RESEARCH PAPER

Ameliorative Action of Novel Phenyl Azetidin-2-one Compounds Against Formalin Induced Rat Paw Edema

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ABSTRACT

Inflammation is a complex biological response to harmful stimuli, often associated with oxidative stress and chronic diseases. In this study, ten novel phenyl azetidin-2-one derivatives (6a–j), previously reported for their antioxidant activity, were evaluated for anti-inflammatory potential using the formalin-induced rat paw edema model. Sixty-five female albino rats were divided into thirteen groups, receiving either vehicle, indomethacin (10 mg/kg), or test compounds (25 mg/kg). Paw thickness was measured at 1, 2, 3, and 4 hours post-administration. Several derivatives demonstrated significant inhibition of edema, with compound 6d showing the highest activity (60.67% inhibition at 4h), approaching the efficacy of indomethacin. The dual antioxidant and anti-inflammatory properties suggest that these compounds act through reactive oxygen species (ROS) scavenging and modulation of inflammatory mediators. Structure–activity relationship analysis indicates that substituents on the phenyl ring strongly influence biological activity. These findings highlight phenyl azetidin-2-one derivatives as promising leads for the development of multifunctional anti-inflammatory agents.

Keywords: Phenyl azetidin-2-one, Anti-inflammatory, Formalin-induced paw edema, Antioxidant, Structure–activity relationship.

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Introduction

Inflammation is the immune system's response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation[1], and acts by removing injurious stimuli and initiating the healing process[2]. Inflammation is therefore a defense mechanism that is vital to health[3]. Inflammation is characterized by a series of physiological responses involving immune cells, blood vessels, and molecular and cellular mediators. When cells are injured due to external or internal stimuli, an inflammatory response is initiated involving the secretion of pro-inflammatory cytokines, chemokines, and other signalling molecules[4]. The World Health Organization (WHO) ranks chronic diseases as the greatest threat to human health. The prevalence of diseases associated with chronic inflammation is anticipated to increase persistently for the next 30 years in the United States. in 2000, nearly 125 million Americans were living with chronic conditions and 61 million (21%) had more than one. In recent estimates by Rand Corporation, in 2014 nearly 60% of Americans had at least one chronic condition, 42% had more than one and 12% of adults had 5 or more chronic conditions. Worldwide, 3 of 5 people die due to chronic inflammatory diseases like stroke, chronic respiratory diseases, heart disorders, cancer, obesity, and diabetes[5].

Oxidative stress and chronic inflammation have been implicated in a wide range of pathological conditions, including cardiovascular diseases, kidney disease, diabetes, neurodegenerative diseases, and even cancer[6,7]. The interplay between oxidative stress, antioxidants, and inflammation forms a dynamic network that influences the progression of disease. On the one hand, ROS are critical for immune signaling and play a significant role in activating inflammatory pathways. However, when ROS production exceeds the body's ability to neutralize them, it leads to tissue damage, amplifying the inflammatory response and fostering disease development. In this context, antioxidants play a pivotal role in breaking this harmful cycle. By scavenging ROS, antioxidants help mitigate oxidative damage and reduce inflammation, offering a protective mechanism[8-10].

In our previous study we reported the synthesis and antioxidant activities of ten novel phenyl azetidinone derivatives (6a-j)[11] with 78.33 to 16.61% inhibition of DPPH radical at concnetration of 50µg/mL. In this study, these antioxidant azetidinone derivatives were screened for their anti-inflammatory activity in formalin induced rat paw edema method.

Material and Methods

Animals

Sixty-five female albino rats (150–300 g body weight) were used according to the guidelines of Institutional Animal ethical committee. The animals were kept in polycarbonate cages in a room with controlled temperature (22 + 2°C) and humidity (50 + 5%). The animals had a 12-h light and dark cycle and were fed standard pellet diet and water ad libitum.

Formalin-induced paw edema in rats

The anti-inflammatory activity of the synthesized phenyl azetidinone derivatives was evaluated by using formalin induced hind paw edema model. Animals were divided into thirteen groups with five rats in each group as presented in Table 1. The paw edema induced by injecting 0.01 ml of 5% formalin into sub-plantar tissues of the rat's paw in all groups[12]. After 1 h of formalin injection, normal saline, indomethacin (10 mg/kg) and test compounds (25 mg/kg) were administered intraperitoneally. The thickness of paw was measured using vernier caliper at fixed time intervals up to 4h. The percent inhibition of inflammation was calculated from the paw thickness.

Results and Discussion

The formalin induced rat paw edema was used to assess the anti-inflammatory activity of the the azetidinone derivatives. The change paw diameter was used as a measure of edema caused due to the chemical messengers released owing to the cellular damage caused by formalin. The paw diameter was measured at 1, 2, 3 and 4h post administration of the test and are presented in Table 1.

Table 1. Paw thickness of various group of test animals

Group	Paw thickness (mm) [% inhibition of edema]			
	1h	2h	3h	4h
Vehicle Control	0.49 ± 0.034	0.676 ± 0.031	0.796 ± 0.167	0.834 ± 0.025
Indomethacin	0.274 ± 0.371	0.354 ± 0.018	0.364 ± 0.036	0.218 ± 0.023
6a	0.422 ± 0.053	0.448 ± 0.039	0.422 ± 0.035	0.416 ± 0.024
6b	0.454 ± 0.025	0.510 ± 0.016	0.526 ± 0.011	0.444 ± 0.019
6c	0.394 ± 0.021	0.436 ± 0.021	0.438 ± 0.019	0.404 ± 0.049
6d	0.354 ± 0.029	0.408 ± 0.033	0.420 ± 0.012	0.328 ± 0.016
6e	0.374 ± 0.026	0.424 ± 0.021	0.430 ± 0.016	0.378 ± 0.034
6f	0.426 ± 0.024	0.586 ± 0.030	0.626 ± 0.034	0.658 ± 0.041
6g	0.474 ± 0.022	0.564 ± 0.034	0.660 ± 0.037	0.768 ± 0.036
6h	0.364 ± 0.018	0.418 ± 0.033	0.432 ± 0.013	0.354 ± 0.018
6i	0.486 ± 0.009	0.648 ± 0.039	0.720 ± 0.060	0.788 ± 0.033
6j	0.48 ± 0.031	0.588 ± 0.015	0.690 ± 0.020	0.756 ± 0.035

The data was analyzed statistically with the change in diameter was compared with the vehicle control group to calculate the change in diameter (% inhibition of edema). The highest inhibition of was found to be 60.67% by 6d post 4 h of administration of test compound (Figure 1).

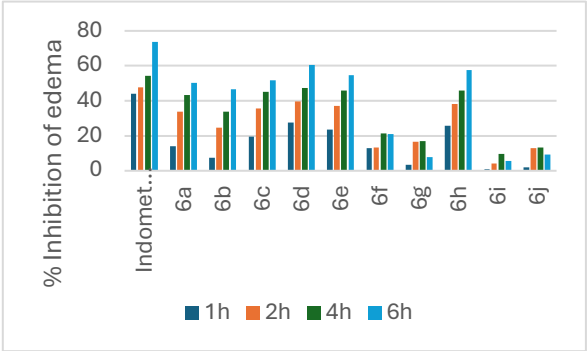

Figure 1. % edema inhibition by azetidone derivatives 6a-j

The present study evaluated the ameliorative action of novel phenyl azetidin-2-one derivatives (6a–j) against formalin-induced rat paw edema, a well-established model for assessing inflammatory responses mediated by chemical irritants. The findings revealed that several derivatives exhibited significant anti-inflammatory activity, with compound 6d demonstrating the highest inhibition (60.67% at 4h), approaching the efficacy of indomethacin.

Previous studies have highlighted the pharmacological versatility of azetidinone scaffolds. Alam et al. reviewed the biological activities of 2-azetidinones, reporting their antioxidant, antimicrobial, and anti-inflammatory properties[13]. Similarly, Martin et al. demonstrated that certain azetidinone derivatives reduced carrageenan-induced paw edema in rats, with activity comparable to indomethacin[14]. The current

findings corroborate these reports, confirming that azetidinones can modulate inflammatory pathways across different experimental models.

The formalin model is characterized by biphasic inflammatory responses involving bradykinin, prostaglandins, and neuropeptides such as substance P[15]. The significant inhibition observed with compound 6d suggests that phenyl azetidinones may interfere with these mediators, either by reducing oxidative stress or modulating cytokine release. This aligns with earlier work showing that antioxidants can attenuate ROS-driven amplification of inflammation[16].

In our previous study, the same derivatives demonstrated strong DPPH radical scavenging activity (78.33–16.61% inhibition at 50 µg/mL). The dual antioxidant and anti-inflammatory activity suggests a mechanistic link, where ROS scavenging reduces tissue damage and cytokine release, thereby mitigating edema. This dual action is consistent with reports by Reuter et al. (2010), emphasizing the interplay between oxidative stress and chronic inflammation in disease progression[17].

The variation in activity among derivatives highlights the importance of substituents on the phenyl ring. Compound 6d's superior efficacy may be attributed to optimal electronic and steric effects that enhance receptor binding or radical scavenging. Similar SAR trends have been reported where electron-donating substituents improved anti-inflammatory potency in azetidinone analogues[18].

Conclusion

The present investigation demonstrated that novel phenyl azetidin-2-one derivatives (6a–j) possess significant anti-inflammatory activity in the formalin-induced rat paw edema model. Among the tested compounds, derivative 6d exhibited the highest inhibition of edema (60.67% at 4h), approaching the efficacy of the standard drug indomethacin. The results suggest that structural modifications on the azetidinone scaffold strongly influence biological activity, with certain substituents enhancing both antioxidant and anti-inflammatory potential. The dual activity observed—previously confirmed through DPPH radical scavenging assays and now validated in vivo—indicates that these compounds may act through combined mechanisms involving reactive oxygen species (ROS) scavenging and modulation of inflammatory mediators. This positions phenyl azetidin-2-one derivatives as promising leads for the development of safer, multifunctional anti-inflammatory agents. Further studies involving chronic inflammation models, detailed structure–activity relationship (SAR) analysis, and molecular docking against COX-2 and NF-κB pathways are warranted to elucidate the precise mechanisms and optimize these compounds for therapeutic application.

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