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*Proceeding Paper*

# Benzo[*a*]phenoxazines as Potential Anti-Inflammatory Drugs †

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**Abstract:** The chemical diversity of natural products provides a promising source of anti-inflammatory drugs, notably targeting COX-2, whose activity is associated with inflammatory processes and carcinogenesis. The COX-2 and LOX inhibitory capacity of several benzo[*a*]phenoxazines, *N*-, *O*-heterocyclic compounds was studied. Kinetic assay results revealed that these molecules significantly inhibit COX-2 activity, highlighting their promise as potent anti-inflammatory agents.

**Keywords:** Benzo[*a*]phenoxazines; anti-inflammatory agents; COX-2; LOX enzymes

## 1. Introduction

Inflammation is a complex and essential biological response to harmful stimuli, such as pathogens, damaged cells, or irritants. It is designed to eliminate the initial cause of cell injury, clear out damaged cells and tissues, and initiate tissue repair. While acute inflammation is beneficial and crucial for healing, chronic inflammation can contribute to the development and progression of several chronic diseases, including rheumatoid arthritis, cardiovascular diseases, and cancer. The chronic inflammatory state involves a prolonged immune response that can lead to tissue damage and various pathologies [1,2].

Two critical enzymes involved in the inflammatory process are cyclooxygenases (COX) and lipoxygenases (LOX). COX-2, an inducible isoform of cyclooxygenase, plays a significant role in the synthesis of prostaglandins, which mediate inflammation, pain, and fever. Its expression is often upregulated in response to inflammatory stimuli and has been linked to carcinogenesis. On the other hand, LOX enzymes are involved in the metabolism of arachidonic acid to leukotrienes, which are potent inflammatory mediators. Both COX-2 and LOX are therefore important targets for anti-inflammatory drug development, as their selective inhibition can mitigate the inflammatory response with potentially fewer side effects compared to non-selective inhibitors [3–5].

Natural products have long been a rich source of pharmacologically active compounds, providing a diverse chemical library for drug discovery. Among these, benzo[*a*]phenoxazines, *N*-,*O*-heterocyclic compounds, have garnered attention for their notable therapeutic potential [6,7]. These compounds have demonstrated significant anti-inflammatory and anti-cancer properties, largely due to their ability to inhibit COX-2 enzymatic activity. This inhibition can reduce the production of pro-inflammatory prostaglandins, thereby alleviating inflammation and its associated symptoms [8–10].

Considering these facts, the present study explores the anti-inflammatory properties of various benzo[*a*]phenoxazines from our library specifically focusing on their potential as novel inhibitors of COX-2 and LOX enzymes. By evaluating the activity of these enzymes, we aim to elucidate the efficacy of benzo[*a*]phenoxazines in reducing inflammatory pathways. The results could pave the way for the development of new anti-

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inflammatory drugs that offer enhanced efficacy and safety, potentially benefiting patients with chronic inflammatory conditions and related diseases [11–13].

## 2. Results and Discussion

The series of benzo[*a*]phenoxazine derivatives shown in Figure 1, previously synthesized by our research group [14,15], was evaluated for their enzyme inhibition properties.

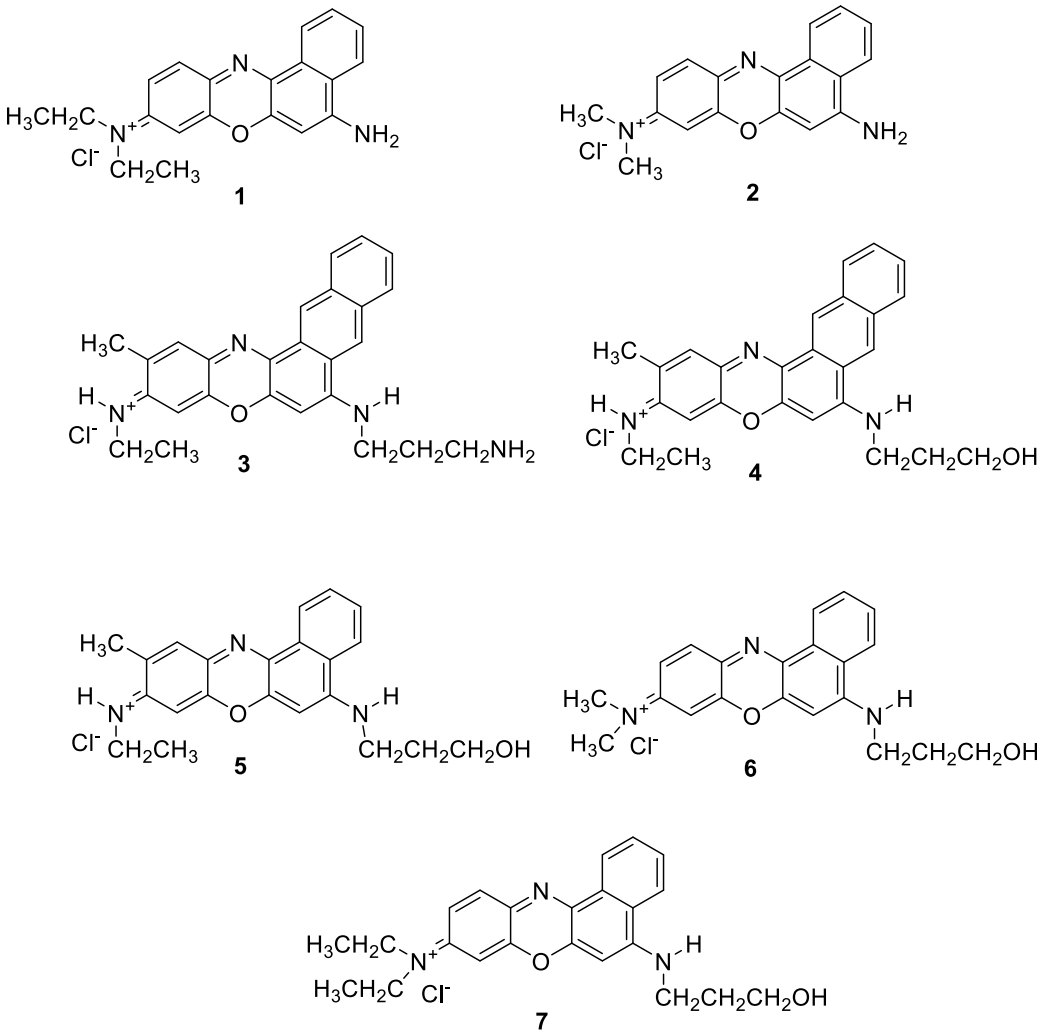


**Figure 1.** Structures of the benzo[a]phenoxazine derivatives **1–7** used in this study.

### *2.1. LOX Inhibition*

The evaluation of benzo[*a*]phenoxazines in terms of their potential as LOX inhibitors revealed results that were not statistically significant. This fact suggests that these compounds do not effectively inhibit LOX activity (Figure 2).

The lack of statistical significance implies that no substantial differences in LOX activity were observed between the groups treated with the compounds and the control groups. Therefore, we can infer that the compounds studied do not have a relevant impact on LOX inhibition.

In this study, quercetin was used as a positive control because of its specific ability to inhibit LOX. Quercetin, a flavonoid, has a structure rich in hydroxyl groups and a conformation that allows it to act as an iron chelator in the LOX active site, blocking the enzyme's catalytic activity. In addition, its antioxidant properties are also protective for this prevention, as they help to neutralize the free radicals generated during the process.

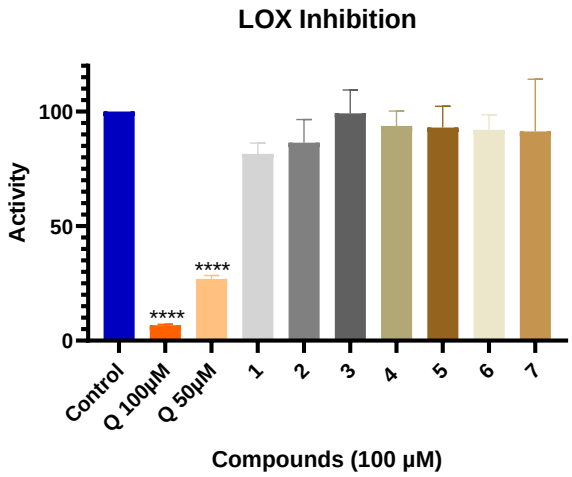


**Figure 2.** LOX inhibition in compounds **1–7** at 100 µM. Q 100 µM and Q 50 µM refer to quercetin, used as positive control, at concentrations of 100 µM and 50 µM, respectively. **** $p < 0.0001$.

The lack of significant inhibition by benzo[*a*]phenoxazines **1–7** may be related to the absence of structural characteristics that are essential for effective interaction with the LOX active site. Unlike quercetin, which binds to iron and interferes with the catalytic process, benzo[*a*]phenoxazines **1–7** may not have the functional groups or conformation needed to compete with the enzyme's substrates, which could explain their inefficiency in inhibiting LOX.

### *2.2. COX-2 Inhibition*

The benzo[*a*]phenoxazines tested at a concentration of 100 µM significantly inhibited COX-2 enzymatic activity. Kinetic assays showed a marked reduction in COX-2 activity with the presence of these compounds, indicating their strong inhibitory potential.

The significant inhibition of COX-2 by benzo[*a*]phenoxazines **1–7** at 100 µM demonstrates their potential as effective anti-inflammatory agents. COX-2 is a crucial enzyme in the inflammatory response, responsible for the production of pro-inflammatory prostaglandins. The ability of benzo[*a*]phenoxazines to markedly reduce COX-2 activity suggests they could play a valuable role in managing inflammation (Figure 3).

The structural properties of benzo[*a*]phenoxazines **1–7** may contribute to their effectiveness in inhibiting COX-2. The robust inhibition observed in this study supports further exploration of these compounds for therapeutic use in inflammatory conditions. The findings highlight the promise of benzo[*a*]phenoxazines as potent COX-2 inhibitors, providing a strong foundation for future research and development.

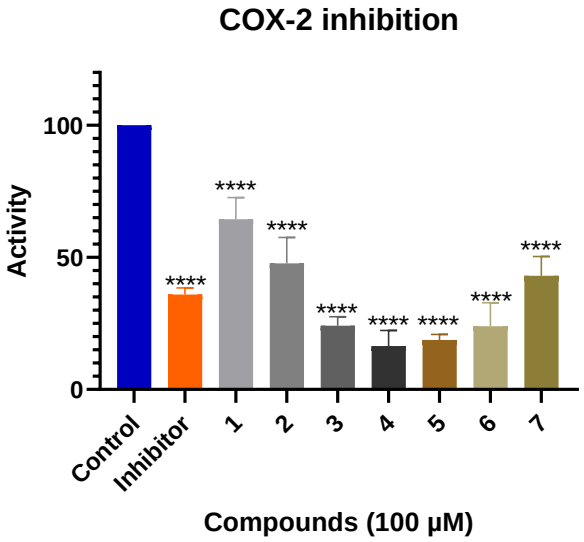


**Figure 3.** COX-2 inhibition in compounds **1–7** at 100 μM. **** $p < 0.0001$.

Given the strong inhibitory effects observed, compound **4**, one of the most promising, was tested at five lower concentrations (50 μM, 25 μM, 12.5 μM, 6.25 μM and 3.13 μM). Even at these reduced concentrations, significant inhibition of COX-2 activity was maintained, demonstrating the potency of this compound (Figure 4).

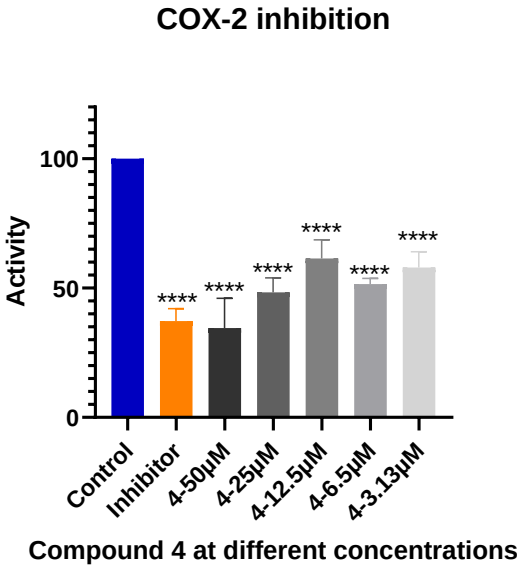


**Figure 4.** Inhibition of COX-2 at lower concentrations of compound **4**. **** $p < 0.0001$.

The consistent and significant inhibition of COX-2 by all benzo[*a*]phenoxazines at 100 μM highlights their potential as potent anti-inflammatory agents. The robust inhibition observed with these compounds suggests a strong interaction with the COX-2 active site.

Remarkably, compound **4** continued to show significant inhibition of COX-2 activity at five reduced concentrations, which indicates that this benzo[*a*]phenoxazine is a highly potent COX-2 inhibitor, capable of achieving substantial inhibition even at lower doses.

These results underline the potential of benzo[*a*]phenoxazines **1–7** as effective COX-2 inhibitors, with the ability to maintain their anti-inflammatory effects over a range of concentrations. This potency makes them promising candidates for further development and optimization in the treatment of inflammatory conditions.

## 3. Experimental

### *3.1. Typical Procedure for the LOX Inhibition*

The assay to determine lipoxygenase (LOX) activity was based on the oxidation of linoleic acid, the enzyme's natural substrate. The reaction was monitored spectrophotometrically, following the formation of conjugated hydroperoxides, which exhibit an absorption peak at 234 nm. The procedure was carried out at 25 °C in a sodium phosphate buffer solution (pH 9.0).

In the wells of a UV microplate, the sample (20 μL) was added. In the control wells, buffer (20 μL) was added. Then, phosphate buffer (pH 9.0) (20 μL) was added to all wells. Subsequently, the enzyme (20 μL), previously dissolved in phosphate buffer, was added to the respective wells. The microplate was incubated at room temperature for 5 min. After incubation, linoleic acid (20 μL) was added to each well, and the readings were taken immediately after.

### *3.2. Typical Procedure for the COX Inhibition*

The test to evaluate the inhibitory activity of the enzyme cyclooxygenase-2 (COX-2) was carried out with the aim of determining the effectiveness of compounds in inhibiting the action of this enzyme, which is involved in the inflammatory process. The colorimetric method used was based on quantifying the production of prostaglandins, one of the products of the reaction catalyzed by COX-2.

To carry out the test, samples of the compounds were prepared and added to an enzyme system containing COX-2 and arachidonic acid as a substrate. The reaction was incubated at 37 °C and the production of prostaglandins was monitored using a colorimetric method, with the absorbance read on a spectrophotometer at 535/587 nm. The decrease in prostaglandin production compared to the control indicated the degree of inhibition of COX-2 activity by the compounds tested.

In each well of the plate, arachidonic acid (5 μL) and the sample (5 μL) were added, except for the control, which received buffer (5 μL). Prior to the reading, master mix (40 μL) was added to each well. The master mix contained buffer (38 μL), enzyme (0.5 μL), cofactor (1 μL), and probe (0.5 μL) per well.

## 4. Conclusions

The benzo[*a*]phenoxazines **1–7** tested did not show significant inhibition of LOX, as evidenced by the absence of statistical significance in the results. In contrast, they demonstrated significant inhibition of COX-2. This persistent inhibition of COX-2 even at low concentrations underlines the therapeutic potential of the benzo[*a*]phenoxazines **1–7** as effective anti-inflammatory agents. Significant inhibition of this enzyme is particularly relevant as it plays a crucial role in mediating inflammatory processes and in the production of prostaglandins, which are associated with pain and inflammation. These results suggest that the benzo[*a*]phenoxazines tested have promising applicability in the development of new anti-inflammatory treatments.

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## References


1. Atanasov, A.G.; Zotchev, S.B.; Dirsch, V.M.; Stürtz, A.R.; Maier, M.; Graham, D.L.; Hirz, M.; Reichelt, M.; Mack, M.; König, W.; et al. Natural products in drug discovery: Advances and opportunities. *Nat. Rev. Drug Discov.* **2021**, *20*, 200–216.
2. Ekiert, H.M.; Szopa, A. Biological activities of natural products. *Molecules* **2020**, *25*, 5769.
3. Mashima, R.; Okuyama, T. The role of lipoxygenases in pathophysiology; new insights and future perspectives. *Redox Biol.* **2015**, *6*, 297–310.
4. Hajdú, I.; Kardos, J.; Major, B.; Fabó, G.; Lőrincz, Z.; Cseh, S.; Dormán, G. Inhibition of the LOX enzyme family members with old and new ligands. Selectivity analysis revisited. *Bioorganic Med. Chem. Lett.* **2018**, *28*, 3113–3118.
5. Smith, W.L.; Langenbach, R. Why there are two cyclooxygenase isozymes. *J. Clin. Invest.* **2001**, *107*, 1491–1495.
6. Martinez, V.; Henary, M. Nile Red and Nile Blue: Applications and Syntheses of Structural Analogues. *Chem. Eur. J.* **2016**, *22*, 13764–13782.
7. Jose, J.; Ueno, Y.; Burgess, K. Water-soluble Nile Blue derivatives: Syntheses and photophysical properties. *Chemistry* **2009**, *5*, 418–423.
8. Odin, E.M.; Onojah, P.K.; Akpanisi, L.E.S.; Akabueze, B.O. Synthesis of Pent-aza Phenoxazines: A New Group of Anti-inflammatory Heterocycles. Their Effect and Other Analgesics on Pain. *ACSJ* **2016**, *16*, 1–12.
9. Katsamakas, S.; Zografos, A.L.; Vasiliki, S. Advances of Phenoxazines: Synthesis, Reactivity and Their Medicinal Applications. *Curr. Med. Chem.* **2016**, *23*, 2972–2999.
10. Shukla, S.; Dwivedi, J.; Yaduvanshi, N.; Jain, S. Medicinal and Biological Significance of Phenoxazine Derivatives. *Mini Rev. Med. Chem.* **2021**, *21*, 1541–1555.
11. FitzGerald, G.A.; Patrono, C. The coxibs, selective inhibitors of cyclooxygenase-2. *N. Engl. J. Med.* **2001**, *345*, 433–442.
12. Anwar, Y.; Sabir, J.S.M.; Qureshi, M.I.; Saini, K.S. 5-Lipoxygenase: A Promising Drug Target Against Inflammatory Diseases-Biochemical and Pharmacological Regulation. *Curr. Drug Targets* **2014**, *15*, 410–422.
13. Warner, T.D.; Mitchell, J.A. Cyclooxygenases: New forms, new inhibitors, and lessons from the clinic. *FASEB J.* **2004**, *18*, 790–804.
14. Frade, V.H.J.; Gonçalves, M.S.T.; Coutinho, P.J.G.; Moura, J.C.V.P. Synthesis and spectral properties of long-wavelength fluorescent dye. *J. Photochem. Photobiol. A Chem.* **2007**, *185*, 220–230.
15. Frade, V.H.J.; Coutinho, P.J.G.; Moura, J.C.V.P.; Gonçalves, M.S.T. Functionalised benzo[*a*]phenoxazine dyes as long-wavelength fluorescent probes for amino acids. *Tetrahedron* **2007**, *63*, 1654–1663.