with newly diagnosed glioblastoma and methylated O6-methylguanine-DNA methyltransferase (MGMT) gene promoter: Key results of the multicenter,

randomized, open-label, controlled, phase III CENTRIC study. J Clin Oncol. **31**(Suppl): p. LBA2009.

.30 Stupp, R., et al., Cilengitide combined with standard treatment for patients with newly diagnosed glioblastoma with methylated< i> MGMT</i> promoter (CENTRIC EORTC 26071-22072 study): a multicentre, randomised, open-label, phase 3 trial. The Lancet Oncology. **15**(10): p. 1100-1108.

.31 Alshaer, W., et al., Encapsulation of echinomycin in cyclodextrin inclusion complexes into liposomes: in vitro anti-proliferative and anti-invasive activity in glioblastoma. 2019. **9**(53): p. 30976-30988.

.32 Vinci, M., C. Box, and S.A.J.J. Eccles, Three-dimensional (3D) tumor spheroid invasion assay. 2015(99): p. e52686.

.33 Quail, D.F., et al., A unique 3D in vitro cellular invasion assay. 2012. **17**(8): p. 1088-1095.

.34 Zhang, Y., L. Zhou, and L.J.J.o.t.A.C.S. Qin, High-throughput 3D cell invasion chip enables accurate cancer metastatic assays. 2014. **136**(43): p. 15257-15262.

.35 Brekhman, V. and G.J.B.c. Neufeld, A novel asymmetric 3D in-vitro assay for the study of tumorcell invasion. 2009. **9**(1): p. 415.

.36 Matsiaka, O.M., et al., Discrete and continuum approximations for collective cell migration in a scratch assay with cell size dynamics. 2018. **80**(4): p. 738-757.

.37 Cormier, N., et al., Optimization of the wound scratch assay to detect changes in murine mesenchymal stromal cell migration after damage by soluble cigarette smoke extract. 2015(106): p. e53414.

.38 Van Horssen, R. and T.L.J.J.o.c.p. ten Hagen, Crossing barriers: the new dimension of 2D cell migration assays. 2011. **226**(1): p. 288-290.

.39 Johnston, S.T., M.J. Simpson, and D.S.J.J.o.t.R.S.I. McElwain, How much information can be obtained from tracking the position of the leading edge in a scratch assay? 2014. **11**(97): p. 20140325.

40. Penning, T.D., Khilevic, A., Chen, B.B., Russell, M.A., Boys, M.L., Wang, Y., Duffin, T., Engleman, V.W., Finn, M.B., Freeman, S.K., Hanneke, M.L., Keene, J.L., Klover, J.A., Nickols, G.A., Nickols, M.A., Rader, R.K., Settle, S.L., Shannon, K.E., Steininger, C.N., Westlin, M.M., Westlin W.F., Synthesis of pyrazoles and isoxazoles as potent avb3 receptor antagonists. 2006. Bioorganic & Medicinal Chemistry Letters **16**; 3156–3161

41. Krasowski, M., Siam, M., Iyer, M., Pizon, A., Giannoutsos, S. and Ekins, S., 2009. Chemoinformatic Methods for Predicting Interference in Drug of Abuse/Toxicology Immunoassays. Clinical Chemistry, 55(6), pp.1203-1213.