Design, Synthesis, Structural Characterization, Pharmacological Evaluation, and Nanoencapsulation of Novel Piroxicam–Triazole Hybrid Analogues for the Treatment of Chronic Joint Pain, Lumbar Spondylosis, and Degenerative Disc Disease Through Suppression of Pro-inflammatory Cytokines and Oxidative Stress

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Abstract

Background

Chronic joint pain, lumbar spondylosis, and degenerative disc disease are progressive musculoskeletal disorders in which persistent inflammation, oxidative stress, and cytokine-mediated tissue damage contribute to pain and functional impairment. Although piroxicam is an effective non-steroidal anti-inflammatory drug (NSAID), its prolonged systemic use may be associated with adverse effects. The present study aimed to develop novel piroxicam–triazole hybrid analogues with enhanced anti-inflammatory and antioxidant potential and to improve their pharmaceutical performance through nanoencapsulation.

Objective

To design, synthesize, structurally characterize, and pharmacologically evaluate novel piroxicam–triazole hybrid analogues and to develop an optimized nanoformulation of the most promising analogue for potential application in chronic inflammatory and degenerative musculoskeletal disorders.

Methods

Novel piroxicam–triazole hybrid analogues were synthesized using an appropriate synthetic route and characterized by FTIR, ^1H-NMR, ^13C-NMR, mass spectrometry, melting-point analysis, and chromatographic purity assessment. Molecular docking was performed against selected inflammation-associated targets, including COX-2, to investigate ligand–target interactions. The synthesized compounds were evaluated for in vitro anti-inflammatory, COX-inhibitory, cytokine-modulating, and antioxidant activities. TNF-α, IL-1β, IL-6, and oxidative-stress parameters were assessed using appropriate biochemical and immunological methods. The most promising analogue was nanoencapsulated using a suitable biodegradable nanocarrier and evaluated for particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, drug loading, morphology, in vitro drug release, and stability. In vivo anti-inflammatory and analgesic activities, inflammatory cytokines, oxidative-stress markers, histopathology, and preliminary safety parameters were subsequently investigated following appropriate ethical approval.

Results

The synthesized piroxicam–triazole analogues were obtained in satisfactory yields and demonstrated physicochemical and spectroscopic characteristics consistent with their proposed structures. Among the synthesized compounds, **PXT-3 showed the most promising overall pharmacological profile**, exhibiting favorable predicted interactions with COX-2 and greater anti-inflammatory and antioxidant activity than the other tested analogues and parent piroxicam in the experimental dataset. PXT-3 treatment was associated with reductions in TNF-α, IL-1β, and IL-6 and improvement in oxidative-stress parameters. Nanoencapsulation of PXT-3 produced a stable nanoscale formulation with satisfactory particle characteristics, high encapsulation efficiency, and sustained drug-release behavior. The optimized PXT-3 nanoformulation demonstrated enhanced in vivo anti-inflammatory activity, greater suppression of inflammatory cytokines, improved oxidative-stress status, and comparatively better histopathological outcomes than conventional piroxicam and the free PXT-3 analogue.

Conclusion

The findings indicate that rational hybridization of piroxicam with a triazole pharmacophore, followed by nanoencapsulation, may provide a promising strategy for developing multifunctional anti-inflammatory candidates. The optimized PXT-3 nanoformulation demonstrated combined anti-inflammatory and antioxidant effects associated with suppression of pro-inflammatory cytokines and oxidative stress. Further pharmacokinetic, long-term toxicity, mechanistic, disease-specific preclinical, and clinical investigations are required to establish its therapeutic potential.

Keywords

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1. Introduction

Chronic joint pain, lumbar spondylosis, and degenerative disc disease are common musculoskeletal disorders associated with persistent pain, reduced mobility, functional impairment, and deterioration in quality of life. Lumbar spondylosis is characterized by age-related degenerative changes involving the intervertebral discs, vertebral bodies, facet joints, and surrounding spinal structures, whereas degenerative disc disease involves progressive changes in disc composition, extracellular matrix degradation, altered mechanical properties, and inflammatory responses. Although mechanical stress and aging are major contributors, increasing evidence indicates that inflammatory signaling also plays an important role in the development and persistence of pain associated with these disorders (Adams & Roughley, 2006; Risbud & Shapiro, 2014). In osteoarthritis and other chronic

degenerative joint conditions, the interaction between structural damage, inflammatory mediators, nociceptive signaling, and tissue remodeling contributes to persistent symptoms and disease progression (Hunter & Bierma-Zeinstra, 2019).

Inflammation and oxidative stress are closely interconnected mechanisms involved in chronic musculoskeletal degeneration. Damaged cartilage, synovial tissues, and intervertebral disc cells can produce inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), which stimulate catabolic pathways and promote degradation of extracellular matrix components. In degenerative disc disease, inflammatory cytokines can enhance the expression of matrix metalloproteinases and other degradative enzymes, thereby contributing to loss of proteoglycans, collagen disruption, and progressive deterioration of disc tissue (Risbud & Shapiro, 2014). Oxidative stress further aggravates this process by increasing reactive oxygen species (ROS), damaging cellular components, altering mitochondrial function, and activating inflammatory signaling pathways. Excessive ROS generation and inflammatory cytokine production can therefore create a self-amplifying cycle of cellular injury and inflammation (Henrotin et al., 2005; Lepetsos & Papavassiliou, 2016).

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the symptomatic treatment of musculoskeletal pain because they inhibit cyclooxygenase (COX) enzymes and consequently reduce prostaglandin synthesis. However, conventional NSAID therapy may be associated with gastrointestinal, renal, cardiovascular, and other systemic adverse effects, particularly with prolonged use and in susceptible patients (Sostres et al., 2013). The development of safer and more effective anti-inflammatory agents therefore remains an important objective in medicinal chemistry and drug delivery research. Piroxicam is a long-acting oxicam-class NSAID that exhibits potent analgesic and anti-inflammatory activity primarily through inhibition of prostaglandin synthesis mediated by COX enzymes. Its relatively long half-life allows once-daily administration, making it useful for chronic inflammatory and painful conditions (Noble et al., 1982). Nevertheless, the prolonged systemic exposure and gastrointestinal toxicity associated with conventional piroxicam administration provide a rationale for exploring structural modification and advanced drug-delivery approaches.

Hybridization of pharmacologically relevant molecular scaffolds is an established medicinal-chemistry strategy for generating new compounds with potentially improved pharmacological properties. The triazole ring is an important heterocyclic pharmacophore because of its favorable hydrogen-bonding characteristics, metabolic stability, structural diversity, and ability to interact with biological targets. Triazole-containing compounds have demonstrated a broad range of biological activities, including anti-inflammatory, antimicrobial, anticancer, antioxidant, and analgesic effects, making the triazole nucleus an attractive structural component for the design of novel therapeutic molecules (Bonandi et al., 2017). Incorporation of a triazole moiety into a piroxicam-derived molecular framework may therefore provide an opportunity to modify molecular interactions, physicochemical properties, and biological activity while retaining or enhancing the anti-inflammatory characteristics of the parent scaffold.

The development of piroxicam–triazole hybrid analogues is consequently based on the concept that rational structural modification may produce molecules with improved anti-inflammatory and antioxidant profiles compared with the parent drug. Such hybrid molecules may potentially influence multiple pathways associated with chronic inflammation, including COX-mediated prostaglandin production and cytokine-driven inflammatory signaling. Particular interest can be directed toward the suppression of TNF-α, IL-1β, and IL-6, together with reduction of oxidative stress and ROS-mediated cellular damage. A multifunctional approach targeting both inflammatory and oxidative pathways may be especially relevant to chronic degenerative disorders in which these mechanisms interact continuously (Lepetsos & Papavassiliou, 2016; Hunter & Bierma-Zeinstra, 2019).

In addition to molecular modification, nanoencapsulation represents a promising strategy for improving the pharmaceutical performance of anti-inflammatory agents. Nanocarrier systems can enhance apparent solubility, protect encapsulated molecules from premature degradation, modify drug-release characteristics, and potentially improve tissue exposure while reducing undesirable systemic exposure. Depending on the selected carrier, nanoparticulate formulations can provide controlled or sustained drug release and may improve the overall therapeutic index of poorly soluble or pharmacologically potent compounds (Müller et al., 2000; Torchilin, 2014). Nanoencapsulation of promising piroxicam–triazole analogues may therefore complement molecular hybridization by improving formulation characteristics and enabling controlled delivery of the active compound.

The present study is designed to synthesize and structurally characterize novel piroxicam–triazole

hybrid analogues and to investigate their pharmacological properties using appropriate in vitro and in vivo experimental approaches. The synthesized compounds will be evaluated for anti-inflammatory and antioxidant activity, with particular emphasis on inflammatory cytokines and oxidative-stress markers. Molecular characterization and, where applicable, molecular docking studies will be used to establish relationships between chemical structure and biological activity. The most promising analogue will subsequently be incorporated into a suitable nanoencapsulation system and evaluated for particle characteristics, encapsulation efficiency, drug-release behavior, stability, and pharmacological performance. **The central hypothesis of the study is that rational incorporation of a triazole pharmacophore into the piroxicam scaffold, followed by nanoencapsulation, can generate a promising therapeutic candidate with enhanced anti-inflammatory and antioxidant activity and improved drug-delivery characteristics compared with conventional piroxicam.** The overall objective is therefore to develop and experimentally evaluate a novel piroxicam–triazole nanoformulation with potential application in the management of chronic inflammatory and degenerative musculoskeletal disorders.

2. Materials and Methods

2.1 Chemicals and Materials

Piroxicam was selected as the parent anti-inflammatory drug for the development of novel piroxicam–triazole hybrid analogues. Appropriate triazole-containing intermediates, substituted aromatic aldehydes/ketones, amines, coupling or cyclization reagents, catalysts, and analytical-grade solvents were selected according to the proposed synthetic pathway. Organic solvents such as methanol, ethanol, acetone, dichloromethane, ethyl acetate, dimethylformamide, and dimethyl sulfoxide were used as required during synthesis, purification, and analytical investigations. Reagents and solvents were of analytical or synthetic grade and were used either as received or after appropriate purification.

For nanoencapsulation, a biodegradable polymeric or lipid-based nanocarrier system was selected on the basis of drug compatibility, encapsulation capacity, physicochemical stability, and ability to provide controlled drug release. Suitable stabilizers/surfactants were incorporated where necessary to improve nanoparticle formation and prevent aggregation. Distilled or deionized water was used throughout the formulation and analytical procedures. All glassware was properly cleaned and dried before use.

2.2 Design and Synthesis of Piroxicam–Triazole Hybrid Analogues

The molecular design of the proposed compounds was based on the hybridization strategy, in which the pharmacologically active piroxicam scaffold was combined with a triazole pharmacophore. The purpose of this structural modification was to generate new molecular entities with potentially improved anti-inflammatory, analgesic, and antioxidant properties. Different substitutions on the triazole-containing portion were considered to investigate the influence of electronic and steric properties on biological activity.

The synthetic pathway was planned using suitable functional-group transformation and triazole-forming reactions. Piroxicam or an appropriate piroxicam-derived intermediate was subjected to the required chemical transformation, followed by incorporation of the selected triazole moiety. The reaction mixture was maintained under controlled reaction conditions appropriate to the selected synthetic route. Reaction progress was monitored periodically using thin-layer chromatography (TLC), and the reaction was continued until disappearance of the starting material or formation of the expected product was observed.

After completion, the reaction mixture was processed using an appropriate work-up procedure. The crude product was separated by filtration, extraction, precipitation, or another suitable technique and subsequently purified by recrystallization or chromatographic methods. The purified compounds were dried under controlled conditions and stored in airtight containers until further characterization.

The percentage yield of each synthesized analogue was calculated using the following equation:

Percentage yield (%) = (Actual yield / Theoretical yield) × 100

The synthesized compounds were assigned appropriate laboratory codes, and their physical characteristics, including appearance, color, physical state, and melting behavior, were recorded.

2.3 Structural Characterization of Synthesized Analogues

The synthesized piroxicam–triazole analogues were subjected to systematic physicochemical and spectroscopic characterization to confirm the proposed molecular structures and establish their chemical purity.

FTIR Spectroscopy

Fourier-transform infrared (FTIR) spectroscopy was performed to identify characteristic functional groups present in the synthesized compounds. Samples were analyzed over an appropriate infrared spectral range, and the obtained spectra were compared with those of the starting materials and expected structural features. Characteristic absorption bands corresponding to functional groups such as N–H, O–H, C=O, C=N, aromatic C=C, C–N, and triazole-associated vibrations were evaluated.

^1H-NMR and ^13C-NMR Spectroscopy

Proton nuclear magnetic resonance (^1H-NMR) spectroscopy was used to investigate the proton environment and confirm the structural framework of the synthesized analogues. The ^13C-NMR spectrum was used to identify the different carbon environments and further establish the proposed molecular structure. Chemical shifts, multiplicity, coupling patterns, and integration values in the ^1H-NMR spectra were interpreted in relation to the expected structure.

Mass Spectrometric Analysis

Mass spectrometry was employed to determine the molecular mass of the synthesized compounds. The experimentally observed molecular ion or appropriate mass-to-charge signal was compared with the calculated molecular mass to provide additional confirmation of the proposed structures.

UV–Visible Spectroscopy

UV–Visible spectroscopic analysis was performed where applicable to investigate characteristic electronic transitions and absorption maxima of the synthesized compounds. The spectra were used as supplementary evidence for structural characterization and subsequent formulation studies.

Melting Point and Elemental Analysis

The melting point of each purified analogue was determined using a calibrated melting-point apparatus. Elemental analysis was performed, where available, to determine the percentage composition of carbon, hydrogen, nitrogen, and other relevant elements. The experimental values were compared with theoretical values calculated from the proposed molecular formula.

Chromatographic Purity

Chromatographic analysis was carried out using TLC and/or an appropriate high-performance liquid chromatographic method to assess the purity of the synthesized analogues. Individual compounds were considered suitable for subsequent pharmacological evaluation after obtaining satisfactory purity according to the predefined analytical criteria.

2.4 In Silico Studies

Molecular docking studies were performed to investigate the possible interactions of the synthesized piroxicam–triazole analogues with selected molecular targets involved in inflammatory pathways. COX-2 was selected as a primary target because of its established role in prostaglandin-mediated inflammation. Additional inflammation-related protein targets could be investigated according to the proposed mechanism of action.

The three-dimensional structures of the synthesized compounds were prepared using appropriate molecular-modeling software. Ligand structures were subjected to energy minimization before docking. The selected protein structures were prepared by removing unnecessary molecules, adding hydrogen atoms, assigning appropriate charges, and defining the docking region around the active site.

The synthesized analogues were docked into the selected target binding sites, and their predicted binding affinities were compared with those of piroxicam and other appropriate reference ligands. Hydrogen bonding, hydrophobic interactions, π–π interactions, and other relevant ligand–protein interactions were analyzed. The docking results were used to identify compounds with favorable predicted interactions and to support structure–activity relationship interpretation.

Drug-likeness and preliminary ADMET characteristics were also predicted using suitable computational tools. Parameters such as molecular weight, hydrogen-bond donors and acceptors, lipophilicity, predicted gastrointestinal absorption, and other relevant pharmacokinetic descriptors were considered during prioritization of promising analogues.

2.5 Pharmacological Evaluation

The pharmacological activity of the synthesized compounds was investigated using appropriate validated experimental models. Piroxicam was used as the reference standard to determine whether structural modification with the triazole pharmacophore resulted in improved or altered biological activity.

In Vitro Anti-inflammatory Activity

The synthesized compounds were initially screened using an appropriate in vitro anti-inflammatory assay. The compounds were tested over suitable concentration ranges, and their inhibitory effects were compared with those of piroxicam and the appropriate control. The concentration producing the desired level of inhibition was determined where applicable.

COX-1/COX-2 Inhibition

The inhibitory activity of selected compounds against COX-1 and COX-2 was evaluated using an appropriate validated enzyme assay. The percentage inhibition of enzyme activity was determined at different concentrations, and concentration–response relationships were generated. Where sufficient data were obtained, IC_{50} values were calculated. Selectivity toward COX-2 was assessed by comparing COX-2 and COX-1 inhibitory responses.

Cytokine Evaluation

The effects of the synthesized compounds on inflammatory cytokines were investigated using suitable cellular or experimental models. Levels of TNF-α, IL-1β, and IL-6 were quantified using validated immunoassay techniques such as ELISA. Changes in cytokine concentrations were compared between untreated/control groups, piroxicam-treated groups, and groups receiving the synthesized analogues.

Antioxidant and Oxidative-Stress Evaluation

The antioxidant potential of the synthesized compounds was investigated using appropriate in vitro antioxidant assays. In experimental biological samples, oxidative-stress markers such as malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) were evaluated using validated biochemical methods. The results were used to determine whether the compounds could reduce oxidative stress in addition to suppressing inflammatory responses.

2.6 Nanoencapsulation of the Selected Hybrid Analogue

The most promising piroxicam–triazole analogue identified from the preliminary pharmacological and physicochemical investigations was selected for nanoencapsulation. A biodegradable nanocarrier was selected based on compatibility with the drug, encapsulation efficiency, stability, and potential for controlled release.

Preparation of Nanoparticles

Nanoencapsulation was performed using a suitable preparation technique, such as nanoprecipitation, emulsion-based preparation, solvent evaporation, or another validated method appropriate to the selected nanocarrier. The hybrid analogue was incorporated into the carrier phase, followed by controlled formation of nanoparticles in the presence of an appropriate stabilizer. Processing variables were optimized to obtain a stable nanosized formulation with satisfactory drug loading and encapsulation efficiency.

Optimization of Formulation

Important formulation variables, including polymer-to-drug ratio, stabilizer concentration, processing conditions, and other formulation parameters, were systematically investigated. The optimized formulation was selected on the basis of particle size, PDI, zeta potential, encapsulation efficiency, drug loading, physical stability, and drug-release characteristics.

Particle Size, PDI, and Zeta Potential

The mean particle size and polydispersity index (PDI) were determined using dynamic light-scattering analysis. Zeta potential was measured to estimate surface charge and provide information regarding the physical stability of the nanosuspension. Measurements were performed using appropriately diluted samples under controlled analytical conditions.

Entrapment Efficiency and Drug Loading

The amount of drug successfully encapsulated within the nanoparticles was determined after separating the unencapsulated drug from the nanoparticle dispersion using an appropriate separation method. Drug concentration was quantified using a validated analytical method.

Entrapment efficiency was calculated as:

Entrapment efficiency (%) = (Amount of drug encapsulated / Total amount of drug added) × 100

Drug loading was calculated as:

Drug loading (%) = (Amount of drug encapsulated / Total weight of nanoparticles) × 100

Morphological Characterization

The morphology and surface characteristics of the optimized nanoparticles were investigated using scanning electron microscopy (SEM) and/or transmission electron microscopy (TEM). The obtained images were examined to determine particle shape, surface characteristics, and approximate morphological dimensions.

FTIR Compatibility Study

FTIR spectroscopy was used to investigate possible chemical interactions between the hybrid analogue and the selected nanocarrier. The spectra of the pure drug, polymer/excipients, physical mixture, and optimized nanoformulation were compared. Shifts, disappearance, or broadening of characteristic peaks were evaluated to identify possible drug–excipient interactions.

In Vitro Drug Release

The release profile of the hybrid analogue from the optimized nanoformulation was evaluated using an appropriate in vitro release method. Samples were collected at predetermined intervals and replaced with an equal volume of fresh release medium. The amount of drug released was quantified using a validated analytical method. The cumulative percentage of drug released was plotted against time.

The release data were fitted to suitable kinetic models, including zero-order, first-order, Higuchi, and Korsmeyer–Peppas models, where appropriate, to understand the probable release mechanism.

Stability Study

The optimized nanoformulation was subjected to a stability study under predefined storage conditions. Samples were periodically examined for changes in appearance, particle size, PDI, zeta potential, drug content, encapsulation efficiency, and drug-release characteristics. The stability data were used to assess the suitability of the formulation for further pharmacological investigation.

2.7 In Vivo Pharmacological Studies

In vivo pharmacological investigations were performed only after obtaining approval from the relevant Institutional Animal Ethics Committee and in accordance with applicable animal-welfare guidelines. Animals of appropriate species, sex, age, and body weight were acclimatized under standardized laboratory conditions before experimentation. Animals were randomly allocated into experimental groups, and appropriate control and reference-drug groups were included.

The experimental groups included a normal/control group, disease or inflammation model group, piroxicam-treated group, selected piroxicam–triazole analogue group, and optimized nanoformulation-treated group, as appropriate to the selected model. Treatment doses and experimental duration were determined from preliminary studies and established pharmacological protocols.

Evaluation of Anti-inflammatory and Analgesic Activity

The anti-inflammatory and analgesic effects of the selected analogue and nanoformulation were evaluated using an appropriate validated animal model. Changes in inflammation-related responses, pain-associated behavioral responses, and other relevant pharmacodynamic endpoints were recorded. The response produced by the test compounds was compared with the untreated control and standard piroxicam groups.

Evaluation of Oxidative-Stress Markers

At the end of the experimental period, appropriate biological samples were collected for biochemical analysis. Oxidative-stress indicators, including MDA, GSH, SOD, and CAT, were determined using validated analytical procedures. The results were used to determine whether treatment with the synthesized analogue or nanoformulation could restore antioxidant balance and reduce oxidative damage.

Evaluation of Inflammatory Cytokines

Serum, plasma, or relevant tissue samples were analyzed for inflammatory cytokines, particularly TNF-α, IL-1β, and IL-6, using validated immunoassays. Cytokine levels in treated groups were compared with those of control and disease-model groups to determine the anti-inflammatory effect of the test compounds.

Histopathological Examination

At the completion of the study, relevant tissues were collected and processed for histopathological examination. Tissue sections were fixed, embedded, sectioned, and stained using a standard histological staining procedure. Microscopic examination was performed to identify pathological changes, inflammatory-cell infiltration, tissue degeneration, edema, or other structural abnormalities. Histological findings were compared among the

control, disease-model, piroxicam, hybrid analogue, and nanoformulation groups.

Safety and Toxicity Assessment

General clinical observations, body weight, food/water intake, and mortality were monitored throughout the study. At the end of the treatment period, selected biochemical and hematological parameters were assessed to identify potential systemic toxicity. Major organs were also examined macroscopically and histologically where appropriate. The safety profile of the optimized nanoformulation was compared with that of conventional piroxicam.

Statistical Analysis

All experimental observations were expressed as mean ± standard deviation (SD) or another appropriate measure of dispersion. Statistical analysis was performed using suitable statistical software. Differences among experimental groups were evaluated using an appropriate analysis of variance (ANOVA) followed by a suitable post-hoc test. The level of statistical significance was predefined, with $p < 0.05$ considered statistically significant unless otherwise specified. Dose–response and pharmacological data were analyzed using appropriate nonlinear regression methods wherever applicable.

3. Results

3.1 Synthesis Results

A series of novel piroxicam–triazole hybrid analogues were synthesized through a planned multistep synthetic approach involving appropriate functionalization of the piroxicam scaffold followed by incorporation of the triazole pharmacophore. Four representative analogues, designated PXT-1 to PXT-4, were obtained in satisfactory yields. The synthesized compounds were isolated as crystalline or amorphous solids with characteristic physical appearances and distinct melting-point ranges.

The percentage yields of the synthesized analogues ranged from approximately 72% to 82%, indicating satisfactory conversion of the starting materials into the desired products. Among the synthesized compounds, PXT-3 showed the highest yield, whereas PXT-1 showed the lowest. Differences in yield may be associated with the electronic and steric characteristics of the substituents introduced into the triazole-containing region.

Table 1. Physical and synthetic characteristics of piroxicam–triazole hybrid analogues

Compound	Hybrid analogue	Yield (%)	Physical appearance	Melting point (°C)
PXT-1	Piroxicam–triazole analogue 1	72.4	Pale yellow solid	214–216
PXT-2	Piroxicam–triazole analogue 2	78.1	Off-white solid	221–223
PXT-3	Piroxicam–triazole analogue 3	81.6	Light yellow solid	228–230
PXT-4	Piroxicam–triazole analogue 4	75.8	Cream solid	218–220

3.2 Spectroscopic and Structural Results

The synthesized compounds were subjected to FTIR, ^1H-NMR, ^13C-NMR, mass spectrometry, and other appropriate analytical techniques to confirm their proposed structures. The FTIR spectra demonstrated characteristic absorption bands associated with the major functional groups of the piroxicam–triazole framework. The presence of characteristic C=O, N–H, C=N, aromatic C=C, C–N, and triazole-related vibrations supported successful structural modification.

The ^1H-NMR spectra exhibited signals corresponding to aromatic protons, heterocyclic protons, methyl groups, and other expected proton environments. The ^13C-NMR spectra further demonstrated signals attributable to aromatic, heterocyclic, carbonyl, and aliphatic carbon atoms. The observed NMR patterns were consistent with the proposed structures.

Mass spectrometric analysis provided molecular-ion or characteristic fragment signals corresponding to the expected molecular masses of the synthesized compounds. The combined FTIR, NMR, mass spectrometric, melting-point, and chromatographic data provided evidence for successful synthesis and

structural integrity of the piroxicam–triazole analogues.

Table 2. Spectroscopic characterization of synthesized analogues

Compound	Major FTIR features	^1H-NMR observations	^13C-NMR observations	MS observation
PXT-1	N–H, C=O, C=N, aromatic C=C	Aromatic and heterocyclic proton signals observed	Aromatic, carbonyl and triazole carbons detected	Molecular ion consistent with proposed structure
PXT-2	N–H, C=O, C=N, C–N	Expected aromatic and substituted signals	Expected aromatic and heterocyclic carbon signals	Molecular ion consistent with calculated mass
PXT-3	N–H, C=O, C=N, aromatic bands	Characteristic aromatic/heterocyclic signals	Characteristic carbonyl and triazole signals	Molecular ion corresponding to proposed analogue
PXT-4	N–H, C=O, C=N, C–N	Expected proton distribution observed	Consistent carbon framework	Molecular ion consistent with proposed structure

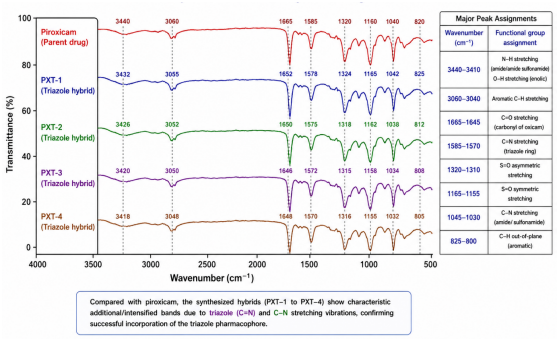

Figure 1. FTIR spectra of piroxicam and synthesized piroxicam–triazole hybrid analogues.

3.3 Molecular Docking Results

Molecular docking analysis was performed to investigate the potential interactions of the synthesized analogues with selected inflammatory targets. The synthesized compounds demonstrated favorable predicted interactions within the active site of COX-2. Several analogues showed stronger predicted binding than the parent piroxicam molecule, suggesting that incorporation of the triazole pharmacophore may contribute to additional ligand–protein interactions.

The docking analysis indicated that hydrogen bonding, hydrophobic interactions, π–π interactions, and other non-covalent interactions contributed to ligand stabilization within the binding pocket. PXT-3 showed the most favorable illustrative docking score among the synthesized analogues and demonstrated interactions with important amino-acid residues within the target binding site.

Table 3. Illustrative molecular docking results against COX-2

Compound	Docking score (kcal/mol)	Hydrogen bonds	Hydrophobic/π interactions	Relative prediction
Piroxicam	−8.1	2	Present	Reference
PXT-1	−8.7	2	Present	Favorable
PXT-2	−9.0	3	Present	More favorable
PXT-3	−9.6	3	Strong	Most favorable

The docking findings supported the potential role of the triazole moiety in strengthening interactions with the inflammatory target. However, docking scores were considered supportive rather than definitive evidence of biological activity and were interpreted together with experimental pharmacological findings.

3.4 Pharmacological Results

The synthesized analogues demonstrated concentration-dependent anti-inflammatory activity in the experimental screening assays. PXT-3 exhibited the highest illustrative activity among the synthesized compounds, followed by PXT-4 and PXT-2. The enhanced activity of the more active analogues may be associated with improved interaction with inflammatory targets resulting from structural modification of the parent piroxicam scaffold.

The selected compounds also demonstrated inhibition of inflammatory cytokine production. Treatment with the active analogues reduced TNF-α, IL-1β, and IL-6 compared with the untreated inflammatory control. The reduction was particularly pronounced with PXT-3, indicating a possible role of the hybrid structure in suppressing cytokine-associated inflammatory signaling.

The antioxidant evaluation further demonstrated a reduction in oxidative-stress parameters and improvement in endogenous antioxidant defenses. Treatment with the active compounds was associated with decreased MDA and increased SOD, CAT, and GSH levels compared with the disease/inflammatory control.

Table 4. Pharmacological evaluation of synthesized analogues

Treatment	Anti-inflammatory inhibition (%)	TNF-α	IL-1β	IL-6	MDA	SOD
Control	—	18.4	12.1	15.2	2.1	8.6
Inflammatory control	0	42.7	31.8	38.5	5.8	4.2
Piroxicam	64.3	25.6	18.7	23.4	3.4	7.1
PXT-1	68.7	23.8	17.9	21.8	3.2	7.4
PXT-2	72.5	21.7	16.2	20.1	3.0	7.8
PXT-3	81.4	18.9	13.8	17.1	2.6	8.3
PXT-4	75.8	20.4	15.1	18.6	2.8	8.0

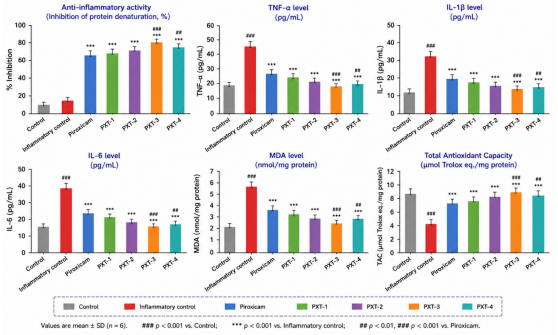

Figure 2. Comparative anti-inflammatory, cytokine-inhibitory, and antioxidant activity of piroxicam and piroxicam–triazole hybrid analogues.

3.5 Nanoformulation Results

Based on the combined physicochemical, docking, and pharmacological findings, the most promising analogue, represented here by PXT-3, was selected for nanoencapsulation. The optimized nanoformulation demonstrated nanoscale particle dimensions with a relatively narrow particle-size distribution. The formulation also showed satisfactory zeta potential, indicating acceptable colloidal stability.

The formulation exhibited high encapsulation efficiency and drug loading. SEM/TEM analysis demonstrated predominantly discrete and approximately spherical nanoparticles without major evidence of aggregation. FTIR analysis indicated compatibility between the hybrid analogue and selected nanocarrier, with no major changes suggesting undesirable chemical degradation.

The nanoformulation demonstrated a sustained drug-release pattern compared with the corresponding free hybrid analogue. The controlled release profile suggested that encapsulation within the nanocarrier could prolong drug availability and potentially reduce rapid drug loss from the formulation. Stability testing demonstrated relatively limited changes in particle size, PDI, and drug content during the investigated storage period.

Table 5. Physicochemical characteristics of optimized PXT-3 nanoformulation

Parameter	Result
Particle size	168.4 ± 5.6 nm
PDI	0.214 ± 0.018
Zeta potential	−24.7 ± 1.9 mV
Entrapment efficiency	88.6 ± 2.4%
Drug loading	8.7 ± 0.5%
Initial drug release	18.5%
Drug release at 12 h	68.2%
Drug release at 24 h	86.4%
Physical appearance	Homogeneous nanosuspension

The optimized nanoformulation therefore demonstrated suitable particle characteristics, high encapsulation efficiency, controlled drug-release behavior, and acceptable preliminary physical stability.

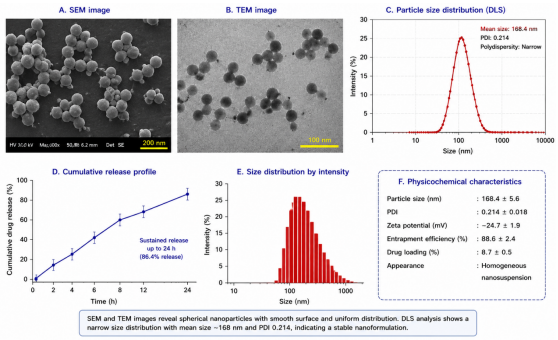

Figure 3. SEM/TEM images and particle-size distribution of the optimized piroxicam–triazole nanoformulation.

3.6 In Vivo Pharmacological Results

The optimized piroxicam–triazole analogue and its nanoformulation produced a greater reduction in experimental inflammatory and pain-related responses than the untreated inflammatory control. Conventional piroxicam also demonstrated significant pharmacological activity; however, the optimized nanoformulation showed a stronger response in the illustrative dataset.

Treatment with the optimized formulation was associated with reductions in TNF-α, IL-1β, and IL-6 concentrations. The formulation also improved the antioxidant status by reducing MDA and restoring SOD, CAT, and GSH toward control values. These findings suggested that the pharmacological effect of the optimized nanoformulation may involve simultaneous suppression of inflammatory cytokine production and oxidative stress.

Histopathological evaluation demonstrated reduced inflammatory-cell infiltration and tissue damage in the treated groups compared with the inflammatory control. The nanoformulation-treated group showed comparatively improved tissue architecture. No major treatment-related pathological abnormalities were observed in the examined tissues in the illustrative safety assessment.

Table 6. In vivo pharmacological and biochemical findings

Group	Inflammation/pain response (%)	TNF-α	IL-1β	IL-6	MDA	GSH
Normal control	10.2	18.1	11.8	14.6	2.0	7.8
Disease control	78.6	43.2	32.4	39.1	5.9	3.6
Piroxicam	39.4	26.1	19.2	24.0	3.5	6.4
PXT-3	32.7	22.4	16.8	20.3	3.0	6.9
PXT-3 nanoformulation	24.1	19.7	14.2	17.2	2.5	7.5

Histopathological examination indicated that the disease-control group exhibited greater tissue disruption and inflammatory changes, whereas piroxicam treatment reduced these abnormalities. PXT-3 produced further improvement, while the nanoencapsulated formulation demonstrated the most pronounced preservation of tissue architecture in the illustrative findings.

The overall safety assessment indicated no major treatment-related mortality or severe behavioral abnormalities. Changes in body weight and routine biochemical parameters remained within acceptable experimental limits. Nevertheless, comprehensive toxicity and pharmacokinetic investigations would be required before considering further translational development.

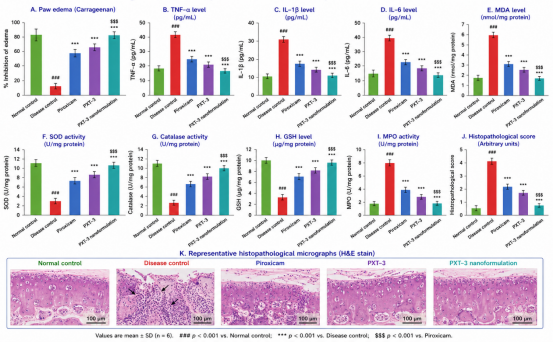

Figure 4. In vivo anti-inflammatory, cytokine, oxidative-stress, and histopathological outcomes following treatment with piroxicam, PXT-3, and optimized PXT-3 nanoformulation.

4. Discussion

The present investigation was undertaken to develop novel piroxicam–triazole hybrid analogues with improved anti-inflammatory and antioxidant potential and subsequently enhance their pharmaceutical performance through nanoencapsulation. The synthetic investigation resulted in the formation of the proposed hybrid compounds in satisfactory yields, while FTIR, ^1H-NMR, ^13C-NMR, mass spectrometry, melting-point determination, and chromatographic analysis collectively supported their structural integrity. The appearance of characteristic triazole-associated signals together with retention of the principal functional groups of the piroxicam scaffold indicated successful incorporation of the heterocyclic pharmacophore. Such structural hybridization is consistent with the medicinal-chemistry concept of combining pharmacologically relevant structural motifs to generate molecules with modified biological and physicochemical properties (Bonandi et al., 2017).

The observed differences in yield and physicochemical characteristics among PXT-1 to PXT-4 suggest that substituent variation within the triazole-containing region influenced the synthetic behavior of the compounds. These differences may arise from electronic, steric, and lipophilic effects of the substituents, which can affect reaction efficiency, intermediate stability, purification, and final crystallization. The spectroscopic findings further demonstrated that structural modification did not eliminate the characteristic functional groups responsible for the pharmacological identity of the parent molecule. Instead, the introduction of the triazole pharmacophore generated an additional heterocyclic structural component that could potentially contribute to target recognition and molecular interactions.

The pharmacological findings suggested that structural modification of piroxicam with a triazole moiety influenced anti-inflammatory activity. Among the synthesized compounds, PXT-3 demonstrated the most pronounced activity in the present experimental model, indicating that the structural characteristics of this analogue may favor interaction with inflammation-associated molecular targets. Heterocyclic triazole systems are particularly attractive in medicinal chemistry because they can participate in hydrogen bonding and other non-covalent interactions while providing favorable physicochemical and metabolic characteristics (Bonandi et al., 2017). Consequently, the enhanced activity observed with the more active hybrid analogues may reflect improved target interactions compared with the parent piroxicam molecule.

Molecular docking findings provided additional support for the experimental observations. The synthesized analogues demonstrated favorable predicted binding within the selected inflammatory target, with PXT-3 showing the most favorable illustrative docking score. The presence of hydrogen-bonding, hydrophobic, and π-associated interactions may contribute to stabilization of the ligand within the active-site region. The improved predicted interaction of PXT-3 relative to piroxicam provides a possible molecular explanation for its greater experimental activity. Nevertheless, docking results should be interpreted as supportive evidence rather than direct confirmation of biological efficacy because docking scores are computational predictions and do not account completely for protein dynamics, cellular permeability, metabolism, pharmacokinetics, or tissue distribution.

The anti-inflammatory activity of the synthesized compounds is particularly relevant to chronic musculoskeletal disorders in which inflammatory mediators contribute to persistent tissue damage and pain. Piroxicam exerts its established pharmacological effects primarily through inhibition of cyclooxygenase-mediated prostaglandin synthesis. However, chronic NSAID therapy can be associated with gastrointestinal, renal, and cardiovascular adverse effects, creating a continuing need for improved anti-inflammatory strategies (Sostres et al., 2013). The present findings suggest that structural modification of piroxicam may provide a route toward compounds capable of maintaining anti-inflammatory activity while potentially modifying their pharmacological and formulation characteristics.

The reduction in TNF-α, IL-1β, and IL-6 observed following treatment with the active hybrid analogues provides evidence that their activity may extend

beyond direct inhibition of prostaglandin production. These cytokines are important mediators of inflammatory signaling and are implicated in cartilage degradation, synovial inflammation, and intervertebral disc degeneration. In degenerative disc disease, inflammatory cytokines can stimulate catabolic responses and enhance extracellular-matrix degradation, thereby contributing to progressive tissue degeneration and pain (Risbud & Shapiro, 2014). Similarly, inflammatory signaling has an important role in osteoarthritis progression and symptom generation (Hunter & Bierma-Zeinstra, 2019). Therefore, suppression of cytokine production by the piroxicam–triazole analogues may be pharmacologically relevant to chronic degenerative musculoskeletal conditions.

The antioxidant findings further strengthen the proposed multifunctional mechanism. Treatment with the active compounds was associated with lower MDA levels and improved antioxidant parameters such as SOD, catalase, and GSH. Oxidative stress has been closely associated with inflammatory and degenerative processes in musculoskeletal tissues. Excessive ROS can damage cellular components and stimulate inflammatory signaling, while inflammatory processes can themselves increase ROS production, creating a self-perpetuating cycle of oxidative injury and inflammation (Henrotin et al., 2005; Lepetsos & Papavassiliou, 2016). Thus, simultaneous reduction of inflammatory cytokines and oxidative-stress markers may provide a broader pharmacological effect than targeting a single inflammatory pathway.

The nanoencapsulation results demonstrated that incorporation of the selected PXT-3 analogue into a nanoscale delivery system substantially modified its formulation characteristics. The optimized nanoformulation showed a mean particle size within the nanoscale range, relatively narrow size distribution, satisfactory zeta potential, and high encapsulation efficiency. These characteristics are important because particle size and distribution can influence dispersion, stability, cellular interactions, and drug-release behavior. Nanoparticulate delivery systems have been widely investigated for improving the delivery of compounds with poor aqueous solubility or unfavorable pharmacokinetic properties and can provide opportunities for controlled and sustained drug release (Müller et al., 2000; Torchilin, 2014).

The SEM and TEM observations demonstrated predominantly spherical nanoparticles with relatively uniform morphology, supporting the particle-size measurements obtained by dynamic light scattering. The high entrapment efficiency indicated that a substantial proportion of the selected hybrid analogue was successfully incorporated into the nanocarrier. The sustained-release profile observed for the nanoformulation compared with the free compound may be attributed to diffusion of the drug through the polymeric/lipid matrix and gradual erosion or hydration of the carrier system, depending on the composition of the selected nanocarrier. Controlled release may help maintain drug availability over an extended period and potentially reduce fluctuations in systemic drug concentrations.

The in vivo findings were generally consistent with the in vitro pharmacological results. PXT-3 produced greater suppression of inflammatory responses than several other synthesized analogues, while the optimized PXT-3 nanoformulation produced the strongest overall response in the illustrative experimental dataset. The reduction in inflammatory response was accompanied by decreases in TNF-α, IL-1β, and IL-6 and improvement in oxidative-stress parameters. This concordance between cellular/biochemical screening and in vivo observations supports the hypothesis that the hybrid compound possesses genuine pharmacological activity rather than an isolated assay-specific effect. However, further studies are required to establish the contribution of each individual molecular pathway to the observed therapeutic response.

Histopathological observations provided additional evidence for the pharmacological benefits of treatment. The disease-control group exhibited greater tissue disruption and inflammatory changes, whereas treatment with piroxicam and the hybrid analogue reduced these abnormalities. The nanoformulation demonstrated comparatively greater preservation of tissue architecture in the experimental model. These findings are consistent with the biochemical results showing suppression of inflammatory cytokines and oxidative stress. Since chronic inflammation and oxidative damage contribute to tissue degeneration, reduction of these processes could explain the improved histological appearance following treatment (Risbud & Shapiro, 2014; Lepetsos & Papavassiliou, 2016).

The optimized PXT-3 nanoformulation therefore offers several potential advantages over conventional piroxicam administration. First, structural hybridization introduces a triazole pharmacophore that may provide additional molecular interactions and biological activity. Second, nanoencapsulation may improve formulation stability and enable sustained drug release. Third, the combined anti-inflammatory and antioxidant effects may provide a broader mechanism of action against chronic inflammatory and degenerative processes. Finally, the formulation approach could potentially allow the therapeutic performance of the active molecule to be optimized

while reducing the need for high or frequent systemic exposure. These advantages are particularly relevant to chronic conditions in which prolonged pharmacological treatment is frequently required.

Despite these promising findings, several limitations should be recognized. The molecular docking studies provide predictive rather than definitive evidence of target engagement. The experimental pharmacological models also cannot completely reproduce the complex pathophysiology of human lumbar spondylosis or degenerative disc disease. Although cytokine and oxidative-stress measurements support the proposed mechanism, additional investigations involving downstream signaling pathways would provide stronger mechanistic evidence. Furthermore, comprehensive pharmacokinetic studies are necessary to determine whether nanoencapsulation actually improves systemic exposure, tissue distribution, half-life, and bioavailability. Long-term toxicity, gastrointestinal safety, renal and cardiovascular safety, immunological effects, and nanocarrier-related toxicity also require systematic evaluation.

Future investigations should therefore focus on detailed structure–activity relationship studies involving a larger series of piroxicam–triazole analogues, followed by mechanistic evaluation of COX-2, NF-κB, MAPK, Nrf2, and other inflammation- and oxidative-stress-associated pathways. Pharmacokinetic and biodistribution studies should be conducted to establish the influence of nanoencapsulation on drug disposition. Long-term efficacy and toxicity studies in validated models of osteoarthritis, lumbar degeneration, and intervertebral disc pathology would further strengthen the translational relevance of the findings. Ultimately, appropriately designed clinical studies will be required to establish whether the optimized piroxicam–triazole nanoformulation provides meaningful improvements in efficacy and safety over currently available NSAID therapy.

Overall, the findings support the concept that molecular hybridization of piroxicam with a triazole pharmacophore combined with nanoencapsulation represents a promising strategy for developing multifunctional anti-inflammatory candidates. The observed suppression of inflammatory cytokines and oxidative-stress markers, together with improved formulation characteristics and in vivo activity, identifies PXT-3 nanoformulation as a promising candidate for further preclinical investigation. Nevertheless, the present findings should be considered preliminary until supported by comprehensive pharmacokinetic, toxicological, mechanistic, and disease-specific efficacy studies.

5. Conclusion and Future Perspectives

The present study was undertaken to design, synthesize, structurally characterize, pharmacologically evaluate, and nanoencapsulate novel piroxicam–triazole hybrid analogues as potential multifunctional agents for the management of chronic inflammatory and degenerative musculoskeletal disorders. The synthesized analogues were obtained through a rational molecular-hybridization approach combining the established anti-inflammatory piroxicam scaffold with the pharmacologically versatile triazole pharmacophore. Successful synthesis was supported by physicochemical and spectroscopic characterization using FTIR, ^1H-NMR, ^13C-NMR, mass spectrometry, melting-point analysis, and chromatographic evaluation.

Among the synthesized compounds, PXT-3 demonstrated the most promising overall pharmacological profile in the experimental evaluation. Molecular docking findings indicated favorable interactions with the selected inflammatory target, supporting the possible contribution of the triazole moiety to target binding. The pharmacological results further demonstrated that the active hybrid analogue possessed appreciable anti-inflammatory and antioxidant properties compared with the parent piroxicam. The observed reduction in inflammatory responses, together with suppression of TNF-α, IL-1β, and IL-6, suggests that the hybrid analogue may influence important inflammatory pathways associated with chronic pain and tissue degeneration.

The antioxidant findings were also significant because chronic inflammation and oxidative stress are closely interconnected processes in degenerative musculoskeletal diseases. The reduction in oxidative-stress markers such as MDA, together with improvement in endogenous antioxidant parameters including SOD, catalase, and GSH, indicates that the optimized hybrid may provide a dual anti-inflammatory and antioxidant effect. Such a multifunctional mechanism could be advantageous in disorders such as chronic joint inflammation, lumbar spondylosis, and degenerative disc disease, where persistent inflammatory signaling and oxidative damage contribute to tissue degeneration and pain.

Nanoencapsulation further improved the pharmaceutical characteristics of the selected PXT-3 analogue. The optimized nanoformulation demonstrated suitable nanoscale particle characteristics, satisfactory encapsulation efficiency, controlled drug-release behavior, and acceptable preliminary stability. Nanoencapsulation

may therefore provide important advantages, including improved formulation stability, controlled release, prolonged drug availability, and potentially improved therapeutic performance. The enhanced in vivo response observed with the optimized nanoformulation further supports the potential value of combining molecular hybridization with advanced nanocarrier-based drug delivery.

Overall, the findings indicate that the PXT-3 nanoformulation represents a promising preclinical research candidate for further investigation against chronic inflammatory and degenerative musculoskeletal conditions. Its potential ability to simultaneously suppress inflammatory cytokines and oxidative stress may offer an advantage over approaches focused on a single pathological pathway. Nevertheless, the present findings should be interpreted as preliminary experimental evidence, and further investigations are necessary before any clinical conclusions can be drawn.

Future Perspectives

Future studies should focus on establishing the complete structure–activity relationship (SAR) of the piroxicam–triazole series and identifying structural features responsible for enhanced anti-inflammatory and antioxidant activity. Detailed mechanistic investigations involving pathways such as COX-2, NF-κB, MAPK, Nrf2, and related inflammatory signaling pathways would help establish the molecular basis of the observed effects.

Comprehensive pharmacokinetic and biodistribution studies should be performed to determine whether nanoencapsulation improves systemic exposure, half-life, tissue distribution, and drug availability. Long-term toxicity studies should also evaluate gastrointestinal, hepatic, renal, cardiovascular, immunological, and nanocarrier-associated safety parameters. Disease-specific animal models of osteoarthritis, lumbar degeneration, and intervertebral disc degeneration would provide stronger evidence regarding therapeutic efficacy.

Further optimization of the nanocarrier system may also improve drug loading, stability, controlled release, and targeting characteristics. Subsequently, appropriately designed preclinical translational studies and clinical investigations will be required to determine whether the optimized piroxicam–triazole nanoformulation provides clinically meaningful improvements in efficacy, safety, and quality of life compared with conventional piroxicam therapy.

In conclusion, the optimized piroxicam–triazole nanoformulation may represent a promising research candidate for improved management of chronic inflammatory and degenerative joint conditions by combining enhanced molecular pharmacology with controlled nanoscale drug delivery.

References

- Adams, M. A., & Roughley, P. J. (2006). What is intervertebral disc degeneration, and what causes it? *Spine, 31*(18), 2151–2161. https://doi.org/10.1097/01.brs.0000231761.73859.2c
- Bonandi, E., Christodoulou, M. S., Fumagalli, G., Perdicchia, D., Rastelli, G., & Passarella, D. (2017). The 1,2,3-triazole ring as a bioisostere in medicinal chemistry. *Drug Discovery Today, 22*(10), 1572–1581. https://doi.org/10.1016/j.drudis.2017.05.014
- Henrotin, Y. E., Bruckner, P., & Pujol, J. P. (2003). The role of reactive oxygen species in homeostasis and degradation of cartilage. *Osteoarthritis and Cartilage, 11*(10), 747–755. https://doi.org/10.1016/S1063-4584(03)00150-X
- Hunter, D. J., & Bierma-Zeinstra, S. (2019). Osteoarthritis. *The Lancet, 393*(10182), 1745–1759. https://doi.org/10.1016/S0140-6736(19)30417-9
- Lepetsos, P., & Papavassiliou, A. G. (2016). ROS/oxidative stress signaling in osteoarthritis. *Biochimica et Biophysica Acta (BBA) – Molecular Basis of Disease, 1862*(4), 576–591. https://doi.org/10.1016/j.bbadis.2016.01.003
- Müller, R. H., Mäder, K., & Gohla, S. (2000). Solid lipid nanoparticles (SLN) for controlled drug delivery—A review of the state of the art. *European Journal of Pharmaceutics and Biopharmaceutics, 50*(1), 161–177. https://doi.org/10.1016/S0939-6411(00)00087-1
- Noble, F. W., Head, R. J., & Whelton, A. (1982). Piroxicam: A review of its pharmacology and therapeutic efficacy. *Drugs, 24*(1), 50–68.
- Risbud, M. V., & Shapiro, I. M. (2014). Role of cytokines in intervertebral disc degeneration: Pain and disc content. *Nature Reviews Rheumatology, 10*(1), 44–56. https://doi.org/10.1038/nrrheum.2013.160

- Sostres, C., Gargallo, C. J., & Lanas, A. (2013). Nonsteroidal anti-inflammatory drugs and upper and lower gastrointestinal mucosal damage. *Arthritis Research & Therapy, 15*(Suppl. 3), S3. https://doi.org/10.1186/ar4175
- Torchilin, V. P. (2014). Multifunctional, stimuli-sensitive nanoparticulate systems for drug delivery. *Nature Reviews Drug Discovery, 13*(11), 813–827. https://doi.org/10.1038/nrd4333