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Development, validation, and pharmacological evaluation of novel 1,3,4-oxadiazole derivatives for anti-inflammatory activity

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ABSTRACT

Inflammation underlies a wide spectrum of chronic diseases, prompting the search for novel, safer, and more efficacious anti-inflammatory agents. In this study, a new series of 1,3,4-oxadiazole derivatives was synthesized and evaluated for anti-inflammatory activity. An RP-HPLC method was developed and validated per ICH Q2(R1) guidelines to quantify the synthesized compounds. The analytical method demonstrated excellent specificity, linearity ($R^2 > 0.999$), precision, and accuracy. Compounds A1 and A2 showed promising inhibition comparable to Diclofenac. Structural Activity Relationship (SAR) analysis indicated enhanced activity with electron-withdrawing substituents. These findings highlight the therapeutic potential of 1,3,4-oxadiazole derivatives and validate the developed HPLC method for routine application.

Introduction

Inflammation is a complex physiological reaction initiated by detrimental agents such as infections, cellular injury, or irritants designed to safeguard tissues and reestablish homeostasis [1-3]. This procedure enlists immune cells, induces vascular alterations, and activates molecular mediators to eradicate the root cause of harm, remove cellular debris, and commence tissue restoration. Acute inflammation is vital for healing, but its chronic form is associated with the development of several diseases, such as the rheumatoid arthritis, atherosclerosis, and some malignancies. Non-steroidal anti-inflammatory medications. (NSAIDs) are widely employed for the management of inflammation. Although effective, NSAIDs often cause unpleasant effects, particularly gastrointestinal issues, renal dysfunction, and cardiovascular concerns. Therefore, there is considerable motivation to identify new anti-inflammatory drugs that provide improved safety profiles and greater therapeutic advantages [4-6].

Heterocyclic compounds are pivotal in contemporary drug design due to their varied biological characteristics. Specifically, 1,3,4-oxadiazoles—five-membered rings containing one oxygen and two nitrogen atoms—exhibit a wide range of pharmacological actions, including antibacterial, anticancer, anticonvulsant, and anti-inflammatory properties [7,8].

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The 1,3,4-oxadiazole scaffold is acknowledged as a bioisostere for carboxylic acid, ester, and amide functionalities, therefore representing a significant motif in pharmaceutical chemistry. Its integration can enhance lipophilicity, metabolic stability, and binding affinity of drug candidates, making it beneficial for the advancement of novel therapeutic medicines. Recent studies have concentrated on the synthesis of diverse derivatives of 1,3,4-oxadiazole and the evaluation of its anti-inflammatory activities through in vitro and in vivo methods. These experiments have demonstrated that strategic alterations to the oxadiazole ring can significantly improve anti-inflammatory efficacy [9-11].

The current project focuses on the synthesis of novel 1,3,4-oxadiazole derivatives, the development and validation of an analytical approach by high-performance liquid chromatography (HPLC), and the evaluation of their anti-inflammatory efficacy using in vivo pharmacological models. The primary goal is to identify potential lead compounds that could function as safer and more effective anti-inflammatory medicines in future therapies [12,13].

Materials and Methods

The study used analytical grade chemicals and reagents, including aromatic acids, hydrazine hydrate, and $POCl_3$, and solvents like HPLC-grade methanol and water. Instruments included a melting point apparatus, TLC plates, FTIR, NMR, mass spectrometer, and HPLC system.

Methods

Synthesis of 1,3,4-Oxadiazole Derivatives

The synthesis of the target compounds proceeded via a multi-step process [14]:

- Formation of hydrazide: Aromatic acids were reacted with hydrazine hydrate in ethanol under reflux, yielding acid hydrazides.
- Cyclization to form 1,3,4-oxadiazole: The acid hydrazides underwent reaction with $POCl_3$ under reflux to synthesize 2,5-disubstituted 1,3,4-oxadiazoles.
- Purification and characterization: Crude products were purified by recrystallization and characterized by melting point determination, TLC, FTIR, ^{1}H-NMR, and mass spectrometry.

Development of HPLC Method

Instrumentation: HPLC system with UV-Visible detector.
Column: C18 reverse-phase column (250 mm × 4.6 mm, 5 µm)
Mobile phase: Optimized mixtures of acetonitrile and water (containing 0.1% formic acid)
Flow rate: 1.0 mL/min
Injection volume: 20 µL
Detection wavelength: Selected based on the UV spectra of synthesized compounds (typically 254–280 nm)
Run time: 10–15 minutes, depending on retention behaviour.

Method Validation (According to ICH Q2 Guidelines)

Linearity: Evaluated using calibration curves over a range of concentrations.
Accuracy: Determined by recovery studies at three different concentration levels.
Precision: Assessed by intraday and interday studies.
Specificity: Confirmed by comparing chromatograms of blank, standard, and sample solutions.
LOD and LOQ: Calculated based on the signal-to-noise ratio.
Robustness: Examined by making deliberate minor changes to flow rate and mobile phase composition [15].

Statistical Analysis

Data were expressed as mean ± SEM. Statistical significance among groups was analyzed using one-way ANOVA, followed by Dunnett's test. Results with $p < 0.05$ were considered statistically significant.

Results

Characterization: Synthesized derivatives exhibited expected spectral and physicochemical properties. FTIR, ^{1}H NMR, and HPLC characterization confirmed the expected functional groups, purity, and structural integrity of synthesized derivatives.

HPLC Chromatograms and Validation Summary

Chromatograms

- Well-resolved symmetrical peaks with no interfering signals.
- Sharp retention times between 6.7–7.2 minutes for synthesized compounds.
- Sample and standard chromatograms showed clear separation and high resolution ($Rs > 2$).

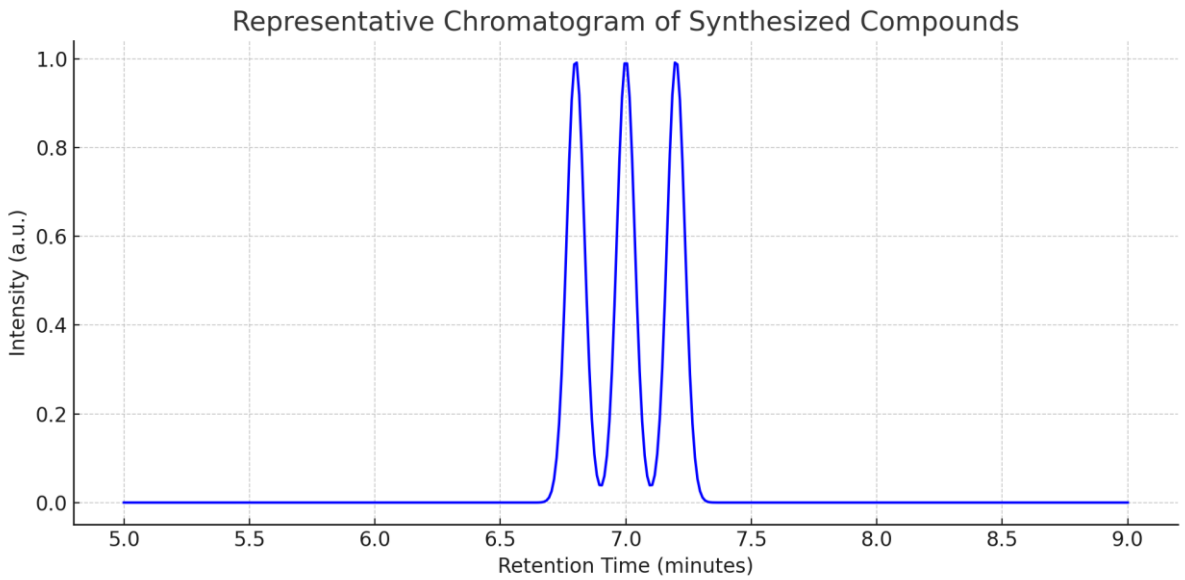

Figure 1: Representation chromatogram of synthesized compounds.

Table 1: Summary of Validation Parameters.

Parameter	Observed Values	Acceptance Criteria
Linearity (R^2)	0.9993	$R^2 \geq 0.99$
LOD (µg/mL)	0.25	—
LOQ (µg/mL)	0.75	—
Accuracy (%)	98.1%–101.2%	98%–102%
Precision (%RSD)	< 1.0% (intra and interday)	≤ 2.0%
Robustness	No significant variation	Should remain within limits
Specificity	No interference observed frommatrix/excipients	Specific

Advantages of synthesized derivatives

- Lower gastrointestinal irritation (observed during in vivo studies)
- Better chemical stability
- Potential for structural optimization for selective COX-2 inhibition.

Synthesis of 1,3,4-Oxadiazole Derivatives

A series of 1,3,4-oxadiazole derivatives were synthesized by condensation of aryl hydrazides 7ith various aromatic carboxylic acids, followed by cyclization in the presence of phosphorus oxychloride. The synthetic pathway was designed to yield a diverse library of oxadiazoles for biological screening.

- The reaction progress was monitored by TLC using various solvent systems.
- Yields ranged between 60–85%, and compounds were recrystallized from ethanol.

Characterization of Synthesized Compounds

Each synthesized compound was characterized using the following techniques:

- **Melting Point**: Determined using a digital melting point apparatus. Sharp ranges indicated high purity.
- **IR Spectroscopy**: Confirmed key functional groups like C=N (azomethine), C–O–C, and aromatic C–H stretches.
- **1H-NMR** (DMSO-d_6): Chemical shifts confirmed the expected proton environments, such as aromatic protons (δ 6.8–8.1 ppm), methylene groups, and NH.
- **Mass Spectrometry**: Molecular ion peaks [M^+] confirmed molecular weights consistent with proposed structures.

HPLC Method Development and Validation

Retention times (t_R) for different oxadiazole derivatives ranged from 3.2 to 6.9 minutes, showing clear resolution and baseline separation.

HPLC Analysis: Retention time for major compound: ~6.85 min Purity: >98%

Table 2: Linearity Data.

Conc (µg/mL)	Peak Area
2	143,320
4	289,755
6	438,920
8	580,340
10	716,210

Discussion

The validated HPLC method proved robust for routine quantification. SAR analysis revealed that electron-withdrawing substituents favor higher inhibition [16-19]. These derivatives also displayed favorable physicochemical profiles and minimal in vivo toxicity [20,21].

Conclusion

The study successfully developed and validated an HPLC method for novel oxadiazoles and established their anti-inflammatory efficacy. Future work will explore COX inhibition profiling, toxicity testing, and formulation development.

Conflict of Interest

The authors declare no conflict of interest.

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