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In Vivo Evaluation of Anti-Inflammatory Activity of Quinoxaline Derivatives Using Carrageenan-Induced Rat Paw Edema Model

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Abstract

Paw swelling induced by carrageenan injection serves as a reliable model for assessing acute inflammatory responses in rats. In this study, we synthesized and evaluated a series of quinoxaline derivatives for their anti-inflammatory activity using the carrageenan-induced rat hind paw edema method. The compounds 5a, 5e, 5f, 5g, 5h, 5l, 5q, and 5u were selected based on their inhibitory activity against p38α MAP kinase. Among these, compound 5f, which possesses a 2-chlorophenyl group at position 4 of the triazole ring, demonstrated the highest inhibition of paw edema, with an 84.15% reduction, comparable to the standard drug diclofenac sodium (83.22%). The structure-activity relationship revealed that the presence of a chloro group at the 6th position of the quinoxaline ring led to reduced anti-inflammatory activity. This study highlights the potential of quinoxaline derivatives as effective anti-inflammatory agents.

Keywords: Quinoxaline derivatives, Carrageenan-induced edema, Anti-inflammatory activity, p38α MAP kinase inhibition, Rat paw edema model, Structure-activity relationship

Introduction

Paw swelling, or footpad edema, is a convenient method for assessing inflammatory responses to antigenic challenges and irritants. Typically, test compounds are assessed for acute anti-inflammatory activity by examining their ability to reduce or prevent the development of carrageenan-induced paw swelling [1]. This model has long been used to assess the anti-inflammatory properties of agents such as nonsteroidal anti-inflammatory drugs (NSAIDs) that inhibit prostaglandin production [2].

Carrageenan, from the Irish word "carraigin" meaning Irish moss, refers not only to a species of red alga *Chondrus crispus* found along rocky areas of the Atlantic coast of the British Isles, Europe, and North America, but also refers to its mucopolysaccharide extract, discovered by the British pharmacist Stanford in 1862 [3]. The name was later changed to carrageenan to comply with the "−an" suffix for polysaccharides [4]. Structurally, the carrageenans are a complex group of polysaccharides made up of repeating galactose-related monomers and are of three main types: lambda, kappa, and iota [5]. Each has its own gel characteristics, all of which are thermally reversible [6]. The lambda form does not gel strongly at room temperature and is injectable to induce an inflammatory response [7].

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Inflammation induced by carrageenan, originally described by Winter [8], is acute, non-immune, well-researched, and highly reproducible [9]. Cardinal signs of inflammation—edema, hyperalgesia, and erythema—develop immediately following subcutaneous injection, resulting from the action of proinflammatory agents such as bradykinin, histamine, tachykinins, complement, and reactive oxygen and nitrogen species [10].

Material and Methods

Materials

Reagents and Solvents

All the reagents and solvents were of laboratory grade and were procured from Merck (Darmstadt, Germany) and S.D. Fine chemicals (Delhi, India).

Experimental Technique

Melting point: Melting points were recorded in open capillaries using Labtronics Digital Auto Melting Point Apparatus (Haryana, India) and are uncorrected.

IR spectrometer: IR spectra were recorded on Perkin-Elmer 1720 FTIR spectrometer (New York, USA).

NMR spectrometer: 1H NMR spectra (400 MHz) and 13C-NMR spectra (100 MHz) were obtained on Bruker Avance- instrument (Zurich, Switzerland) with complete proton decoupling. Chemical shifts were reported in ppm downfield from tetramethylsilane (TMS) as the internal standard.

Mass spectrometer: Mass spectra were recorded on Jeol SX-102/DA-6000 (Tokyo, Japan) spectrometer.

Thin Layer Chromatography: Purity of the compounds was checked by TLC using precoated aluminium TLC plates (Merck) and spots were visualized in a UV/Visible chamber (UV 254nm).

Elemental analysis: Elemental analysis (C, H and N) were conducted using a CHNS Vario EL III (ElementarAnalysensysteme GmbH, Germany) and the results are within ± 0.4 % of theoretical values.

Experimental

The Quinoxaline derivatives were synthesized in laboratory as per the established protocol and were evaluated for their anti-inflammatory activity using the carrageenan-induced rat hind paw edema method.

Animals: Wistar rats of either sex, weighing 150-200 g.

Instrument: Digital Plethysmometer (PLM-01 Plus).

Standard: Diclofenac sodium at an oral dose of 10 mg/kg.

Test Compounds: Administered at an equimolar oral dose relative to 10 mg/kg diclofenac sodium.

Method:

- The animals were randomly allocated into groups of six animals each. They were fasted for 24 hours before the experiment, with free access to water.
- The control group received only a 0.5% CMC solution.
- The standard group received the standard drug, diclofenac sodium, while the test groups received the synthesized compounds.
- One hour after the administration of the test compounds and the standard drug, 0.1 cm^3 of 1% carrageenan solution in saline was injected subcutaneously into the subplantar region of the right hind paw of each rat.
- The right hind paw volume was measured before and 4 hours after carrageenan treatment using a digital plethysmometer.
- The percentage of edema inhibition was calculated from the mean effect in the control and treated animals using the following equation:

$$\textbf{Percent edema inhibition} = [(Vc - Vt) / Vc] \times 100$$

where Vt represents the mean increase in paw volume in rats treated with the test compounds, and Vc represents the mean increase in paw volume in the control group of rats.

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Table 1: In-vivo anti-inflammatory activity of selected quinoxaline derivatives (5a,5e,5f, 5g, 5h, 5l, 5q, 5u) and standard drug

Compound	Body Weight	Initial paw volume	Paw volume after 4 h	Increase in paw volume	Mean	Std Dev	SEM	% Inhibition	Activity relative to standard
	195	0.44	0.59	0.15					
	176	0.39	0.64	0.25					
	182	0.45	0.62	0.17					
5a					0.187	0.051	0.021	73.89	88.80
	165	0.41	0.54	0.13					
	163	0.38	0.55	0.17					
	180	0.41	0.66	0.25					
	175	0.49	0.64	0.15					
	156	0.37	0.49	0.12					
	168	0.44	0.56	0.12					
5e					0.118	0.021	0.009	83.45	100.28
	190	0.48	0.57	0.09					
	181	0.39	0.52	0.13					
	178	0.42	0.52	0.1					
	178	0.45	0.59	0.14					
	180	0.42	0.59	0.17					
	191	0.42	0.53	0.11					
5f					0.113	0.039	0.016	84.15	101.12
	200	0.44	0.51	0.07					
	165	0.39	0.46	0.07					
	155	0.40	0.52	0.12					
	158	0.41	0.6	0.19					
	165	0.44	0.62	0.18					
5g					0.145	0.038	0.016	79.72	95.80
	181	0.45	0.58	0.13					
	163	0.41	0.57	0.16					
	200	0.47	0.59	0.12					

	170	0.38	0.47	0.09					
	155	0.41	0.59	0.18					
	197	0.32	0.52	0.2					
5h	167	0.41	0.55	0.14					
					0.177	0.046	0.019	75.29	90.48
	181	0.37	0.49	0.12					
	154	0.38	0.55	0.17					
	170	0.41	0.66	0.25					
	195	0.48	0.59	0.11					
	176	0.45	0.61	0.16					
	182	0.42	0.67	0.25					
5l					0.217	0.077	0.032	69.70	83.75
	165	0.43	0.75	0.32					
	163	0.41	0.68	0.27					
	180	0.38	0.57	0.19					
	181	0.43	0.64	0.21					
	165	0.41	0.7	0.29					
	173	0.45	0.65	0.2					
5q					0.232	0.040	0.016	67.60	81.23
	198	0.46	0.73	0.27					
	165	0.39	0.58	0.19					

	200	0.44	0.67	0.23					
	167	0.4	0.61	0.21					
	183	0.44	0.72	0.28					
	190	0.45	0.71	0.26					
5u					0.272	0.048	0.020	62.00	74.51
	175	0.39	0.62	0.23					
	181	0.41	0.73	0.32					
	200	0.46	0.79	0.33					
Control	173	0.42	1.18	0.76	0.715	0.117	0.048	-	-
	162	0.39	0.98	0.59					
	170	0.31	1.2	0.89					
	180	0.32	0.94	0.62					
	165	0.36	1.15	0.79					
	165	0.32	0.96	0.64					
	192	0.43	0.54	0.11					
	175	0.42	0.51	0.09					
Diclofenac sodium	167 200	0.45 0.47	0.55 0.58	0.1 0.11	0.120	0.032	0.013	83.22	100
	181	0.38	0.56	0.18					
	165	0.36	0.49	0.13					

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Results and Discussion

The anti-inflammatory activity of the selected compounds (**5a**, **5e**, **5f**, **5g**, **5h**, **5l**, **5q** and **5u**) which showed good p38α MAP kinase inhibitory activity was evaluated by the carrageenan inducedpawedemamethod.Thetestedcompoundssh owedanti-inflammatoryactivityranging from 62.00 to 84.15% (**Table 2**) whereas standard drug diclofenac sodium showed 83.22% inhibition after 4 h. Compound **5f** possessing the 2-chlorophenyl group attached to position 4 of the triazole ring emerged as the most potent compound of the series with 84.15
% inhibition. On replacement of 2-chlorophenyl with 2-fluorophenyl group (**5e**) there was a slight decrease in activity (83.45%). Activity was further decreased when these groups were replaced by 2-bromo (**5g**) and 4-nitrophenyl groups (**5h**) (79.72 and 75.29% respectively). Compound**5a**withunsubstitutedphenylgroupshowe d73.89%activity.Thecompoundswith chloro group at the 6thposition of quinoxaline ring (**5l, 5q and 5u)** showed reduced anti- inflammatory activity (69.70%, 67.60% and 62.00% respectively) in comparison to unsubstitutedquinoxalinering.Amongthesecompou nds,**5q**havingthe2-chlorophenylgroup in position 4 of the triazole ring showed higher activity (67.60%) than **5u** having the 2-methoxyphenylgroupinposition4ofthetriazolering(62.00%).Itwasnotedthatasthesizeofhalogenatomin creases,anti-inflammatoryactivitydecreasesexceptincompound **5f**having 2-chlorogroupwheretheactivityslightlyincreases.Furt hermore,compoundshavingelectron withdrawing groups were found to be more active than electron donating groups.

Table 2: In vivo anti-inflammatory activity of selected quinoxaline derivatives (5a, 5e, 5f, 5g, 5h, 5l, 5q, 5u) and standard drug

Compounds	%inhibition
5a	73.89
5e	83.45
5f	84.15
5g	79.72
5h	75.29
5l	69.70
5q	67.60
5u	62.00
Diclofenac sodium	83.22

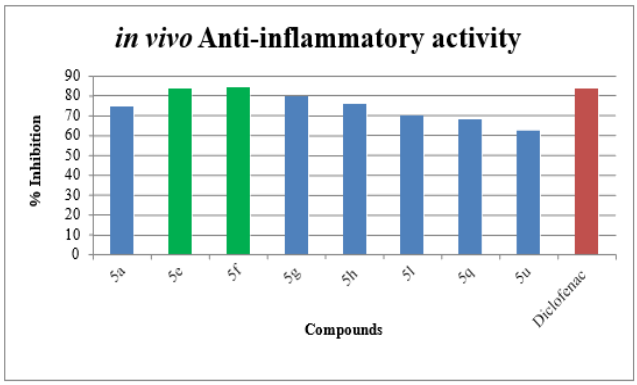

Fig. 1. Graphical representation of *in vivo* anti-inflammatory activity of quinoxaline derivatives

Conclusion

This study demonstrated the potent anti-inflammatory activity of quinoxaline derivatives in the carrageenan-induced rat paw edema model. Among the tested compounds, 5f emerged as the most effective, exhibiting an inhibition rate of 84.15%, which is comparable to the standard drug diclofenac sodium. The SAR analysis revealed that the presence of a 2-chlorophenyl group at position 4 of the triazole ring significantly enhances the anti-inflammatory activity, while substitution at the 6th position of the quinoxaline ring with a chloro group reduces the activity. These findings suggest that quinoxaline derivatives hold promise as potential anti-inflammatory agents and warrant further investigation for their therapeutic applications.

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