

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

Development and Characterization of Tapinarof-Loaded
Nanoformulations for the Treatment of Psoriasis

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2. Introduction

Psoriasis is one of the most common chronic inflammatory skin diseases worldwide. Although numerous studies have investigated the pathophysiology and treatment of psoriasis, most of them have primarily focused on systemic biologic therapies or conventional topical treatments. However, many patients try to avoid using biologic agents and corticosteroids, highlighting the ongoing need for well-tolerated topical therapies that are safe for long-term use. Despite this need, relatively few studies have addressed the challenges and opportunities associated with the development of novel topical formulations.

Psoriasis is a chronic immune-mediated disease affecting approximately 1–3% of the global population, corresponding to more than 100 million individuals worldwide. Although it is primarily considered a dermatological disorder, it is now well established that psoriasis is associated with several comorbidities. This disease increases the risk of cardiovascular disorders, metabolic syndrome, obesity, inflammatory bowel diseases, and psoriatic arthritis. Furthermore, visible skin symptoms place a significant psychological burden on patients, often leading to anxiety, depression, and a significant reduction in quality of life. The development of psoriasis is influenced by genetic, environmental, and immunological factors, while its persistence is maintained by complex inflammatory processes.

Most patients present with mild, localized lesions that do not require systemic treatment. Therefore, topical therapy plays a crucial role in the management of these patients. Unfortunately, no definitive cure for psoriasis is currently available. However, symptom control can significantly improve quality life of the patients. Current topical therapies include corticosteroids, vitamin D analogues, retinoids, and calcineurin inhibitors. These treatments are often effective in reducing inflammation and clinical symptoms, but their long-term use may be associated with various adverse effects. Corticosteroids may cause skin atrophy, striae, and tachyphylaxis, whereas vitamin D analogues and retinoids frequently induce irritation, erythema, or a burning sensation. Systemic drugs and biologic therapies used in moderate-to-severe cases represent major therapeutic advances. On the other hand, their high cost and potential adverse effects limit their suitability and accessibility for many patients. Consequently, there remains a considerable demand for innovative steroid-free topical formulations capable of providing long-term therapeutic efficacy while maintaining a favorable safety profile.

One of the major obstacles to effective dermal drug delivery is the barrier function of the stratum corneum, which is considered one of the most impermeable epithelial barriers in

the human body to exogenous substances. The skin is the largest organ of the human body and consists of three main layers: the epidermis, dermis, and hypodermis. The outermost layer of the epidermis, the stratum corneum, works as the primary line of defense against environmental insults while simultaneously constituting a significant barrier to drug penetration through the skin.

In psoriasis, the skin barrier is impaired, which further complicates effective drug delivery. These challenges may be partially overcome through the use of chemical penetration enhancers. However, their application may be problematic, particularly in chronic conditions, as many of them are related to irritation. In addition, approximately 40% of newly developed drug candidates exhibit hydrophobic characteristics, including tapinarof. The delivery of such poorly water-soluble compounds remains a significant challenge in pharmaceutical development. Therefore, there is a need for innovative dermal delivery systems capable of enhancing drug penetration without the use of chemical permeation enhancers.

Over the past decade, advances in nanotechnology have opened new perspectives in topical drug delivery. Nanoscale drug delivery systems can improve the solubility, stability, and bioavailability of active pharmaceutical ingredients while facilitating their penetration through the skin. These systems are particularly advantageous for poorly soluble lipophilic compounds, which often exhibit limited therapeutic performance in conventional formulations.

Nanoemulsions offer considerable potential for topical drug delivery, unfortunately, their low viscosity limits their applicability on the skin. The incorporation of nanoemulsions into gel matrices using suitable gelling agents enables the development of nanogels, thereby improving their topical applicability. Owing to their unique physicochemical properties, nanogels enhance drug solubility and dermal penetration while simultaneously providing moisturizing effects. Moreover, they enable controlled drug release and may reduce systemic adverse effects, making them particularly attractive for the treatment of chronic inflammatory skin disorders such as psoriasis. Although nanotechnology-based drug delivery systems are increasingly gaining attention in dermatology, the incorporation of tapinarof into nanogel formulations remains a relatively unexplored area.

In the present work, tapinarof was selected as the active pharmaceutical ingredient due to its promising therapeutic potential and its ability to target key pathways involved in the pathophysiology of psoriasis. Tapinarof is an aryl hydrocarbon receptor (AhR) agonist that represents a novel therapeutic approach for the treatment of psoriasis. Activation of the AhR contributes to the restoration of immune homeostasis in the skin, reduces the production of pro-inflammatory cytokines, and improves skin barrier function. Through AhR activation,

inflammatory responses mediated by IL-17 and IL-23 may be attenuated, targeting one of the central molecular mechanisms involved in psoriasis pathogenesis. In addition, tapinarof possesses antioxidant properties that may also contribute to the modulation of inflammatory processes.

However, achieving therapeutic efficacy requires not only the anti-inflammatory potential of the active ingredient but also its efficient delivery into the deeper layers of the skin and adequate biological availability. By combining the advantageous properties of hydrogels and nanocarriers, nanogels provide a promising platform for stable drug incorporation and enhanced dermal penetration. Furthermore, by minimizing adverse effects and reducing the frequency of administration, they may improve patient adherence. Based on these considerations, tapinarof-loaded nanogels may represent a promising novel approach for the topical treatment of psoriasis and contribute to the development of advanced therapeutic systems that combine efficacy, safety, and patient-friendly application.

3. Aims

The aim of my research was to develop innovative nanogel formulations capable of improving the bioavailability of tapinarof and enhancing its skin penetration, thereby providing a more effective therapeutic response in the treatment of psoriasis. Throughout my work, I focused on the comprehensive pharmaceutical technological and biological characterization of the developed formulations, with particular emphasis on their physicochemical properties, biocompatibility, and therapeutic potential in psoriasis model systems.

In the first phase of the study, tapinarof-loaded nanogel formulations were developed and subjected to pharmaceutical dosage form characterization. Following the optimization of the formulations, texture profile and rheological analyses were performed, as these parameters are important determinants of spreadability and the biological availability of the active pharmaceutical ingredient. The particle size distribution of the formulations was determined using dynamic light scattering (DLS). Furthermore, biocompatibility and cellular effects were evaluated on the HaCaT cell line using the MTT assay, while wound healing assays were performed on fibroblast cells. In parallel, the in vitro release of tapinarof was investigated using the Franz diffusion cell method.

In the second phase of the study, the anti-inflammatory effects and molecular mechanisms of action of the developed nanogels were investigated using an imiquimod (IMQ)-induced HaCaT–THP-1 co-culture psoriasis model, which reliably mimics the inflammatory microenvironment of psoriasis in vitro. Cytokine profiles were determined by enzyme-linked immunosorbent assay (ELISA), while the expression of inflammation-related genes was quantified by quantitative polymerase chain reaction (qPCR). In addition, the effects of the nanoformulations on inflammatory cellular responses were assessed using real-time cell analysis (RTCA) and wound healing assay. Finally, activation of the NF- κ B signaling pathway in THP-1 monocytes was evaluated by determining the extent of p65 translocation.

4. Materials and methods

4.1. Materials

Tapinarof, Triacetin, Tween 80, Dulbecco's Modified Eagle Medium (DMEM), phosphate-buffered saline (PBS), RPMI 1640 medium, dimethyl sulfoxide (DMSO), Trypan Blue dye, and trypsin–EDTA were purchased from Sigma-Aldrich (St. Gallen, Switzerland). Ethanol (96%), glycerol (85%), and oleic acid were obtained from Molar Chemicals Ltd. (Budapest, Hungary). Kollisolv PEG 400 and Kolliphor EL were purchased from BASF (Ludwigshafen, Germany). Carbopol 940 and Carbopol 934 were supplied by B.F. Goodrich Chemical Company (Charlotte, NC, USA), while triethanolamine was obtained from VWR Chemicals. Labrafil M and Transcutol HP were kindly provided by Gattefossé (Lyon, France). Imiquimod was purchased from MedChemExpress through Bio-Kasztel Ltd. (Budapest, Hungary).

4.2. Methods

4.2.1. Preparation of tapinarof-loaded nanogels

First, the required amount of tapinarof was dissolved in Transcutol HP and ethanol. The oily surfactants (Labrafil M, Triacetin, Tween 80, Kolliphor EL, and oleic acid) were combined with the hydrophilic surfactants (Transcutol HP, ethanol, and PEG 400) to improve stability and texture, as well as to facilitate transdermal penetration of the active pharmaceutical ingredient. The excipients were mixed using a magnetic stirrer to prepare the oil phase. The stirring speed was maintained at 400 rpm for 15 min. Subsequently, the aqueous phase was added to obtain the SNEDDS formulation, followed by vortex mixing for 5 min. Carbopol was incorporated as the gelling agent. For the preparation of Carbopol gels, the required amount of Carbopol powder was dispersed in a small volume of purified water and allowed to swell uniformly for 24 h. In the case of nanogel I, glycerol was added as a humectant. The SNEDDS formulations were then added dropwise to the gel matrix while stirring continuously at 500 rpm. To achieve nanosized droplets, an UP200Ht handheld homogenizer (200 W, 26 kHz) was applied before gelation. Finally, the pH of the gels was adjusted to match the physiological pH of the skin by adding a few drops of triethanolamine, resulting in homogeneous nanogel formulations.

4.2.2. Physicochemical characterization

4.2.2.1. Particle size determination

Particle size analysis was performed using a Malvern Zetasizer Nano ZSP instrument (Malvern Panalytical, Malvern, United Kingdom) to verify that the tapinarof-loaded nanogels maintained their stable particle size distribution after gel formation. Prior to analysis, the formulations were diluted 1000-fold with purified water to avoid multiple light scattering and ensure accurate measurements. The samples were equilibrated at 25 °C for 5 min, followed by three measurements, each consisting of 13 runs.

4.2.2.2. Rheological characterization

Rheological measurements were carried out using a RheolabQC rotational rheometer (Anton Paar, Graz, Austria) to determine the rheological properties of the formulations. Viscosity and flow curves were evaluated using RheoPlus Rheometer software (32 V3.10 21003407-33024). Viscosity profiles were obtained through rotational tests under controlled shear-rate conditions. A total of 30 g of each formulation was placed into the cup of the concentric cylinder measuring system of the rheometer. Both viscosity and flow behavior were measured at room temperature (24 °C) and skin temperature (35 °C). The shear rate was gradually increased from 0.01 s⁻¹ to 50 s⁻¹ and subsequently decreased back to 0.01 s⁻¹ to evaluate hysteresis behavior.

4.2.2.3. Texture analysis

The resistance of the nanogels to compression was determined using a CT3 Texture Analyzer (Brookfield, Middleboro, MA, USA). For analysis, the container filled with the tested formulation was positioned approximately 5.50 cm below the probe of the texture analyzer. Prior to measurement, the probe was lowered to the sample surface at a speed of 1 mm/s. The instrument was equipped with a TA5 cylindrical probe (12.7 mm diameter, 35 mm length). Compression testing was performed using the following settings: test speed of 0.50 mm/s, trigger force of 4.0 g, and target distance of 10.0 mm. All measurements were conducted at room temperature (25 °C). The force acting on the probe was recorded using Texture Pro CT software version 1.3 (Brookfield Engineering Laboratories, MA, USA). Each measurement was performed in triplicate, and the mean values and standard deviations were calculated.

4.2.2.4. In vitro release study

The in vitro release of tapinarof from gels with different compositions was determined using a Franz diffusion cell system. A membrane was placed between the donor and receptor compartments. The concentration profile of the investigated compound was determined based on samples collected at predetermined time points. Samples weighing 500 mg were placed onto an artificial cellulose acetate membrane (0.5 μm pore size) in the donor compartment, while a pH 5 buffer was selected as the receptor medium to simulate the physiological pH of the skin. The receptor phase was maintained at 32 °C. Prior to the experiment, the membrane was pretreated with isopropyl myristate for 15 min to mimic the lipophilic properties of the skin. The stirring speed of the magnetic stirrer was set to 450 rpm. Samples were collected from the receptor phase after 15, 30, 60, 90, 120, 150, 180, 240, and 300 min. Analytical determination was performed using a spectrophotometer. The amount of diffused active pharmaceutical ingredient was expressed as a percentage and plotted as a function of time.

4.2.3. In vitro cellular studies

4.2.3.1. Monoculture studies

4.2.3.1.1 Cell viability assay on HaCaT cells

The safety of the excipients and active ingredients used in the SNEDDS formulations was evaluated using the MTT assay. Cell viability studies were performed on HaCaT cells to assess cytotoxicity. Cells were seeded into 96-well plates at a density of 10,000 cells/well. The cells were allowed to grow for 7 days until complete confluence was achieved. The culture medium was removed, and after washing with PBS, 200 μl /well of sample solution was added to the cells. This was followed by a 30 min incubation period for short-term experiments and a 24 h incubation period for long-term experiments. Subsequently, the samples were removed, and an MTT dye solution (tetrazolium bromide) at a concentration of 5 mg/ml was added to the cells. The cells were then incubated for 3 h at 37 °C.

Viable cells convert the yellow, water-soluble tetrazolium bromide into purple formazan crystals. At the end of the incubation period, the formazan precipitate was dissolved in hydrochloric acid–isopropanol solution (25:1). The absorbance of the samples was then measured using a spectrophotometer (Fluostar Optima). The obtained absorbance values were directly proportional to the number of viable cells.

4.2.3.1.2 Wound healing assay on fibroblast cells

Fibroblast cells were seeded into 12-well plates (Thermo SC NUNC, Roskilde, Denmark) at a density of 10,000 cells/well. Confluent monolayers were synchronized in DMEM containing 0.5% FBS for 2 h. A wound area measuring approximately 500–800 μm in width was created using a standard 200 μl pipette tip. The wound cultures were washed with PBS to remove detached and excess cells. Subsequently, the monolayers were treated with free tapinarof, blank gels, or tapinarof-loaded gels at concentrations of 5, 10, 20, and 40 $\mu\text{g/ml}$. The wound area was observed and photographed at 0 and 24 h using an optical microscope (Axiovert, ZEISS, Jena, Germany).

4.2.3.2. Co-culture studies

4.2.3.2.1 Real-time cell analysis (RTCA)

The most effective concentration of imiquimod, previously determined by Váradi et al., was applied during the experiments. The aim of the study was to determine the optimal concentration of tapinarof-loaded nanogels for use in subsequent experiments. The effects of the test samples on cell proliferation, both in the presence and absence of imiquimod, were monitored using the xCELLigence Real-Time Cell Analysis (RTCA) system, which enables real-time monitoring of cell proliferation and viability. An E-Plate 16 coated with gold microelectrodes for electrical impedance measurement was used. HaCaT cells were seeded at a density of 10,000 cells/well. After 6 h, following cell attachment, 1,000 THP-1 cells were added to each well to establish the co-culture model. Cells were maintained at 37 °C in a humidified incubator containing 5% CO_2 . After 24 h, the cells were treated with serial dilutions of tapinarof, tapinarof-loaded nanogels, or blank nanogels (0.1 μM , 1 μM , and 10 μM). Cell culture medium served as the negative control, while imiquimod-containing medium served as the positive control. Cell proliferation was monitored for 96 h, and data were recorded every 30 min. The results were expressed as normalized cell index values calculated by the RTCA software.

4.2.3.2.2 Wound healing assay in the co-culture model

An in vitro wound healing assay was performed to evaluate cell proliferation and migration in the co-culture model. A 24-well plate equipped with wound-making inserts (0.5 mm width) was used to generate the scratch area. HaCaT cells were seeded into the two compartments of each insert at a density of 280,000 cells/well, corresponding to 140,000

cells/compartment. THP-1 cells were added 6 h later at a density of 28,000 cells/well. DMEM medium was used throughout the experiment. The co-cultures were incubated at 37 °C, and after 24 h the inserts were carefully removed to create the wound area. To preserve non-adherent THP-1 cells, no washing step (e.g., PBS washing) was performed before treatment. The cells were then treated with 10 µM free tapinarof in the presence or absence of imiquimod, 10 µM tapinarof-loaded nanogel in the presence or absence of imiquimod, or a drug-free nanogel equivalent to 10 µM formulation concentration. The wound area was examined and documented at 0, 6, 12, and 24 h using light microscopy.

After 24 h, the experiment was terminated. The wells were washed with PBS, and the cells were fixed with a fixation solution for 15 min at room temperature. Following a second PBS washing step, the cells were stained with Trypan Blue for 15 min at room temperature. Images were acquired using a Zeiss Axioscope A1 microscope (Carl Zeiss AG, Germany).

Cell migration rates were determined by quantitative analysis of wound closure based on the captured images. The wound area measured at the final time point (24 h) was compared with the initial wound area (0 h), and the migration rate was calculated as the percentage of wound closure.

4.2.3.2.3 ELISA assays

ELISA assays were also performed using the HaCaT–THP-1 co-culture model. A 12-well Transwell system equipped with a permeable polycarbonate membrane (0.4 µm pore size) was used for the experiments. The basal chamber was filled with 1500 µl of DMEM, while HaCaT cells were seeded into the apical chamber at a density of 300,000 cells/well.

After 6 h, THP-1 cells were added to the apical chamber at a density of 30,000 cells/well to establish the co-culture model. The cells were incubated at 37 °C for 3 days to reach approximately 100% confluence. Subsequently, the cultures were treated with the following formulations: 10 µM free tapinarof in the presence or absence of imiquimod, and 10 µM tapinarof-loaded nanogel in the presence or absence of imiquimod. Untreated culture medium served as the negative control, while 1 µM imiquimod was used as the positive control. After 24 h of treatment, samples were collected from the apical chamber. The concentrations of TNF- α , IFN- γ , IL-17A, and IL-23 in the supernatants were determined using ELISA kits according to the manufacturer's instructions.

4.2.3.2.4 Gene expression analysis by qPCR

For qPCR analysis, 500,000 HaCaT cells were seeded into each well of a 12-well plate. After allowing the cells to attach for 6 h at 37 °C, 50,000 THP-1 cells were added to each well, and the co-cultures were maintained in an incubator containing 5% CO₂. The co-cultures were cultured under identical conditions for 72 h. After 3 days, the cells were treated with 1 µM imiquimod, 10 µM tapinarof-loaded nanogel, 10 µM free tapinarof, or combinations of these. Following 24 h of treatment, the culture medium containing the non-adherent THP-1 cells was collected. The cell suspension was centrifuged, the supernatant was removed, and the cell pellet was washed with PBS. Simultaneously, the adherent HaCaT cells remaining in the wells were also washed with PBS. Subsequently, 1 ml of TRIzol reagent (Invitrogen, Cat. No. 15596026) was added directly to each well to lyse the adherent cells. The THP-1 cell pellet was resuspended in the TRIzol lysate, and the combined samples were transferred into 1.5 ml RNase-free microcentrifuge tubes (Eppendorf, Invitrogen, Cat. No. F23488) for total RNA isolation. Following cell lysis, chloroform was added to the samples for phase separation. After vigorous vortexing, the samples were centrifuged at maximum speed for 15 min at 4 °C. The upper aqueous phase was carefully collected and mixed with isopropanol. The samples were then centrifuged again at maximum speed for 10 min at 4 °C. The supernatant was removed, and the RNA pellet was washed with 70% ethanol. After a further centrifugation step (maximum speed, 4 °C, 5 min), the supernatant was discarded, the pellet was air-dried, and finally dissolved in RNase-free water.

RNA concentrations were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific), and RNA quality was assessed using ND-1000 Software V3.3.0.

Complementary DNA (cDNA) was synthesized using the TaqMan Reverse Transcription Reagent Kit (Applied Biosystems, USA) according to the manufacturer's instructions.

The expression levels of *Tnf-α*, *Ifn-γ*, *Il-17a*, and *Il-23* were analyzed using PrimePCR SYBR Green Assays according to the manufacturer's protocol. Human GAPDH was used as the endogenous control. Relative gene expression levels were determined by quantitative real-time PCR using a CFX96 Touch Real-Time PCR Detection System (Bio-Rad). The PCR program consisted of an initial denaturation step at 95 °C for 2 min, followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s. All measurements were performed in triplicate. Normalized gene expression levels were calculated using the $\Delta\Delta C_t$ method.

4.2.3.2.5 NF- κ B translocation

To investigate activation of the NF- κ B signaling pathway, nuclear translocation of the p65 subunit was analyzed in THP-1 cells by immunofluorescence staining. Prior to cell seeding, sterile glass coverslips were placed into the wells of a 16-well plate. THP-1 cells were suspended in RPMI 1640 medium, and 2 μ l of a 100 ng/ml PMA solution was added to 20 ml of cell suspension to induce differentiation. PMA treatment promotes the differentiation of THP-1 monocytes into macrophage-like adherent cells, thereby facilitating cell attachment and reliable immunofluorescence analysis. The cells were then seeded at a density of 50,000 cells/well and incubated for 30 h at 37 °C in a humidified atmosphere containing 5% CO₂. Following incubation, the PMA-containing medium was removed and replaced with PMA-free RPMI 1640 culture medium. The cells were incubated for an additional 24 h under the same conditions. Subsequently, the culture medium was removed and replaced with fresh RPMI 1640 medium, and the cells were treated with 10 μ M tapinarof-loaded nanogel, 10 μ M free tapinarof, and 10 μ M free tapinarof. After 24 h of treatment, 1 μ M imiquimod was added to induce inflammation, and the cells were incubated for a further 1 h. The cells were then washed twice with 500 μ l PBS. Fixation was performed using 4% paraformaldehyde (PFA) solution for 15 min at room temperature. Permeabilization was carried out using 0.1% Triton X-100 solution to facilitate antibody access to the nucleus. Non-specific binding sites were blocked using 5% FBS solution. The cells were then incubated for 1 h with Alexa Fluor 488-conjugated NF- κ B p65 (F-6) antibody (Cat. No. sc-8008 AF488) at a concentration of 2 μ g/ml, followed by three washes with PBS. Nuclear staining was performed using a nuclear dye diluted 1:10,000 for 10 min. The staining solution was removed, and the cells were washed twice more with PBS. Finally, the glass coverslips were mounted onto microscope slides.

Fluorescence imaging was performed using a Zeiss Axioscope A1 fluorescence microscope (Carl Zeiss AG, Jena, Germany) and Zeiss Immersol™ 518F immersion oil (Cat. No. 201006). Image analysis was carried out using ZEN 2012 software (v3.8.99.07000, Carl Zeiss Microscopy GmbH, Göttingen, Germany).

5. Results

5.1. Physicochemical characterization

5.1.1. Particle size determination

The particle size distribution and polydispersity index (PDI) of both formulations were determined. Based on dynamic light scattering (DLS) measurements, both compositions were found to be within the nanometer size range. To achieve this, the gels were subjected to manual ultrasonication prior to gelation. In the case of nanogel II, sonication at 50% amplitude for 2 min was sufficient to obtain the desired nanoscale particle size, which was maintained after gel formation. For nanogel I, this treatment time was not sufficient. Therefore, the sonication time was increased to 3 min, resulting in nanometer-sized gels. Furthermore, all formulations exhibited low polydispersity index values.

5.1.2. Rheological characterization

The rheological properties of the formulations were investigated to determine which preparation was the most suitable for topical application, as viscosity significantly influences the spreadability of semisolid formulations as well as the bioavailability of incorporated active pharmaceutical ingredients. To characterize the rheological behavior of the formulations, changes in viscosity and flow behavior were evaluated as a function of shear rate. The results demonstrated that the viscosity of the gels depended on both the magnitude and duration of the applied mechanical stress (shear rate). Initially, high viscosity values were observed, which gradually decreased with increasing shear rate, indicating pseudoplastic behavior. In all cases, increasing shear stress resulted in an increase in shear rate, suggesting that greater force was required to deform the nanogel structures. Furthermore, the measurements clearly showed that nanogel I exhibited higher viscosity and a steeper flow curve compared to nanogel II. Measurements performed at 24 °C yielded slightly higher viscosity values than those obtained at 35 °C, indicating that viscosity decreased with increasing temperature. During the experiment, the shear rate was first increased and subsequently decreased to investigate hysteresis behavior. Under the experimental conditions, hysteresis was not observed. The internal structure of the investigated samples did not undergo permanent changes upon exposure to shear stress. The absence of hysteresis and the limited thixotropic behavior of the gels indicate that they were capable of recovering their original structure after the removal of shear forces.

5.1.3. Texture analysis

The investigated formulations were also subjected to texture analysis. Based on the results, the samples exhibited no significant resistance to compression. The consistency of both gels proved to be appropriate from a pharmaceutical formulation perspective, as low consistency facilitates the release of the active pharmaceutical ingredient. Furthermore, low mechanical resistance is considered a favorable property for topical application.

5.1.4. In vitro release study

The diffusion profiles of the formulations were investigated through an isopropyl myristate-impregnated cellulose acetate membrane. The mean cumulative percentage of tapinarof permeating through the membrane was determined as a function of time. Based on the permeation studies, the active pharmaceutical ingredient began to be released immediately, and the dissolution curves exhibited a continuous increase throughout the study period. The results demonstrated that nanogel II exhibited superior performance compared to the other formulation. After 5 h, 81% of the incorporated tapinarof had permeated through the membrane, which was considerably higher than the 52% observed for nanogel I. In addition, the flux of nanogel II was $0.1935 \text{ mg/cm}^2 \cdot \text{h}^{-1}$, whereas nanogel I exhibited a lower flux value of $0.1255 \text{ mg/cm}^2 \cdot \text{h}^{-1}$. Correlation coefficients associated with the fitting of the release data to kinetic models were determined for both zero-order and first-order models. Correlation coefficient values (R^2) closer to 1 indicate a better fit to the respective kinetic model. Based on the obtained results, both gels could be adequately described by zero-order and first-order kinetic models.

5.2. In vitro cellular studies

5.2.1. Monoculture studies

5.2.1.1. Cell viability assay on HaCaT cells

Cytotoxicity studies were performed using HaCaT cells in all cases. The experiment was conducted with the various excipients used in the formulations to ensure that they were non-toxic to the cells. Labrafil M, Transcutol HP, and Tween 80 were evaluated after dilution in PBS at different concentrations. In addition, the active pharmaceutical ingredient and the nanogel formulations, both with and without tapinarof, were dissolved in 9% NaCl solution at various concentrations. Triton X-100 was used as the positive control, while PBS served as the negative control. Cell viability was expressed as a percentage relative to the negative control.

The results demonstrated that only Tween 80 did not prove to be safe at the tested concentrations. However, since it enhances drug release and improves penetration, its use may still be considered. All other investigated samples proved to be safe and non-cytotoxic, as cell viability remained above 70% in all cases. The effects of free tapinarof and the different nanogel formulations on HaCaT cell viability were evaluated in both short-term (30 min incubation) and long-term (24 h incubation) experiments. The results of the short-term studies showed that all formulations, including free tapinarof, were non-toxic to HaCaT cells. Similar results were obtained in the long-term experiments. However, a slight reduction in cell viability was observed for some formulations following 24 h of incubation. Overall, both studies demonstrated that tapinarof and the developed nanogel formulations can be safely applied to HaCaT cells.

5.2.1.2. Wound healing assay on fibroblast cells

The effects of tapinarof-loaded nanogels on fibroblast cell migration were investigated using a scratch wound healing assay. Wound closure was assessed after 24 h of treatment at a concentration of 40 µg/ml. The effects of formulations with and without the active pharmaceutical ingredient were evaluated relative to the wound closure rate obtained with free tapinarof. In cells treated with nanogel II containing tapinarof, fewer fibroblasts were observed within the scratched area compared with cells treated with the corresponding blank nanogel. In the case of the blank formulations, complete closure of the scratch area was observed. Treatment with tapinarof-loaded nanogel I also resulted in wound closure within 24 h in the fibroblast cell culture. In summary, the results suggest that the incorporation of tapinarof into nanogel II enhances its antiproliferative and antimigratory activity.

5.2.2. Co-culture studies

5.2.2.1. Real-time cell analysis (RTCA)

The potential anti-psoriatic effects of tapinarof and nanogel-based delivery systems were evaluated using real-time cell analysis in the co-culture model. The inflammatory environment induced by IMQ resulted in enhanced cell proliferation, consistent with previous findings reported in the literature. Free tapinarof alone moderately reduced IMQ-induced proliferation, indicating its anti-inflammatory activity. Both tapinarof-loaded nanogel formulations reduced the cell proliferation index more effectively than free tapinarof,

particularly in the presence of IMQ. When incorporated into the nanogel formulations, tapinarof attenuated IMQ-induced cell proliferation in a dose-dependent manner. According to the experimental results, nanogel I also significantly reduced IMQ-induced proliferation; however, nanogel II exhibited a more pronounced effect. Based on the RTCA results, 10 μ M tapinarof and the tapinarof-loaded nanogels produced the most significant biological effects; therefore, this concentration was selected for subsequent experiments.

5.2.2.2. Wound healing assay in the co-culture model

Cell migration was evaluated using an in vitro wound healing assay in the co-culture model. In untreated (control) samples, cell migration proceeded as expected. Complete wound closure was also observed in IMQ-treated samples, as IMQ promotes keratinocyte proliferation. Treatment with blank nanogels did not affect wound closure, further confirming their biocompatibility. Free tapinarof moderately reduced cell proliferation, while tapinarof-loaded nanogel I also decreased the rate of wound closure in the absence of IMQ. However, the strongest antimigratory effect was observed for the tapinarof-loaded nanogel II formulation. Interestingly, tapinarof, both in its free form and when incorporated into nanogels, inhibited cell migration more effectively in an inflammatory microenvironment (in the presence of IMQ) than under physiological conditions. Overall, the tapinarof-loaded nanogel II formulation proved to be the most effective in reducing abnormal keratinocyte migration, highlighting its potential for modulating inflammatory cellular responses.

5.2.2.2.1 ELISA assays

The levels of pro-inflammatory cytokines (TNF- α , IFN- γ , IL-17A, and IL-23) were determined using ELISA in the HaCaT–THP-1 co-culture model. IMQ treatment significantly increased the secretion of all investigated cytokines compared to the control group, confirming the successful induction of an inflammatory response. Both free tapinarof and the nanogel formulations exhibited anti-inflammatory effects. However, the tapinarof-loaded nanogel formulations proved to be more effective than the free drug. Under inflammatory conditions, free tapinarof moderately reduced IL-23 secretion but did not exhibit inhibitory effects on the other cytokines; in some cases, it even resulted in higher cytokine levels than IMQ treatment alone. In the presence of IMQ, tapinarof-loaded nanogel I reduced TNF- α and IL-23 levels and slightly decreased IL-17A secretion, whereas no substantial change was observed in IFN- γ levels. The strongest anti-inflammatory effect was observed with tapinarof-loaded nanogel II,

which reduced the levels of all investigated cytokines, including IL-23. Although no detectable changes in IL-17A and IL-23 gene expression were observed by qPCR, ELISA analysis revealed significant modulation at the protein level. These findings suggest that tapinarof-loaded nanogels effectively reduce IMQ-induced inflammatory cytokine production in this in vitro co-culture model.

5.2.2.2.2 Gene expression analysis by qPCR

The expression levels of the *Tnf- α* , *Ifn- γ* , *Il-17a*, and *Il-23* genes were determined by qPCR. *Tnf- α* expression was increased in IMQ-treated samples compared with the control group, confirming the successful induction of an inflammatory response. Among the investigated formulations, tapinarof-loaded nanogel I exerted the most pronounced anti-inflammatory effect in the presence of IMQ, as it significantly reduced IMQ-induced *Tnf- α* expression. Free tapinarof and tapinarof-loaded nanogel II also reduced *Tnf- α* expression; however, nanogel I exhibited the greatest inhibitory effect. The expression of *Ifn- γ* showed a slight increase following IMQ treatment. Treatment with free tapinarof and tapinarof-loaded nanogels in the presence of IMQ resulted in a modest reduction in *Ifn- γ* expression. Overall, *Ifn- γ* gene expression remained relatively stable under the applied experimental conditions. The mRNA expression levels of *Il-17a* and *Il-23* remained below the detection limit in all experimental groups after 24 h of treatment. Neither IMQ stimulation nor treatment with free tapinarof or tapinarof-loaded nanogels induced measurable transcriptional changes in *Il-17a* or *Il-23* expression in the HaCaT–THP-1 co-culture model. These findings differ from the cytokine level alterations observed by ELISA, suggesting that cytokine secretion and gene expression were not directly correlated under the applied experimental conditions.

5.2.2.2.3 NF- κ B translocation

Activation of the NF- κ B signaling pathway was investigated by immunofluorescence staining in the THP-1 monocytic cell line through monitoring the nuclear translocation of the p65 subunit. IMQ treatment resulted in the nuclear localization of the p65 subunit, indicating activation of the NF- κ B pathway. Treatment with free tapinarof resulted in a slight reduction in p65 nuclear translocation, whereas tapinarof-loaded nanogel I exhibited a moderate inhibitory effect. The most effective inhibition of IMQ-induced p65 nuclear localization was observed in cells treated with tapinarof-loaded nanogel II, indicating a stronger suppression of NF- κ B activation compared with both free tapinarof and tapinarof-loaded nanogel I.

6. Discussion

6.1. Evaluation of formulation development and dosage form characterization

In the first phase of my experimental work, the gels were evaluated from several aspects, including cell viability assessment and particle size determination. The nanoscale size of the formulations was confirmed by dynamic light scattering (DLS). Based on the measurements, both formulations remained within the nanometer size range even after gel formation.

The formulations were also subjected to rheological and texture analyses, as these parameters are directly related to the composition of the formulations and consequently to their consistency and stability. In particular, the temperature- and shear rate-dependent changes in viscosity and shear stress influence drug release and, consequently, skin penetration.

Rheological measurements demonstrated that the viscosity of both gels decreased with increasing temperature; at 35 °C, viscosity was slightly lower than at room temperature. Similar findings were reported by Zeynep Ay Şenyiğit and co-workers, who observed pseudoplastic behavior in Carbopol 934-based gels developed for the dermal delivery of mupirocin. In another study, Rajinikanth et al. also reported shear-thinning behavior in Carbopol 934-based gel formulations. The work of Giuseppe and colleagues further supports these findings, demonstrating that the viscosity of Carbopol-containing gels decreases with increasing temperature.

As a consequence of the reduced viscosity, the gels flow more readily at body temperature, which may result in faster drug release. Nanogel II exhibited slightly lower viscosity, suggesting that this formulation may provide more rapid drug release. Lower viscosity also implies improved spreadability at body temperature, facilitating topical application.

Texture analysis revealed no significant difference between the two formulations in terms of compressive force. Nanogel I exhibited an average compressive force of approximately 65 N·mm, whereas nanogel II showed a slightly lower value of approximately 60 N·mm. The investigated samples exhibited no noticeable resistance, indicating that their consistency was suitable for gel-based dosage forms, which may contribute to improved patient compliance.

During the in vitro drug release studies, nanogel II exhibited faster and more extensive tapinarof release over time. After 11 minutes, the amount of released tapinarof approached 14%, whereas nanogel I showed a more moderate release profile, reaching approximately 8%. Both formulations remained stable throughout the experiment; however, a clear difference was

observed in their drug release rates. This difference is likely attributable to variations in formulation composition and rheological properties, which influence permeation through the skin.

Among the two gels, nanogel II demonstrated a significantly superior release profile, which is consistent with the results of the rheological studies. Such rapid drug release may be particularly advantageous when a rapid therapeutic effect is desired, as in the treatment of psoriasis.

During formulation development, we aimed to select excipients that promote drug solubility and release. According to the literature, nonionic surfactants possess the lowest irritation potential; therefore, they were chosen for this study. Labrafil M, Tween 80, and Transcutol HP are well-known solvents or co-solvents that are frequently used to enhance the penetration of topical formulations.

According to Yue Zhang and co-workers, tapinarof exhibits high solubility in Tween 80 and ethanol. Based on the findings reported by Jasmine Musakhanian, Transcutol HP is capable of dissolving substantial amounts of both hydrophilic and lipophilic compounds and is therefore suitable for incorporation into hydrogel systems. Accordingly, tapinarof was dissolved in these solvents during formulation development.

The biocompatibility of the tapinarof-loaded formulations and the selected excipients was evaluated using the HaCaT cell line. The effects of tapinarof and the different nanogels on cell viability were characterized through both short-term and long-term studies to obtain a comprehensive understanding of their biocompatibility profiles. Based on the results of the MTT assay, free tapinarof, the nanogel formulations, as well as Labrafil M and Transcutol HP, showed appropriate biocompatibility within the investigated concentration range. In contrast, Tween 80 exhibited cytotoxic effects and proved to be the only investigated component that resulted in a reduction in cell viability.

However, these findings are not consistent with the observations of Farzaneh Forouz and co-workers, who also investigated the toxicity of Labrafil M and Transcutol HP using the MTT assay. Their results indicated that, although these substances were less toxic than Triton X-100, they could not be considered non-toxic excipients based on pharmacological or toxicological evaluations.

In our experiments, only Tween 80 exhibited toxicity. In contrast, numerous studies in the literature have reported different findings. For example, Caroline Maupas and colleagues investigated the toxicity of Tween 80 and other surfactants incorporated into lipid nanoparticles and observed pronounced surfactant type-dependent toxicity in HaCaT cells. However, Tween

80 was not identified as the most toxic surfactant. The toxicity of surfactants is primarily attributed to their amphipathic structure. Conversely, Maiulle T. Pacheco and co-workers investigated nanoemulsions containing sucupira oil and attributed the observed toxicity not to the surfactants but to the oil itself. Furthermore, Katja Steine and colleagues identified Tween 80 as one of the most skin-friendly surfactants.

According to the literature, tapinarof possesses antiproliferative properties. This was also supported by our in vitro wound healing studies, in which the extent of wound closure primarily reflects cell migration. While faster wound closure is generally considered beneficial in classical wound healing models, the reduction of increased cell migration and proliferation may be regarded as a potentially beneficial effect in the context of psoriasis. Tapinarof alone inhibited wound closure more effectively after 24 h than the blank gels. Furthermore, nanogel I containing tapinarof moderately inhibited cell migration, whereas treatment with nanogel II containing tapinarof resulted in slower wound closure, indicating that fibroblast migration within the scratched area was inhibited within 24 h. This may be beneficial in the treatment of psoriasis, as the disease is characterized by increased keratinocyte proliferation.

6.2. Evaluation of the results of in vitro cellular studies

In the second phase of my experimental work, tapinarof-loaded nanogels were evaluated using the HaCaT–THP-1 co-culture model based on several parameters. The antiproliferative effects of the nanoformulations were investigated using real-time cell analysis (RTCA) and in vitro wound healing assays. Since no scientific data are currently available regarding the application of RTCA for the evaluation of tapinarof, our experiment can be considered unique. Measurements were performed both in the presence and absence of the active pharmaceutical ingredient under inflammatory (IMQ-induced) and non-inflammatory conditions.

The results demonstrated that both nanogel formulations significantly reduced IMQ-induced cell proliferation, whereas free tapinarof exhibited a more moderate effect. Blank nanogels did not promote cell proliferation. Among all tested samples, the 10 μ M tapinarof concentration proved to be the most effective; therefore, subsequent experiments were conducted using this concentration.

During the in vitro wound healing assay, the 10 μ M tapinarof concentration resulted in a migration profile different from that observed in our previous experiment performed on human dermal fibroblasts, where the active ingredient was tested at a concentration of 40 μ M in the absence of an inflammatory stimulus. In the inflammatory keratinocyte–macrophage

model, IMQ accelerated the closure of the gap between cells; however, treatments containing tapinarof significantly slowed this process. Among the two nanogel formulations, nanogel II exhibited a more favorable trend than nanogel I. In the presence of IMQ, however, all treatments significantly slowed gap closure compared with free tapinarof. This property may be particularly advantageous in diseases such as psoriasis, which are characterized by hyperproliferation and inflammation and where the therapeutic goal is to reduce abnormal keratinocyte proliferation.

ELISA assays were performed to evaluate the effects of tapinarof-loaded nanogel formulations compared with free tapinarof on the secretion of key pro-inflammatory cytokines (TNF- α , IFN- γ , IL-17A, and IL-23) in the HaCaT–THP-1 co-culture model. Our results demonstrated that tapinarof-loaded nanogels induced a more pronounced reduction in cytokine levels than free tapinarof. Under inflammatory conditions, nanogel I reduced the levels of TNF- α and IL-23 and moderately decreased IL-17A secretion, whereas nanogel II exhibited the most effective anti-inflammatory profile, significantly reducing the levels of all four investigated cytokines. In contrast, free tapinarof resulted only in a moderate reduction in IL-23 and showed no detectable inhibitory effect on the other cytokines. Moreover, in some cases, elevated cytokine levels were observed in the presence of IMQ.

These findings are consistent with previous studies demonstrating the anti-inflammatory effects of tapinarof. Smith and colleagues observed that tapinarof reduced the expression of inflammatory cytokines and improved skin barrier function. Both ELISA and qPCR analyses demonstrated reductions in IL-17A, IL-17F, IL-22, and IL-23 levels, supporting the anti-inflammatory activity of tapinarof. Similar results were reported by Zhao and co-workers, who measured reduced IL-17 production in tapinarof-treated dendritic cells using ELISA and also observed corresponding changes at the mRNA level. Likewise, Bissonnette and colleagues clearly demonstrated that tapinarof treatment reduced the levels of inflammatory cytokines (IL-17A, IL-22, IL-23, and IL-1) in a psoriatic mouse model. However, it should be noted that most of these studies were conducted using different biological models, which limits their direct comparability with the present in vitro co-culture model.

The qPCR analysis showed partial discrepancies compared with the ELISA results. Tapinarof-containing formulations reduced Tnf- α mRNA expression compared with free tapinarof, whereas Ifn- γ transcription remained largely unchanged across the experimental groups; therefore, no clear tapinarof-related effect could be demonstrated for this marker in the present model. The mRNA levels of Il-17a and Il-23 remained below the detection limit in all samples. Consequently, under the applied experimental conditions, no direct correlation could

be established between cytokine secretion measured by ELISA and mRNA expression detected by qPCR. This discrepancy may be explained by differences in transcriptional and post-transcriptional regulation, as well as by the temporal delay between mRNA expression and protein secretion.

In the second phase of my experimental work, tapinarof-loaded nanogels were evaluated using the HaCaT–THP-1 co-culture model based on several biological parameters. The antiproliferative effects of the nanoformulations were investigated using real-time cell analysis (RTCA) and in vitro wound healing assays. Since no scientific data are currently available regarding the application of RTCA for the evaluation of tapinarof, our experiment can be considered unique. Measurements were performed both in the presence and absence of the active pharmaceutical ingredient under inflammatory (IMQ-induced) and non-inflammatory conditions. The results demonstrated that both nanogels significantly reduced IMQ-induced cell proliferation, whereas free tapinarof exhibited a more moderate effect. The unloaded nanogels did not promote cell proliferation. Among all tested samples, the 10 μ M tapinarof concentration proved to be the most effective; therefore, subsequent experiments were performed using this dose.

During the in vitro wound healing assay, the 10 μ M tapinarof concentration resulted in a migration profile different from that observed in our previous experiments performed on human dermal fibroblasts, where the active compound was tested at a concentration of 40 μ M without an inflammatory stimulus. In the inflammatory keratinocyte–macrophage model, IMQ accelerated wound closure; however, tapinarof-containing treatments significantly slowed this process. Among the two nanogel formulations, nanogel II exhibited a more favorable trend than nanogel I. In the presence of IMQ, all treatments markedly reduced wound closure compared with free tapinarof. This property may be particularly advantageous in disorders such as psoriasis, which are characterized by hyperproliferation and inflammation, where the therapeutic goal is to reduce abnormal keratinocyte proliferation.

ELISA assays were performed to evaluate the effects of tapinarof-loaded nanogels on the secretion of key pro-inflammatory cytokines (TNF- α , IFN- γ , IL-17A, and IL-23) in the HaCaT–THP-1 co-culture model compared with free tapinarof. Our results demonstrated that tapinarof-loaded nanogels induced a more pronounced reduction in cytokine levels than the free active compound. Under inflammatory conditions, nanogel I reduced TNF- α and IL-23 levels and slightly decreased IL-17A secretion, whereas nanogel II exhibited the most potent anti-inflammatory profile by significantly reducing all four investigated cytokines. In contrast, free tapinarof only moderately decreased IL-23 secretion and showed no detectable inhibitory effect

on the other cytokines. In some cases, cytokine levels were even higher in the presence of IMQ than in the IMQ-treated control group.

These findings are consistent with previous studies demonstrating the anti-inflammatory effects of tapinarof. Smith et al. reported that tapinarof reduced the expression of inflammatory cytokines and improved skin barrier function. Both ELISA and qPCR analyses revealed decreased levels of IL-17A, IL-17F, IL-22, and IL-23, supporting the anti-inflammatory activity of tapinarof. Similar findings were reported by Zhao et al., who observed reduced IL-17 production in tapinarof-treated dendritic cells using ELISA and also demonstrated corresponding changes at the mRNA level. Likewise, Bissonnette et al. clearly showed that tapinarof treatment reduced the levels of inflammatory cytokines, including IL-17A, IL-22, IL-23, and IL-1, in a psoriasis mouse model. However, it should be noted that most of these studies were conducted using different biological models, which limits direct comparison with the present in vitro co-culture system.

The qPCR analysis revealed partial discrepancies compared with the ELISA results. Tapinarof-containing formulations reduced *Tnf- α* mRNA expression compared with free tapinarof, whereas *Ifn- γ* transcription remained largely unchanged across the experimental groups, indicating that no clear tapinarof-associated effect on this gene could be detected in this model. The mRNA levels of *Il-17a* and *Il-23* remained below the detection limit in all samples. Consequently, under the applied experimental conditions, no direct relationship could be established between cytokine secretion measured by ELISA and mRNA expression determined by qPCR. This discrepancy may be explained by differences in transcriptional and post-transcriptional regulation, as well as by temporal differences between mRNA expression and protein secretion.

6.3. Summary of novel scientific results

New scientific results of my experimental work:

- Tapinarof was successfully incorporated into nanogel-based formulations suitable for topical administration and exhibiting appropriate physicochemical properties.
- Dynamic light scattering measurements confirmed that the nanoformulations maintained their particle size within the nanometer range even after gelation.
- Rheological and texture analyses of the different nanogel formulations demonstrated their suitable viscosity, structural characteristics, and consistency for dermal application.
- In vitro drug release studies confirmed that the nanogel formulations provide controlled release of tapinarof.
- Cytotoxicity studies performed on the HaCaT cell line demonstrated the biocompatibility and safety of the excipients and formulations used during formulation development.
- Real-time cell analysis revealed that tapinarof and tapinarof-loaded nanogels influence keratinocyte proliferation in a concentration-dependent manner under an imiquimod-induced inflammatory environment.
- In vitro wound healing studies demonstrated that tapinarof-containing formulations modulate cell migration. Under non-inflammatory conditions, they slowed fibroblast migration, while in the HaCaT–THP-1 co-culture model they also influenced wound closure processes occurring under inflammatory conditions.
- Using ELISA assay in the HaCaT–THP-1 co-culture model, it was demonstrated that tapinarof and its nanogel formulations reduced the levels of pro-inflammatory cytokines (TNF- α , IFN- γ , IL-17A, and IL-23) more effectively than the free active compound.
- Gene expression studies confirmed that tapinarof-containing formulations modulate the mRNA expression levels of inflammatory cytokines, including *Tnf- α* and *Ifn- γ* .
- Immunofluorescence analysis demonstrated that tapinarof-loaded nanogels inhibit the nuclear translocation of the NF- κ B p65 subunit more effectively than free tapinarof, thereby contributing to the modulation of inflammatory signaling pathways.

7. Summary

Psoriasis is a chronic, immune-mediated inflammatory skin disease that significantly impairs quality of life, and the treatment still remains a challenge. Although several therapeutic options are currently available, there is still a strong need for effective, well-tolerated, and long-term applicable topical formulations. Recently approved tapinarof, as an aryl hydrocarbon receptor (AhR) agonist, represents a novel therapeutic approach due to its anti-inflammatory, antioxidant, and skin barrier-restoring properties. However, conventional formulations limit adequate skin penetration and the bioavailability of the active ingredient.

The aim of my doctoral work was to develop innovative topical drug delivery systems that enhance the therapeutic efficacy of tapinarof. For this purpose, nanoformulations containing tapinarof with different compositions were prepared. My results demonstrated that the developed systems maintained their stable particle size below 200 nm even after gelation, exhibited appropriate rheological and textural properties, so they were suitable for dermal application. Furthermore, cytotoxicity studies confirmed their safety on HaCaT cell lines. In vitro release studies revealed that nanogel II exhibited a more favorable drug release profile, which can be attributed to its advantageous viscosity and structural characteristics.

During the investigation of biological effects, I demonstrated that the incorporation of tapinarof into nanogels enhanced its antiproliferative and antimigratory effects using in vitro scratch assay.

In the next phase of the study, the effects of the formulations were evaluated using an imiquimod-induced HaCaT–THP-1 co-culture psoriasis model. The results showed that nanogel-based drug delivery reduced the levels of key pro-inflammatory cytokines (TNF- α , IFN- γ , IL-17A, and IL-23) and more effectively inhibited cell proliferation and migration compared to the free drug. In addition, inhibition of the NF- κ B signaling pathway was observed, further supporting the anti-inflammatory potential of the system.

Overall, the developed tapinarof-loaded nanogel systems improve the bioavailability and biological efficacy of the active compound while maintaining a favorable safety profile. These findings suggest their potential as a promising non-steroidal alternative for the topical treatment of psoriasis and may contribute to the advancement of current therapeutic strategies.

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