Commagene Journal of Biology

Karış, (2025) *Comm. J. Biol.* 9(1), 12-16.
DOI: 10.31594/commagene.1605070

e-ISSN 2602-456X

Research Article/Araştırma Makalesi

In vitro Antiproliferative Activity of *Lissotriton schmidtleri* (Raxworthy, 1988) Skin Secretion on Human Breast Cancer (MCF7) Cell Line

Mert KARIŞ

Nevşehir Hacı Bektaş Veli University, Acıgöl Vocational School of Technical Sciences, Laboratory Technology Program, Nevşehir, TÜRKIYE
ORCID ID: Mert KARIŞ: https://orcid.org/0000-0003-0468-9290

Received: 21.12.2024 **Accepted:** 27.12.2024 **Published online:** 01.01.2025 **Issue published:** 30.06.2025

Abstract: In this study, the *in vitro* antiproliferative activity of *Lissotriton schmidtleri* skin secretion on estrogen-sensitive human breast cancer (MCF7) cells was reported for the first time. The effects of *L. schmidtleri* skin secretion at concentrations of 0.5, 5, and 50 µg/mL on the MCF7 cell line were evaluated using the MTT assay after 48 hours of incubation. According to the MTT assay results, *L. schmidtleri* skin secretion inhibited MCF7 cell viability by approximately 64% at a concentration of 50 µg/mL, with an IC_{50} value calculated as 20.81 ± 0.87 µg/mL. Based on these findings, it is suggested that *L. schmidtleri* skin secretion may serve as a potential anticancer agent against breast cancer.

Keywords: Amphibian skin secretion, MTT assay, *Lissotriton schmidtleri*, cytotoxicity, MCF7.

Lissotriton schmidtleri (Raxworthy, 1988) Deri Salgısının İnsan Meme Kanseri (MCF7) Hücre Hattı Üzerindeki *in vitro* Antiproliferatif Aktivitesi

Öz: Bu çalışmada, *Lissotriton schmidtleri* deri salgısının östrojen duyarlı insan meme kanseri (MCF7) hücreleri üzerindeki *in vitro* antiproliferatif aktivitesi ilk kez rapor edilmiştir. MCF7 hücre hattına, *L. schmidtleri* deri salgısının 0.5, 5 ve 50 µg/mL konsantrasyonları uygulanarak 48 saat inkübasyon sonundaki etkileri MTT testi ile belirlenmiştir. MTT testi sonucunda, *L. schmidtleri* deri salgısı, MCF7 hücre canlılığını 50 µg/mL konsantrasyonda yaklaşık %64 oranında inhibe etmiş ve IC_{50} değeri 20.81 ± 0.87 µg/mL olarak hesaplanmıştır. Elde edilen veriler ışığında, *L. schmidtleri* deri salgısının meme kanserine karşı potansiyel bir antikanser ajan olabileceği değerlendirilmiştir.

Anahtar kelimeler: Amfibi deri salgısı, MTT testi, *Lissotriton schmidtleri*, sitotoksisite, MCF7.

1. Introduction

Breast cancer is commonly seen among women and is one of the leading causes of cancer-related deaths. The World Health Organization (WHO) has reported that breast cancer is the most prevalent type of cancer among women. Moreover, the number of natural and synthetic substances that could exhibit therapeutic effects for the treatment of breast cancer is increasing (Wild, 2014).

In patients, it has been observed that the use of drugs developed in laboratory conditions also exposes healthy cells to various side effects (Smith et al., 2007). In chemotherapy, not only neoplastic cells but also proliferating healthy cells are affected, resulting in various side effects (such as nausea and vomiting) in patients (Hoskin & Ramamoorthy, 2007). Therefore, animal-derived substances have been a significant source of anticancer agents, with numerous compounds isolated from various animal sources demonstrating promising therapeutic potential (Wang et al., 2017). There are several of studies on the effects of animal-derived compounds and bioactive molecules obtained from such as bees (Kamran et al., 2020), spiders (Gao et al., 2007), snakes (Bradshaw et al., 2016), scorpions (Zargan et al., 2011), and amphibians (Sciani et al., 2013).

Lissotriton schmidtleri, also referred to as the Turkish smooth newt, inhabits regions ranging from northeastern Greece and southeastern Bulgaria through East Thrace, extending across the Bosphorus to western and northwestern Anatolia (Wielstra et al., 2015).

In newts, skin secretions play a crucial role in regulating water loss, acting as a barrier against pathogens, and serving as lubricants against predators (Wanninger et al., 2018). Additionally, newt skin secretions have been identified as promising candidates for treating cancer, HIV, and drug-resistant bacterial infections (Xu & Lai, 2015). The effects of newt skin secretions are not limited to these activities. The skin secretions of newts also possess antioxidant properties that help in neutralizing free radicals and protecting cells from oxidative damage. This antioxidant activity is crucial for maintaining cellular integrity and function (Indriani et al., 2023). Studies have shown that newt skin secretions can also promote wound healing and regulate immune reactions (Kröner et al., 2024). Karış et al. (2018) demonstrated that *Lissotriton vulgaris* (*L. schmidtleri* according to the current taxonomy) skin secretion has a remarkable anticancer effect on MDA-MB-231 (hormone-independent human mammary gland adenocarcinoma) cell line.

Holliday & Speirs (2011) indicated that MCF7 is the most widely used breast cancer cell line in research due to its high hormone sensitivity that is attributed to its

Corresponding author: mert.karis@hotmail.com

elevated expression of estrogen receptors (ER). The primary aim of this study was to evaluate the antiproliferative activity of *L. schmidtleri* skin secretion on the estrogen-dependent MCF7 cell line in order to assess its cytotoxic potential and extend the knowledge of its bioactivities.

2. Material and Method

2.1. Field studies and collection of skin secretions

Field studies were conducted in accordance with the activation periods of the *L. schmidtleri* (Fig. 1) and took place in February 2016 in İzmir province of Türkiye. A total of 7 individuals (3♂♂, 4♀♀) were found and captured by using a mesh dip net. The field study was carried out with the permission of the Republic of Türkiye Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks, permit number: 2014-51946.

Figure 1. General aspect of a male (below) and a female (above) specimen of *Lissotriton schmidtleri*

Skin secretion was collected with a slightly modified method (Tyler et al., 1992) through mild electrical stimulation (5-8 V) using a stimulator (C.F. Palmer, London). For each individual, approximately 20 mL of ultra-pure water was used to rinse the secretions into falcon tubes. The skin secretion of all individuals was pooled, clarified by centrifugation (6000 × rpm for 10 minutes), and the supernatants were snap-frozen using liquid nitrogen. The samples were then lyophilized and stored at +4°C until further bioactivity assays were conducted. Secretion collection took place in the field and the newts were subsequently released unharmed back into their natural habitats. A total of 7 mg of lyophilized skin secretion was obtained.

2.2. Determination of the protein concentration

The protein content of the diluted skin secretion sample (2 mg/mL) in ultra-pure water was measured in triplicate using the BCA assay kit (Thermo Scientific, USA) with bovine serum albumin as the standard. Protein concentrations were determined using a UV/Vis spectrophotometer (Thermo Multiskan Spectrum, Bremen, Germany) at a wavelength of 562 nm.

2.3. Cell culture, MTT assay, and determination of IC_{50}

The MCF7 (human breast adenocarcinoma) cell line, purchased from ATCC (Manassas, VA, USA), was used to assess antiproliferative activity. All cells were cultured in Dulbecco's modified Eagle's medium F12 (DMEM/F12), supplemented with 10% fetal bovine serum (FBS), 2 mM/L glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin (Lonza, Visp, Switzerland). The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2. Cytotoxicity of crude skin secretion was evaluated using a modified colorimetric MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] assay (Mosmann, 1983) based on cell viability. Optical density (OD) was measured in triplicate at 570 nm with a reference wavelength of 620 nm, using a UV/Vis spectrophotometer (Thermo, Bremen, Germany). The cells were seeded at an initial concentration of $1x10^5$ cells/mL in 96-well plates and incubated for 24 hours. A non-cancerous cell line HEK-293 (human embrionic kidney) was screened with *L. vulgaris* skin secretion and all concentrations and incubation time were optimized by screening on cell lines repeatedly in a previously published study (Karış et al., 2018). Afterward, the cells were treated with varying concentrations (0.5, 5, and 50 µg/mL) of skin secretions and incubated for an additional 48 hours at 37 °C. Parthenolide, a plant-derived compound, was used as a positive cytotoxic control at concentrations of 0.125, 1.25, and 12.5 µg/mL. Parthenolide is known as a highly cytotoxic agent and frequently used as a positive control in anti-cancer research (Kreuger et al., 2012). Cell viability was determined by calculating the percentage of surviving cells in each culture following incubation with skin secretions. The viability (%) was calculated using the formula:

%Viable cells = [(absorbance of the treated cells) − (absorbance of blank)] / [(absorbance of control) − (absorbance of blank)] × 100

The IC_{50} values were determined by fitting the data to a sigmoidal curve using a four-parameter logistic model and the results were presented as the average of three independent measurements. The IC_{50} values were reported with a 95% confidence interval and calculations were performed using Prism 5 software (GraphPad5, San Diego, CA, USA). The absorbance values from the blank wells were subtracted from the treated and control cell wells and the half-maximal inhibition of growth (IC_{50}) was calculated relative to the untreated controls.

Morphological examinations of cells exposed to secretions at different concentrations were conducted using an inverted microscope (Olympus, Japan).

3. Results

The total protein and peptide concentration of *L. schmidtleri* skin secretion (2 mg/mL) was measured by using the BCA assay and determined as 1775 µg/mL.

The antiproliferative activity of the skin secretion sample was measured against the MCF7 cell line after 48 h incubation. The crude skin secretion of *L. schmidtleri* exhibited a concentration-dependent inhibitory effect on the viability of the MCF7 cells. The inhibition rates were calculated as 17% at a concentration of 0.5 µg/mL, 28% at 5 µg/mL, and 64% at 50 µg/mL (Fig. 2). Parthenolide (positive cytotoxic control agent), a plant-derived sesquiterpene lactone demonstrated inhibition rates of 20% at 0.125 µg/mL, 32% at 1.25 µg/mL, and 76% at 12.5 µg/mL on the MCF7 cell line.

Half maximal inhibitory concentration (IC_{50}) of *L. schmidtleri* skin secretion was calculated as 20.81 ± 0.87 µg/mL (Table 1). The IC_{50} value of positive control parthenolide was found as 2.74 ± 0.09 µg/mL.

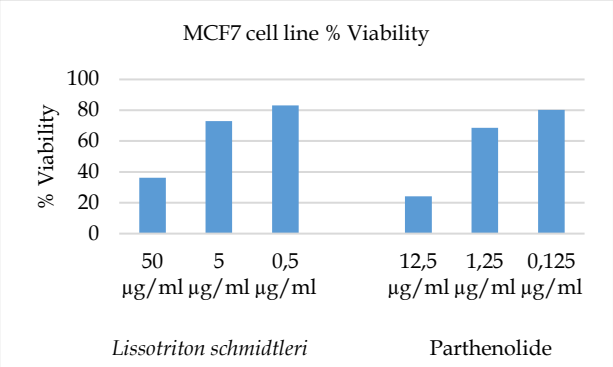

Figure 2. Viability of MCF7 cell line following skin secretion treatment for 48 h. Cell viability was determined by MTT assay, control was exposed to vehicle only which was taken as 100% viability.

Table 1. The IC_{50} values (µg/mL) for MCF7 cell line following 48 h skin secretion exposure by MTT assay. Parthenolide was used as positive control.

Sample	MCF7 (breast)
Parthenolide	2.74 ± 0.09
L. schmidtleri	20.81 ± 0.87

Increasing concentrations led to a greater number of rounded cells, growth inhibition, and a high occurrence of various morphological abnormalities along with larger cell-free areas compared to untreated control cells (Fig. 3).

4. Discussion and Conclusion

The skin secretions of urodele amphibians are complex mixtures consisting of peptides, proteins, and other bioactive compounds (Barros et al., 2022). These secretions serve multiple physiological purposes for the amphibians such as antimicrobial defense, predator deterrence, and environmental adaptation (Clarke, 1997).

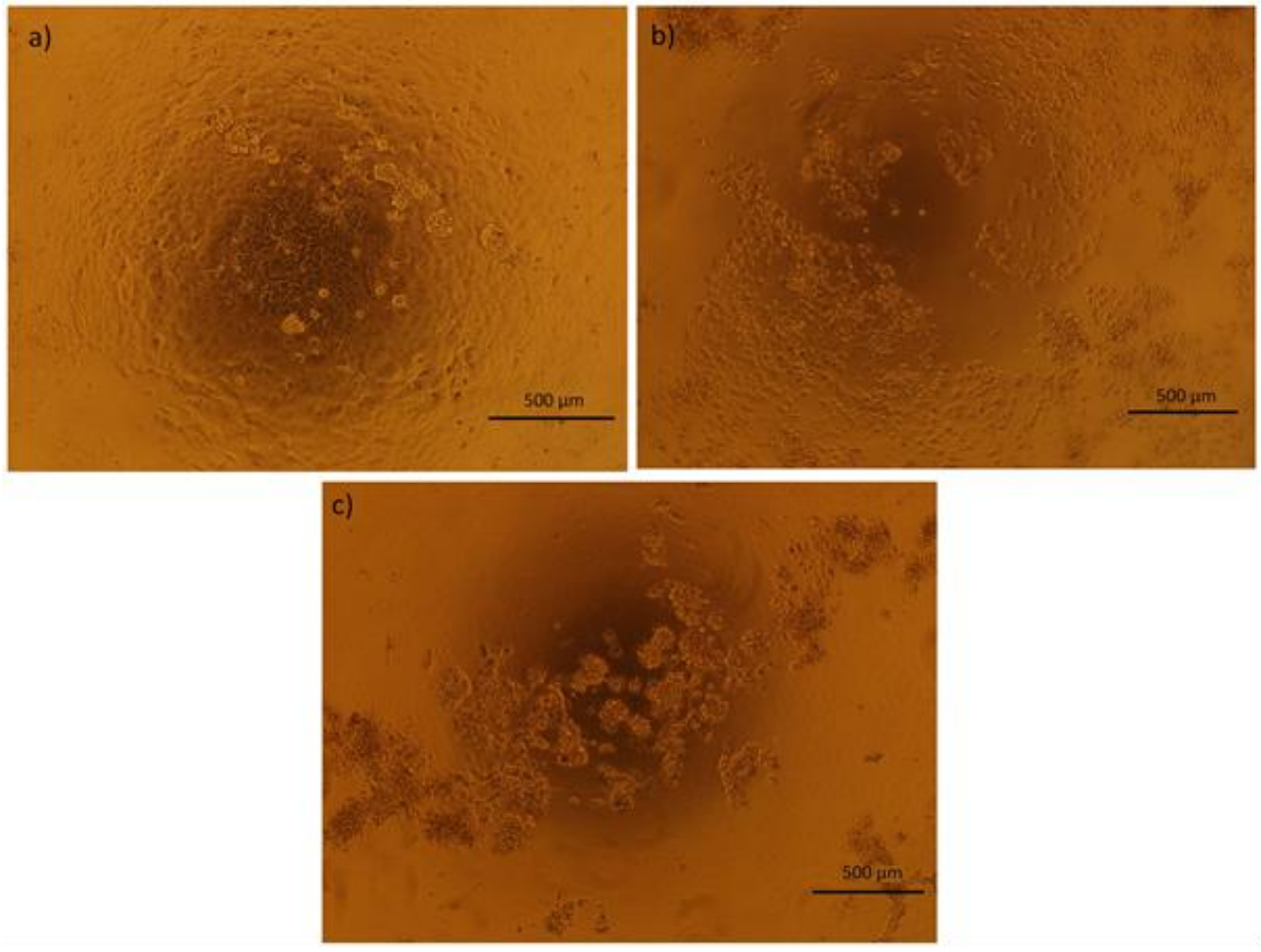

Figure 3. Morphological changes of MCF7 cells following 48 h exposure a) control, b) parthenolide (1.25 µg/mL), c) *L. schmidtleri* skin secretion (50 µg/mL)

As a potential anticancer agent, amphibian-derived dermaseptins are known to insert into the lipid bilayer of the membrane, causing leakage of intracellular contents and eventual cell death. This mechanism is particularly effective against cancer cells, which often have altered membrane characteristics compared to normal cells (Bartels et al., 2019). Molecules in the amphibian skin secretions may induce apoptosis in cancer cells through mitochondrial damage and activation of pro-apoptotic proteins (Oelkrug et al., 2015). For instance, bombinin (a peptide isolated from the skin secretion of *Bombina orientalis*) induces apoptosis in cancer cells by triggering mitochondrial depolarization and caspase activation. By promoting the intrinsic apoptotic pathway, these peptides can selectively target and kill cancer cells while sparing normal tissue (Wang et al., 2024). Temporins (an

antimicrobial peptide isolated from *Hylarana guentheri*) induce cell cycle arrest in cancer cells. This mechanism halts the proliferation of cancer cells by interfering with the progression of the cell cycle, particularly at the G1/S or G2/M checkpoints (Liu et al., 2024).

Karış et al. (2018) determined that *L. vulgaris* (now *L. schmidtleri*) has a high cytotoxic effect with an IC_{50} value of 1.58 µg/mL against MDA-MB-231, a hormone-independent human mammary gland adenocarcinoma cell line and inhibited 85% cell viability at 50 µg/mL concentration. In this study, *L. schmidtleri* showed a lower level of antiproliferative activity against an estrogen-dependent MCF7 (human breast adenocarcinoma) with an IC_{50} value of 20.81 ± 0.87 µg/mL and 65% growth inhibition rate at 50 µg/mL concentration. The altered cytotoxic effects on MCF7 and MDA-MB-231 may occur as the cause of having different subtypes. Breast cancer comprises several molecular subtypes, with the luminal subtype (i.e., MCF7) being the most common and characterized by estrogen receptor-positive (ER+) status. The luminal A subtype, in particular, is defined by ER+, progesterone receptor-negative (PR−), and HER2-negative (HER2−) markers (Perou & Borresen-Dale, 2011). On the other hand, triple negative MDA-MB-231 (ER−, PR− and, HER2−) cells are reported as basal B subtype (claudin-low) breast cancer, which is part of basal-like subtype (Neve et al., 2006).

Sciani et al. (2013) screened the cytotoxic effects of skin secretions of seven species of the genus *Rhinella* on MCF7 cell line resulting in the IC_{50} values ranging between 40-50 µg/mL. *L. schmidtleri* skin secretion showed much higher and more potent activity (20.81 ± 0.87 µg/mL) against MCF7 cells. Wang et al. (2012) performed a cytotoxicity analysis of temporin-1CEa peptide obtained from the skin secretion of *Rana chensinensis* and they found the IC_{50} value on MCF7 cell line as 34.50 µM at 48 h which is also supporting the potential *L. schmidtleri* skin secretion on MCF7 cells. Santana et al. (2020) studied the figainin-1 peptide from the skin secretion of *Boana raniceps* on MCF7 cells and detected IC_{50} value was 13.7 µM. Dermaseptin-PT9 peptide from *Phyllomedusa tarsius* skin secretion showed very similar antiproliferative effect (70%) on MCF7 at 50 µM peptide concentration (Li et al., 2019), which is found 64% in this study with 50 µg/mL *L. schmidtleri* skin secretion exposure. These results support the potential of *L. schmidtleri* skin secretion on MCF7 cell line.

In conclusion, *L. schmidtleri* skin secretion represents a promising and underexplored source of bioactive compounds with potential cytotoxic effects on breast cancer. With their unique biochemistry, selective cytotoxicity, and diverse mechanisms of action, amphibian-derived products could offer significant advantages over conventional therapies. Further research and clinical studies are essential to fully understand and harness the potential of these biological treasures in the fight against breast cancer and other diseases.

Acknowledgment: I would like to extend my thanks to AREL (Research and Education Laboratory, Ege University School of Medicine) for their laboratory facilities during the study. Also, many thanks to Ayşe NALBANTSOY (Ege University) for her vauable suggestions and providing the cell line.

Ethics committee approval: This study was performed in accordance with ethical standards of animal experiments. Legal research ethics committee approval permissions for the study were obtained from the Ege University, Animal Experiments Local Ethics Committee (No: 2014-002).

Conflict of interest: The author declares that there is no conflict of interest.

References

Barros, A.L., Hamed, A., Marani, M., Moreira, D.C., Eaton, P., Plácido, A., ... & Leite, J.R.S. (2022). The arsenal of bioactive molecules in the skin secretion of urodele amphibians. *Frontiers in Pharmacology*, 12, 810821. https://doi.org/10.3389/fphar.2021.810821

Bartels, E.J.H., Dekker, D., & Amiche, M. (2019). Dermaseptins, multifunctional antimicrobial peptides: A review of their pharmacology, effectivity, mechanism of action, and possible future directions. *Frontiers in Pharmacology*, 10, 1421. https://doi.org/10.3389/fphar.2019.01421

Bradshaw, M.J., Saviola, A.J., Fesler, E., & Mackessy, S.P. (2016). Evaluation of cytotoxic activities of snake venoms toward breast (MCF-7) and skin cancer (A-375) cell lines. *Cytotechnology*, 68, 687-700. https://doi.org/10.1007/s10616-014-9820-2

Clarke, B.T. (1997). The natural history of amphibian skin secretions, their normal functioning and potential medical applications. *Biological Reviews*, 72(3), 365-379. https://doi.org/10.1017/S0006323197005045

Gao, L., Yu, S., Wu, Y., & Shan, B. (2007). Effect of spider venom on cell apoptosis and necrosis rates in MCF-7 cells. *DNA and Cell Biology*, 26(7), 485-489. https://doi.org/10.1089/dna.2007.0579

Holliday, D.L., & Speirs, V. (2011). Choosing the right cell line for breast cancer research. *Breast Cancer Research*, 13, 1-7. https://doi.org/10.1186/bcr2889

Hoskin, D.W., & Ramamoorthy, A. (2008). Studies on anticancer activities of antimicrobial peptides. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1778(2), 357-375. https://doi.org/10.1016/j.bbamem.2007.11.008

Indriani, S., Karnjanapratum, S., Nirmal, N.P., & Nalinanon, S. (2023). Amphibian skin and skin secretion: An exotic source of bioactive peptides and its application. *Foods*, 12(6), 1282. https://doi.org/10.3390/foods12061282

Kamran, M.R., Zargan, J., Alikhani, H.K., & Hajinoormohamadi, A. (2020). The Comparative cytotoxic effects of apis mellifera crude venom on MCF-7 Breast Cancer cell line in 2D and 3D cell cultures. *International Journal of Peptide Research and Therapeutics*, 26, 1819-1828. https://doi.org/10.1007/s10989-019-09979-0

Karış, M., Şener, D., Yalçın, H.T., Nalbantsoy, A., & Göçmen, B. (2018). Major biological activities and protein profiles of skin secretions of *Lissotriton vulgaris* and *Triturus ivanbureschi*. *Turkish Journal of Biochemistry*, 43(6), 605-612. https://doi.org/10.1515/tjb-2017-0306

Kröner, L., Lötters, S., & Hopp, M.T. (2024). Insights into caudate amphibian skin secretions with a focus on the chemistry and bioactivity of derived peptides. *Biological Chemistry*, 405(9-10), 641-660. https://doi.org/10.1515/hsz-2024-0035

Kreuger, M.R.O., Grootjans, S., Biavatti, M.W., Vandenabeele, P., & D'Herde, K. (2012). Sesquiterpene lactones as drugs with multiple targets in cancer treatment: focus on parthenolide. *Anti-cancer Drugs*, 23(9), 883-896. https://doi.org/10.1097/CAD.0b013e328356cad9

Li, M., Xi, X., Ma, C., Chen, X., Zhou, M., Burrows, J.F., ... & Wang, L. (2019). A novel dermaseptin isolated from the skin secretion of *Phyllomedusa tarsius* and its cationicity-enhanced analogue exhibiting effective antimicrobial and anti-proliferative activities. *Biomolecules*, 9(10), 628. https://doi.org/10.3390/biom9100628

Liu, Y., Liu, H., Zhang, J., & Zhang, Y. (2024). Temporin-GHaK Exhibits Antineoplastic Activity against Human Lung Adenocarcinoma by Inhibiting the Wnt Signaling Pathway through miRNA-4516. *Molecules*, 29(12), 2797. https://doi.org/10.3390/molecules29122797

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65(1-2), 55-63. https://doi.org/10.1016/0022-1759(83)90303-4

Neve, R.M., Chin, K., Fridlyand, J., Yeh, J., Baehner, F. L., Fevr, T., ... & Gray, J.W. (2006). A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. *Cancer Cell*, 10(6), 515-527. https://doi.org/10.1016/j.ccr.2006.10.008

Oelkrug, C., Hartke, M., & Schubert, A. (2015). Mode of action of anticancer peptides (ACPs) from amphibian origin. *Anticancer Research*, 35(2), 635-643.

Perou, C.M., & Børresen-Dale, A.L. (2011). Systems biology and genomics of breast cancer. *Cold Spring Harbor Perspectives in Biology*, 3(2), a003293. https://doi.org/10.1101/cshperspect.a003293

Santana, C.J.C., Magalhães, A.C.M., dos Santos Júnior, A.C., Ricart, C.A.O., Lima, B.D., Álvares, A.D.C.M., ... & Castro, M.S. (2020). Figainin 1, a novel amphibian skin peptide with antimicrobial and antiproliferative properties. *Antibiotics*, 9(9), 625. https://doi.org/10.3390/antibiotics9090625

Sciani, J.M., de-Sá-Júnior, P.L., Ferreira, A.K., Pereira, A., Antoniazzi, M.M., Jared, C., & Pimenta, D.C. (2013). Cytotoxic and antiproliferative effects of crude amphibian skin secretions on breast tumor cells. *Biomedicine & Preventive Nutrition*, 3(1), 10-18. https://doi.org/10.1016/j.bionut.2012.11.001

Smith, R.A., Cokkinides, V., & Eyre, H.J. (2007). Cancer screening in the United States, 2007: a review of current guidelines, practices, and prospects. *CA: A Cancer Journal for Clinicians*, 57(2), 90-104. https://doi.org/10.3322/canjclin.57.2.90

Tyler, M.J., Stone, D.J., & Bowie, J.H. (1992). A novel method for the release and collection of dermal, glandular secretions from the skin of frogs. *Journal of Pharmacological and Toxicological Methods*, 28(4), 199-200. https://doi.org/10.1016/1056-8719(92)90004-k

Wang, C., Li, H.B., Li, S., Tian, L.L., & Shang, D.J. (2012). Antitumor effects and cell selectivity of temporin-1CEa, an antimicrobial peptide from the skin secretions of the Chinese brown frog (*Rana chensinensis*). *Biochimie*, 94(2), 434-441. https://doi.org/10.1016/j.biochi.2011.08.011

Wang, L., Dong, C., Li, X., Han, W., & Su, X. (2017). Anticancer potential of bioactive peptides from animal sources. *Oncology Reports*, 38(2), 637-651. https://doi.org/10.3892/or.2017.5778

Wang, X., Tang, P., Gong, Y., Yao, H., Liang, M., Qu, H., ... & Jiang, Q. (2024). Bombinin-BO1 induces hepatocellular carcinoma cell-cycle arrest and apoptosis via the HSP90A-Cdc37-CDK1 axis. *iScience*, 27(8), 110382. https://doi.org/10.1016/j.isci.2024.110382

Wanninger, M., Schwaha, T., & Heiss, E. (2018). Form and Function of the skin glands in the Himalayan newt *Tylototriton verrucosus*. *Zoological Letters*, 4, 1-10. https://doi.org/10.1186/s40851-018-0095-x

Wielstra, B., Bozkurt, E., & Olgun, K. (2015). The distribution and taxonomy of *Lissotriton* newts in Turkey (Amphibia, Salamandridae). *ZooKeys*, 484, 11-23. https://doi.org/10.3897/zookeys.484.8869

Wild, C. (2014). *World cancer report 2014* (pp. 482-494). C. P. Wild, & B. W. Stewart (Eds.). Geneva, Switzerland: World Health Organization.

Xu, X., & Lai, R. (2015). The chemistry and biological activities of peptides from amphibian skin secretions. *Chemical Reviews*, 115(4), 1760-1846. https://doi.org/10.1021/cr4006704

Zargan, J., Umar, S., Sajad, M., Naime, M., Ali, S., & Khan, H.A. (2011). Scorpion venom (*Odontobuthus doriae*) induces apoptosis by depolarization of mitochondria and reduces S-phase population in human breast cancer cells (MCF-7). *Toxicology in vitro*, 25(8), 1748-1756. https://doi.org/10.1016/j.tiv.2011.09.002