ORIGINAL ARTICLE

Quinoline-linked Pyrimidine Hybrids: Synthesis, High-performance Liquid Chromatography-based Purity Profiling, and Multimodal Biological Evaluation as a Potent Cyclooxygenase-2-Targeting Anti-Inflammatory Agent

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Abstract

Introduction: Inflammation is a major pathological condition associated with several chronic disorders, and selective cyclooxygenase-2 (COX-2) enzyme inhibition is considered an effective strategy for developing safer anti-inflammatory medications. In this study, novel quinoline-linked pyrimidine derivatives were developed, and their potential to reduce inflammation was studied. **Materials and Methods:** The synthesized derivatives were characterized by analytical techniques. The protein denaturation and COX inhibition assays were performed to assess *in vitro* anti-inflammatory efficacy. The *in vivo* anti-inflammatory potential was assessed using the carrageenan-induced paw edema and cotton pellet-induced granuloma models in Wistar rats. Histopathological evaluation and ulcerogenic assessment were also performed. **Results and Discussion:** Several synthesized derivatives demonstrated potential anti-inflammatory properties in *in vitro* investigations, along with preferential COX-2 inhibition over COX-1. One of the derivatives significantly reduced paw edema volume and granuloma development in experimental models. Histopathological studies demonstrated a near-normal tissue framework, and ulcer evaluation showed a lack of gastric mucosal damage and ulcers. The observed anti-inflammatory activity may be linked with preferential COX-2 inhibition and structural features of quinoline-pyrimidine hybrids. **Conclusion:** The present study suggests that quinoline-linked pyrimidine hybrids possess favorable anti-inflammatory potential without ulcerogenic liability and could be used as possible lead candidates for the creation of safer anti-inflammatory drugs.

Key words: Anti-inflammatory activity, Cyclooxygenase-2 inhibition, Histopathology, Pyrimidine, Quinoline, Ulcerogenic evaluation

INTRODUCTION

Inflammation, a complex biological reaction brought on by infection, tissue damage, or immunological stimulation, takes part in the pathogenesis of many acute and chronic medical conditions, including arthritis, cardiovascular diseases, and cancer. Non-steroidal anti-inflammatory drugs (NSAIDs) continue to be the common treatment for pain and inflammation. However, long-term use of NSAIDs is frequently linked with gastrointestinal irritation, ulceration, renal toxicity, and cardiovascular complications due to non-selective cyclooxygenase (COX) enzyme inhibition. Therefore, the development of safer anti-inflammatory agents with improved selectivity for COX-2 remains an important area of medicinal chemistry research.[1]

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There are two isoforms of COX, namely COX-1 and COX-2. COX-1 is constitutively produced and essential for the physiological protection of gastric mucosa and renal homeostasis, whereas COX-2 is generated in associated with inflammatory response and prostaglandin synthesis at sites of inflammation. Selective COX-2 inhibition is therefore considered a promising strategy for reducing inflammation while minimizing gastrointestinal adverse effects commonly associated with NSAIDs.[2]

Nitrogen-containing heterocyclic compounds represent an important class of pharmacologically active molecules in medicinal chemistry. Among them, because of their wide range of biological activities, which include antibacterial, anticancer, antimalarial, anticonvulsant, and anti-inflammatory qualities, quinoline derivatives have attracted a lot of attention. Quinoline is considered a preferable moiety in drug design due to its favorable pharmacokinetic and pharmacodynamic characteristics.[3] Several quinoline-containing molecules have demonstrated promising anti-inflammatory activity through modulation of inflammatory mediators and COX pathways.[4]

Pyrimidine is another important heterocyclic scaffold extensively explored in medicinal chemistry because of its wide range of pharmacological activities. Pyrimidine-containing scaffolds are known for potent anti-inflammatory effects by blocking mediators of inflammation such as prostaglandins, tumor necrosis factor-α, and nitric oxide. Several pyrimidine-based compounds have also demonstrated significant COX-2 inhibitory activity and improved anti-inflammatory profile.[5]

Molecular hybridization, which combines two or more bioactive pharmacophores into a single molecular framework, has become a successful method in the development of novel medicinal agents. In view of the established anti-inflammatory capability of both quinoline and pyrimidine scaffolds, the development of quinoline-linked pyrimidine hybrids may provide compounds with enhanced biological activity and improved selectivity toward inflammatory targets.

In our previous *in silico* studies, quinoline-linked pyrimidine derivatives demonstrated promising binding interactions and favorable docking scores against the COX-2 active site. Based on these findings, selected quinoline derivatives showing promising predicted anti-inflammatory activity were synthesized and tested for anti-inflammatory potential through *in vitro* assays and animal models.[6]

Therefore, the study was undertaken to synthesize and characterize novel quinoline-linked pyrimidine hybrids and test their anti-inflammatory potential using protein denaturation assays, COX inhibition studies, carrageenan-induced paw edema, and cotton pellet-induced granuloma models.

METHODOLOGY

Synthesis

The compounds selected for synthesis and biological evaluation were shortlisted based on previously reported *in silico* studies conducted by our group.[6] A total of 9 compounds were synthesized in 3 different series, namely QPDG, QPDU and QPDT, which are obtained from the reaction of corresponding chalcone intermediates with guanidine hydrochloride, urea and thiourea respectively, as given in the scheme [Figure 1].

Chemicals and reagents

Sigma-Aldrich, Loba Chemie, and HiMedia Laboratories provided all of the chemicals and reagents used in the synthesis, which were of analytical grade. Solvents were either used exactly as supplied or, if needed, purified using standard methods. Melting points were determined using a digital melting point apparatus (EQ 730, Equiptronics). The absorbance was determined using a ultraviolet-visible spectrophotometer (Merck, Spectroquant Prove 600).

Chemistry

Synthesis of 2-chloroquinoline-3-carbaldehyde

A cold, stirred (400 rpm) N, N-dimethylformamide solution (0.16 moL) was mixed with 0.21 moL $POCl_3$ dropwise. The reaction mixture was further stirred in cold conditions. The above solution was mixed with 0.04 moL of acetanilide while being constantly stirred in a cold environment. After refluxing for 6 h, the reaction completion was ensured using thin-layer chromatography (TLC). The reaction solution was then put in ice-cold water after being neutralized with a 10% $NaHCO_3$ solution. The crude product was recrystallized using ethyl acetate to produce 2-chloroquinoline-3-carbaldehyde.[7]

Synthesis of chalcones

0.01 moL each of amino acetophenone and 2-chloroquinoline-3-carbaldehyde were dissolved in 10 mL of ethanol. A magnetic stirrer was used to stir up the mixture at 600 rpm. Then, while continuously stirring, 10 mL of a 10% NaOH solution was added. TLC was performed to confirm the reaction endpoint following 5 h of non-stop stirring. After being left overnight, the reaction mixture was filtered and dried. Further, it is recrystallized using ethyl alcohol.[8]

Synthesis of quinoline-linked pyrimidine derivatives

From guanidine: Glacial acetic acid (4–5 drops) was added to the mixture of 0.01 moL each of chalcone and guanidine hydrochloride in 20 mL of ethyl alcohol, and the mixture was refluxed for 6 h. TLC was performed to check the formation of the product. After cooling, the reaction mixture was added to crushed ice, filtered, and dried and recrystallized with ethanol.

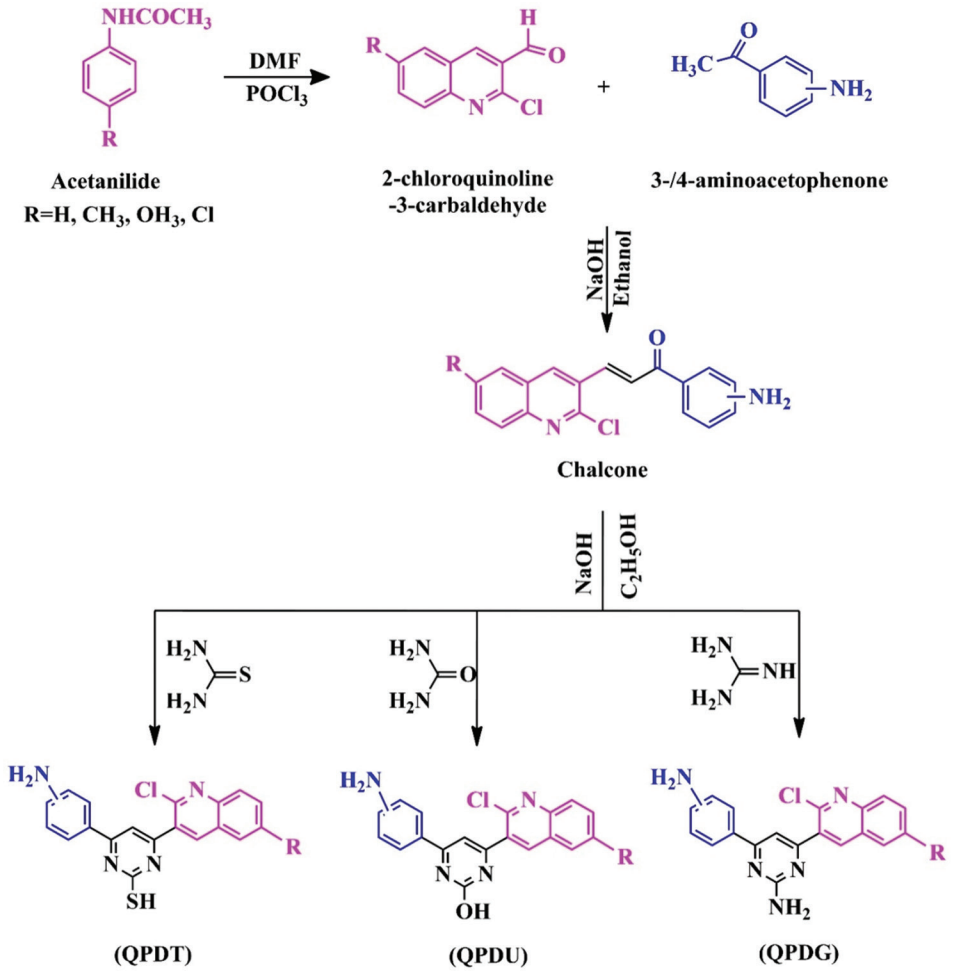

Figure 1: Scheme for the synthesis of quinoline-linked pyrimidine derivatives

From urea: A solution of 0.01 moL each of urea and chalcone in 30 mL of ethyl alcohol was made. This was mixed with 6 mL of glacial acetic acid. The resulting solution was refluxed for 6 h. TLC was used to track the development of the product. The mixture was added to crushed ice, filtered, and dried. To recrystallize, ethanol was utilized.

From thiourea: A stirred solution of 2 mmoL chalcone in 10 mL of ethanol was combined with 2.4 mmoL thiourea. After adding 5 mmoL NaOH solution, the resulting mixture was refluxed for 9 h. To monitor the reaction's completion, TLC was used. Then, the mixture was put in crushed ice, filtered, and dried and recrystallized using ethanol.[9]

4-(4-aminophenyl)-6-(2-chloroquinolin-3-yl)pyrimidin-2-amine (QPDG2): Molecular formula: $C_{19}H_{14}ClN_5$; FTIR (cm^{-1}):3338.61 (N-H), 3051.35 (Ar C-H), 1649.55 (C=N), 1575.87 (Ar C=C), 1173.38 (C-N), 748.36 (C-Cl);^{1}H NMR (400 MHz, DMSO-d6, δ, ppm): 6.31 (s, 2H, Ar-NH_2), 6.05 (s, 2H, Ar-NH_2), Ar-H: 8.56 (s, 1H), 8.15 (d,1H), 8.01(s, 1H), 7.95 (d, 1H), 7.73 (t, 1H), 7.67 (t, 1H), 7.23(d, 2H), 6.69 (d, 2H); ^{13}C NMR (100 MHz, DMSO-d6, δ, ppm): 154.85, 150.20, 147.53, 137.62, 135.68, 132.27, 131.93, 129.03, 128.41, 128.10, 127.61, 125.42, 113.35, 80.26, 65.10, 65.09, 65.05, 65.03, 64.97; LC-MS (m/z): calculated for $C_{19}H_{14}ClN_5$ is 347.80; found: 347.48.

4-(3-aminophenyl)-6-(2-chloro-6-methylquinolin-3-yl) pyrimidin-2-amine (QPDG3): Molecular formula: $C_{20}H_{16}ClN_5$; FTIR (cm^{-1}): 3396.50 (N-H), 3052.03(Ar C-H),2900.80 (C-H), 1663.96 (C=N), 1585.67 (ArC=C), 1332.41 (C-N), and 641.04 (C-Cl0;^{1}H NMR (400 MHz, DMSO-d6, δ, ppm): 6.76 (s, 2H, Ar-NH_2), 5.27 ((s, 2H, Ar-NH_2), 2.69 (s, 3H, Ar-CH_3), Ar-H: 8.64 (s, 1H),8.32 (s, 1H), 8.25 (s, 1H), 7.96 (d, 1H), 7.75 (d, 1H),7.37 (d, 1H), 7.26 (d, 1H), 7.10 (t, 1H), 7.05 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6, δ, ppm): 149.98, 146.34, 144.80, 138.45, 137.88, 137.24, 129.99, 129.43, 127.47, 126.63, 119.43, 116.36, 113.16, 65.45, 65.42, 65.29, 65.15, 65.02, 43.57, 21.59. LC-MS (m/z): calculated for $C_{20}H_{16}ClN_5$ is 361.83; found: 361.59.

4-(4-aminophenyl)-6-(2-chloro-6-methoxyquinolin-3-yl)pyrimidin-2-amine (QPDG6): Molecular formula:

$C_{20}H_{16}ClN_5O$; FTIR (cm^{-1}): 3403.76(N-H), 3086.89(Ar C-H), 2900.14 (C-H), 1660.93(C=N), 1590.95(Ar C=C), 1230.70(C-N), 1175.91(C-O), 646.62(C-Cl); ^{1}H NMR (400 MHz, DMSO-d6, δ, ppm): 6.51 (s, 2H, Ar-NH_2), 5.46 (s, 2H, Ar-NH_2), 3.89 (s, 3H, Ar-OCH_3), Ar-H:8.61 (s,1H), 8.24 (s,1H), 8.09 (s,1H), 7.98 (d,1H), 7.82 (d,1H), 7.37 (d,2H), 7.06 (d,2H); ^{13}C NMR (100 MHz, DMSO-d6, δ, ppm): 154.14, 131.09, 131.08, 125.40, 122.01, 117.94, 114.65, 113.03, 113.02, 113.01, 113.00, 112.97, 112.95, 67.58, 67.53, 67.51, 67.48, 67.43, 55.96, 55.75;LC-MS (m/z): calculated for $C_{20}H_{16}ClN_5O$ is 377.83; found: 377.6637.

4-(4-aminophenyl)-6-(2-chloro-6-methylquinolin-3-yl) pyrimidin-2-ol (QPDU4): Molecular formula: $C_{20}H_{15}ClN_4O$; FTIR (cm^{-1}): 3638.20 (O-H), 3332.71 (N-H), 3053.15 (Ar C-H), 2894.50 (C-H), 1691.17 (C=N), 1579.15 (Ar C=C), 1344.69 (C-N), 645.00 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d6, δ, ppm):11.78 (s, 1H, Ar-OH), 6.05 (s, 2H, Ar-NH_2), 2.69 (s, 3H, Ar-CH_3), Ar-H:8.67 (s, 1H), 8.35 (s, 1H), 8.27 (s, 1H), 7.92 (d, 1H), 7.80 (d, 1H), 7.34 (d, 2H), 6.91(d, 2H); ^{13}C NMR (100 MHz, DMSO-d6, δ, ppm): 160.25, 154.78, 149.25, 146.10, 137.99, 136.82, 135.74, 131.87, 127.91, 127.51, 127.23, 125.36, 113.28, 80.11, 80.00, 65.06, 65.01, 64.99, 64.97, 21.64; LC-MS (m/z): calculated for $C_{20}H_{15}ClN_4O$ is 362.81; found: 362.73.

4-(4-aminophenyl)-6-(2-chloro-6-methoxyquinolin-3-yl) pyrimidin-2-ol (QPDU6): Molecular formula: $C_{20}H_{15}ClN_4O_2$; FTIR (cm^{-1}): 3638.54 (O-H), 3297.73 (N-H), 3102.51 (Ar C-H), 2898.01 (C-H), 1661.26 (C=N), 1590.89 (Ar C=C), 1232.45 (C-N), 1175.92 (C-O), 644.25 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d6, δ, ppm): 11.91 (s, 1H, Ar-OH), 6.04 (s, 2H, Ar-NH_2), 3.94 (s, 3H, Ar-OCH_3), Ar-H: 8.67 (s, 1H), 8.35 (s, 1H), 8.28(s, 1H), 7.88(d, 1H), 7.65(d, 1H), 7.42(d, 2H), 7.08(d, 2H); ^{13}C NMR (100 MHz, DMSO-d6, δ, ppm): 158.48, 154.80, 154.09, 147.51, 143.55, 136.26, 135.73, 131.85, 131.03, 129.65, 128.79, 127.26, 112.92, 80.09, 80.01, 56.17, 56.14, 56.11, 56.06, 56.00; LC-MS (m/z): calculated for $C_{20}H_{15}ClN_4O_2$ is 378.81; found: 378.42.

4-(3-aminophenyl)-6-(2,6-dichloroquinolin-3-yl)pyrimidin-2-ol (QPDU7): Molecular formula: $C_{19}H_{12}Cl_2N_4O$; FTIR (cm^{-1}): 3396.57 (N-H), 3090.85 (Ar C-H), 1662.99 (C=N), 1586.76 (Ar C=C), 1332.13 (C-N), 1256.40 (C-O), 687.13 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d6, δ, ppm): 12.01 (s, 1H, Ar-OH), 6.16 (s, 2H, Ar-NH_2), Ar-H: 8.52 (s, 1H), 8.28 (s, 1H), 8.25 (s, 1H), 7.98 (d, 1H), 7.72 (d 1H), 7.09 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d6, δ, ppm): 149.54, 143.95, 141.86, 137.79, 136.05, 132.00, 129.26, 126.33, 121.30, 119.00, 116.47, 113.06, 80.24, 80.07, 79.95, 62.90, 62.88, 62.86, 62.85; LC-MS (m/z): calculated for $C_{19}H_{12}Cl_2N_4O$ is 383.23; found: 383.48.

4-(4-aminophenyl)-6-(2-chloroquinolin-3-yl)pyrimidine-2-thiol (QPDT2): Molecular formula: $C_{19}H_{13}ClN_4S$; FTIR (cm^{-1}): 3334.69 (N-H), 3049.76 (Ar C-H), 2535.62 (S-H), 1649.72 (C=N), 1594.58 (Ar C=C), 1175.75 (C-N), 753.85 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d6, δ, ppm): 12.27 (s, 1H, Ar-SH), 4.02 (s, 2H, Ar-NH_2), Ar-H: 8.53 (s, 1H), 8.43 (d, 1H), 8.37 (s, 1H),8.17 (d, 1H), 7.87 (t, 1H), 7.79 (d, 2H), 7.55 (t, 1H), 7.14 (d, 2H); ^{13}C NMR (100 MHz, DMSO-d6, δ, ppm): 156.35, 152.02, 148.53, 146.63, 134.45, 130.99, 129.36, 128.54, 127.94, 127.86, 127.49, 117.81, 113.65, 62.32, 62.27, 62.24, 60.75, 60.74, 26.32; LC-MS (m/z): calculated for $C_{19}H_{13}ClN_4S$ is364.85; found: 364.44

4-(4-aminophenyl)-6-(2-chloro-6-methoxyquinolin-3-yl)pyrimidine-2-thiol (QPDT6): Molecular formula: $C_{20}H_{15}ClN_4OS$; FTIR (cm^{-1}): 3349.44 (N-H), 3085.12 (ArC-H), 2895.04 (C-H), 2548.84 (S-H), 1661.31 (C=N), 1588.62 (ArC=C), 1230.27 (C-N), 1175.24 (C-O), 649.50 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d6, δ, ppm): 12.39 (s, 1H, Ar-SH), 4.10 (s, 2H, Ar-NH_2), 3.85 (s, 3H, Ar-OCH_3), Ar-H: 8.49 (s, 1H), 8.41 (s, 1H), 8.31 (s, 1H), 8.06 (d, 1H), 7.84 (d, 1H), 7.75 (d, 2H), 7.11 (d, 2H); ^{13}C NMR (100 MHz, DMSO-d6, δ, ppm): 158.34, 156.35, 152.02, 145.69, 142.57, 134.54, 131.03, 129.35, 128.80, 123.44, 117.82, 113.64, 112.92, 60.68, 60.63, 56.27, 56.13, 56.00, 55.96, 26.31;LC-MS (m/z): calculated for $C_{20}H_{15}ClN_4OS$ is 394.88; found: 394.68.

4-(3-aminophenyl)-6-(2,6-dichloroquinolin-3-yl)pyrimidine-2-thiol (QPDT7): Molecular formula: $C_{19}H_{12}Cl_2N_4S$; FTIR (cm^{-1}): 3337.23 (N-H), 3146.82 (ArC-H), 2519.59 (S-H), 1661.43 (C=N), 1589.65 (ArC=C), 1177.04 (C-N), 692.87 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d6, δ, ppm): 12.35 (s, 1H, Ar-SH), 4.12 (s, 2H, Ar-NH_2), Ar-H: 8.55(s, 1H), 8.38(s, 1H), 8.31 (s, 1H), 8.11(d, 1H), 7.74(d, 1H), 7.41 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d6, δ, ppm): 152.07, 148.58, 146.69, 136.82, 129.41, 128.59, 128.00, 127.55, 119.14, 117.89, 113.72, 113.00, 62.35, 62.34, 62.33, 62.32, 62.31, 62.30, 26.36; LC-MS (m/z): calculated for $C_{19}H_{12}Cl_2N_4S$ is 399.30; found: 399.42.

Determination of percentage purity of synthesized compounds by high-performance liquid chromatography (HPLC)

A HPLC system (Shimadzu, Japan) with a prominence photodiode array detector (SPD-M20A) and a C18 reverse-phase analytical column (250 mm × 4.6 mm, 5 µm) was used to check the purity. Stock solutions of the synthesized compounds were made by mixing methanol and acetonitrile in a 2:3 ratio. The acetonitrile and water (80:20) were employed as the mobile phase, and the flow rate was adjusted to 0.6 mL/min.[10]

In vitro studies

Bovine serum albumin (BSA) denaturation assay

The protein denaturation experiment was conducted using a 0.5 mL reaction mixture that contained aqueous BSA solution (5% w/v, 0.45 mL) and various test samples (0.05 mL). 0.05 mL of distilled water was added as the control. Each

solution's pH was maintained at 6.3 by adding hydrochloric acid (1 N). The resulting solution was heated at 57°C for 3 min following a 20 min incubation period at 37°C. After incubation, 2.5 mL of phosphate-buffered saline (PBS) was added to the cooled solution. Celecoxib served as the reference standard. At 416 nm, the optical density (OD) was measured to quantify the percentage suppression of protein denaturation.[11]

Egg albumin denaturation assay

For this study, albumin was obtained from fresh hen eggs. The egg albumin (0.2 mL), PBS (pH 6, 4.8 mL), and test compounds (2 mL) were taken. After 15 min of incubation at 37°C and 5 min of heating at 70°C, the contents were gently mixed by shaking. After cooling, OD was read at 660 nm. The reference used was celecoxib. Protein denaturation suppression was calculated and reported as a percentage.[12]

In vitro COX assay

An enzyme immunoassay kit (catalog number 760111, Cayman Chemical, Ann Arbor, MI, USA) was used to assess the inhibitory action of quinoline-pyrimidine hybrids on COX-2 and COX-1 enzymes. Assay buffer (160 µL) and hemin (10 µL) were put into background wells. Assay buffer (150 µL), hemin (10 µL), and either COX-2 or COX-1 enzyme (10 µL) were put into control and inhibitor wells. Ten microliters of various doses of the synthesized compounds were put into the inhibitor wells, whereas 10 µL of solvent was placed in the background and control wells. The ELISA plate was gently shaken for a short while before being incubated at 25°C for 5 min. Each well received 20 µL of arachidonic acid and 20 µL of colorimetric substrate solution. The sample was shaken gently and then incubated for 2 min at 25°C. OD was measured at 590 nm.[13]

In vivo studies

Animals

The experimental protocols for the acute oral toxicity study (protocol number NGSMIPS/IAEC/APR-2024/427) and the *in vivo* anti-inflammatory studies (protocol number NGSMIPS/IAEC/APR-2025/489) have been approved by the Institution Animal Ethics Committee of NGSM Institute of Pharmaceutical Science (Reg No. 1781/PO/ReBi/S/14/CCSEA). The animals were kept in separate cages in a regulated laboratory setting at a temperature of 25 ± 2°C, a relative humidity of 50 ± 5%, and a 12-h light/dark cycle. They were given a standard pellet diet and enough water. Before the experiments started, the rats were acclimated for at least a week. Each experiment involved six rats per group. The derivative that showed the most promising action out of the 9 drugs assessed in the *in vitro* investigations was chosen and subsequently examined *in vivo* utilizing animal models.

Acute oral toxicity studies

Non-pregnant, nulliparous, healthy 12-week-old female rats were used in an acute oral toxicity investigation conducted in accordance with Organization for Economic Co-operation and Development guideline 425. Animals that had fasted overnight were administered the synthesized compound orally to assess its acute oral toxic effects. Each rat was constantly monitored over 48 h to assess behavioral and neurological changes, such as convulsions, tremors, diarrhea, sleep, salivation, lacrimation, and feeding behavior. Extra monitoring was done for 14 days to detect any delayed toxicity or mortality.

Carrageenan-induced paw edema

This experiment was conducted on 12-week-old, Wistar rats of both sexes. The rats had acute inflammation when 0.1 mL of fresh carrageenan suspension (1% w/v in 1% carboxymethyl cellulose) was injected on the plantar area of the right rear paw. The test compound QPDT6 was given orally at a dose of 200 mg/kg. Two further groups were maintained as controls: The disease control group given with vehicle (1 mL/kg, p.o.) and the positive control group administered with celecoxib (50 mg/kg). One hour before the carrageenan injection, all treatments were given. A digital plethysmometer (Orchid Scientific, Model: PLM 01 Plus) was used to measure the volume of the paw up to the ankle joint at baseline (0 h), followed by 1, 2, 3, 4, and 5 h following the injection of carrageenan. The percentage suppression of edema in the treated group was determined by comparing the paw volumes with the control group.[14]

COX assay

Paw tissues were obtained after the rats used in the carrageenan-induced paw edema model were sacrificed. The paw tissues were cleaned using tris buffer (pH 7.4). The tissues were homogenized using 5–10 mL of ice-cold buffer (0.1 M Tris-HCl, pH 7.8, supplemented with 1 mM EDTA). The homogenates were centrifuged at 10,000 × g for 15 min at 4°C, and the resulting supernatant was collected. COX peroxidase activity was tested using the Cayman COX Activity Assay Kit (Item No. 760151; Cayman Chemical, Ann Arbor, MI, USA). The experiment was conducted colorimetrically by monitoring the generation of oxidized N, N, N',N'-tetramethyl-p-phenylenediamine at 590 nm.[15]

Cotton pellet-induced granuloma

For this experiment, 12-week-old Wistar rats of both sexes were used. The experiment had four groups of six rats each, namely normal control, disease control, standard, and QPDT6. After the animals were anesthetized, their fur was removed, and a small incision was made so that sterile cotton pellets (10 mg, pre-weighed) could be subcutaneously inserted into the axillary region for the animals in disease control, standard, and QPDT6 groups. For 7 days, the test compound QPDT6 was given orally at a dose of 200 mg/kg body weight. The

disease control and normal control groups were only given the vehicle; the standard group received celecoxib (50 mg/kg, p.o.). The animals were sacrificed after 7 days of treatment, the implanted pellets with the surrounding granulomatous tissue were removed, the adhering tissue was cleaned, and they were dried for 24 h at 60°C. The increase in the pellets' dry weight served as an indicator for the development of granulomas.[16]

Ulcer studies

The test compound QPDT6 was further assessed for ulcerogenic potential after anti-inflammatory screening in the cotton pellet-induced granuloma model. The animals' stomachs were separated and opened along the larger curvature after they were sacrificed. The stomach mucosa was cleaned with regular saline after being rinsed with distilled water. A magnifying lens was used to examine the mucosa for any lesions.[17]

Histopathology

Each rat's stomach, kidney, heart, and liver were removed following the cotton pellet-induced granuloma model experiment. An automated tissue processor was used to process the tissue samples. Following fixation in buffered formalin, the tissues were cleaned, dehydrated with graded alcohols, cleared using xylene, and then fixed in paraffin. Sections were cut into 4–5 µm thickness, placed on slides, and stained with hematoxylin and eosin (H&E). To ascertain whether the test substance caused any harmful effects or preserved normal tissue architecture, H&E-stained sections of gastric, hepatic, renal, and cardiac tissues were examined for histopathological alterations.[18]

Statistical analysis

Experimental data were expressed as mean ± standard error of the mean (SEM). Statistical analysis of carrageenan-induced paw edema data was performed using two-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test, whereas granuloma data were analyzed using one-way ANOVA followed by Dunnett's test. All *in vitro* experiments were performed in triplicate, and the results were expressed as mean ± standard deviation. Statistical analysis was carried out using GraphPad Prism version 8.0.2.

RESULTS

Chemistry and synthesis

The synthesized quinoline-linked pyrimidine derivatives were obtained in moderate to good yields ranging from 27.84 to 95.02%. The compounds showed satisfactory purity on HPLC analysis (> 95%). Melting point and retention factor (Rf) values confirmed the formation of distinct derivatives. The melting points, Rf value, percentage yield, and HPLC purity data are summarized in Table 1.

In vitro studies

Protein denaturation assay

The synthesized compounds inhibited protein denaturation in a concentration-dependent manner, both in the BSA denaturation and egg albumin denaturation assays. Compounds demonstrated varying degrees of inhibitory activity against protein denaturation. The half-maximal inhibitory concentration (IC_{50}) values obtained from BSA denaturation and egg albumin denaturation assays are presented in Table 2.

COX assay

Compounds showed varying degrees of inhibition of COX enzymes; most of the compounds demonstrated preferential suppression of COX-2. The IC_{50} values and selectivity index are presented in Table 3.

In vivo studies

Carrageenan-induced paw edema

Treatment with QPDT6 reduced edema in the rat paw compared to the disease control group at different time intervals. The effect of QPDT6 and celecoxib on carrageenan-induced paw edema is illustrated in Figure 2a.

COX assay

Administration of QPDT6 reduced COX-2 levels in paw tissues of carrageenan-induced paw edema rats, with comparatively lower inhibition of COX-1 activity. The effect of QPDT6 and celecoxib on COX-2 and COX-1 activity in

Table 1: Physicochemical and analytical characterization of synthesized quinoline-linked pyrimidine derivatives

Compound	Melting point	Rf value (n- hexane: ethyl acetate/7:3)	% yield	HPLC % purity
QPDG2	184–187°C	0.58	43.60	96.24
QPDG3	176–178°C	0.77	89.16	96.98
QPDG6	164 –167°C	0.26	90.09	96.89
QPDU4	188–190°C	0.29	95.02	97.88
QPDU6	193–195 °C	0.37	91.20	98.01
QPDU7	201–203°C	0.73	93.60	95.28
QPDT2	207–209°C	0.69	47.22	95.29
QPDT6	194–196°C	0.28	83.33	98.72
QPDT7	207–209°C	0.40	27.84	97.81

HPLC: High-performance liquid chromatography, Rf: Retention factor, QPDG: Quinoline-linked pyrimidine derivatives with a glycine, QPDU: Quinoline-linked pyrimidine derivatives with a urea, QPDT: Quinoline-linked pyrimidine derivatives with a thiosemicarbazone

Table 2: Effect of quinoline-linked pyrimidine derivatives on BSA denaturation and egg albumin denaturation

Compound	BSA assay IC_{50} (µg/mL)	Egg albumin assay IC_{50} (µg/mL)
QPDG2	41.62±0.19	40.57±0.15
QPDG3	37.32±0.18	38.53±0.28
QPDG6	45.14±0.34	42.15±0.32
QPDU4	39.73±0.95	39.91±0.14
QPDU6	34.18±0.42	37.6±0.21
QPDU7	37.62±0.07	39.23±0.12
QPDT2	35.08±0.24	38.46±0.27
QPDT6	30.19±0.09	35.15±0.07
QPDT7	56.15±2.67	43.43±0.7
Celecoxib	26.15±0.11	33.84±0.8

Values are given as mean±standard deviation, *n*=3 in each group. BSA: Bovine serum albumin, IC_{50}: Half-maximal inhibitory concentration, QPDG: Quinoline-linked pyrimidines derived from guanidine, QPDU: Quinoline-linked pyrimidines derived from urea, QPDT: Quinoline-linked pyrimidines derived from thiourea.

Table 3: IC_{50} values and selectivity index of quinoline-linked pyrimidine derivatives against COX-2 and COX-1 enzymes

Compound	IC_{50} (µg/mL)		Selectivity index
	COX-2	COX-1	
QPDG2	25.94±0.23	86.33±0.79	3.33
QPDG3	23.99±0.41	203.61±0.49	8.49
QPDG6	34.41±0.55	84.86±0.27	2.47
QPDU4	32.44±0.11	116.08±3.86	3.58
QPDU6	26.45±0.25	207.54±2.18	7.85
QPDU7	28.92±0.03	229.77±1.48	7.95
QPDT2	24.39±0.23	210.03±0.45	8.61
QPDT6	19.23±0.09	191.35±1	9.95
QPDT7	37.54±0.29	49.25±0.51	1.31
Celecoxib	17.38±0.72	222.02±5.53	12.77

Values are given as mean±standard deviation, *n*=3 in each group. COX: Cyclooxygenase, IC_{50}: Half-maximal inhibitory concentration, QPDG: Quinoline-linked pyrimidines derived from guanidine, QPDU: Quinoline-linked pyrimidines derived from urea, QPDT: Quinoline-linked pyrimidines derived from thiourea.

the paw tissue of rats is given in Table 4.

Cotton pellet-induced granuloma

The QPDT6 and celecoxib-treated group demonstrated reduced granuloma weight in comparison with the disease control group. The effect of QPDT6 and celecoxib on cotton pellet-induced granuloma is illustrated in Figure 2b.

Ulcer study

No visible gastric ulcer or mucosal lesions were observed in the QPDT6 and celecoxib-treated groups during ulcerogenic evaluation carried out following cotton pellet-induced granuloma assays.

Histopathology

The gastric sections from the disease control group showed inflammatory infiltration, whereas the QPDT6 and celecoxib-treated groups demonstrated near-normal gastric architecture. The liver sections of treated animals showed preserved hepatic architecture with reduced inflammatory changes compared with the disease control group, which showed notable inflammatory infiltrate. The cardiac section of disease control showed fibrosed areas, whereas other groups showed normal architecture. The renal sections of all groups showed normal tissue architecture. Representative histopathological sections of gastric, hepatic, cardiac, and renal tissues from different experimental groups are shown in Figure 3.

DISCUSSION

The study demonstrated the successful synthesis and evaluation of quinoline-linked pyrimidine derivatives with potential anti-inflammatory activity. Several compounds exhibited promising activity in both *in vitro* models. The compounds showed preferential suppression of COX-2 over COX-1 along with minimal ulcerogenic effects, suggesting a favorable anti-inflammatory profile.

The quinoline-linked pyrimidine derivatives were synthesized in three different series, namely QPDG, QPDU, and QPDT series. The QPDG series compounds were obtained by reaction of chalcone with guanidine. Similarly, QPDU and QPDT are obtained from urea and thiourea, respectively. QPDG compounds have NH_2 group, QPDU compounds have OH group, and QPDT compounds have SH group on the pyrimidine ring. Structural modification of the quinoline scaffold significantly influenced the anti-inflammatory activity of quinoline-linked pyrimidine derivatives. Among the QPDG series, the compound with methyl group substitution on the quinoline ring showed higher activity. In the QPDU series and QPDT series compounds with a methoxy group substitution on the quinoline ring showed higher activity. The results obtained in the *in vitro* protein denaturation assays and COX assays aligned with our previous *in silico* molecular docking study results.[6]

Protein denaturation is considered one of the major causes of inflammation, and agents that have the potential to suppress protein denaturation may possess anti-inflammatory activity.[19] The synthesized derivatives showed concentration-dependent suppression of protein denaturation, indicating

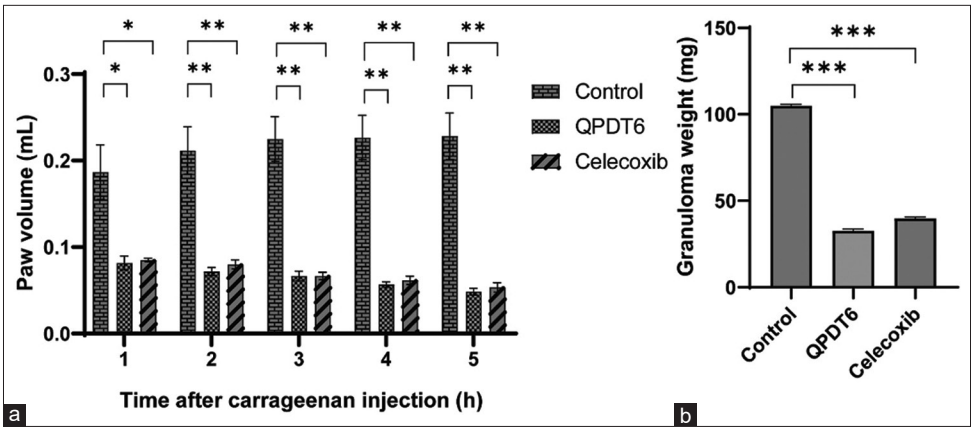

Figure 2: (a) Effect of QPDT6 and celecoxib on paw volume in the carrageenan-induced paw edema model in rats. Paw volume (mL) was measured at different time points after carrageenan injection. Statistical analysis was conducted using two-way analysis of variance (ANOVA). (b) Effect of QPDT6 and celecoxib on granuloma formation in the cotton pellet-induced granuloma model. The bar graph represents the mean dry weight (mg) of granuloma tissue after treatment. Statistical analysis was performed using one-way ANOVA. Data are expressed as mean±standard error of the mean ($n = 6$); *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus control

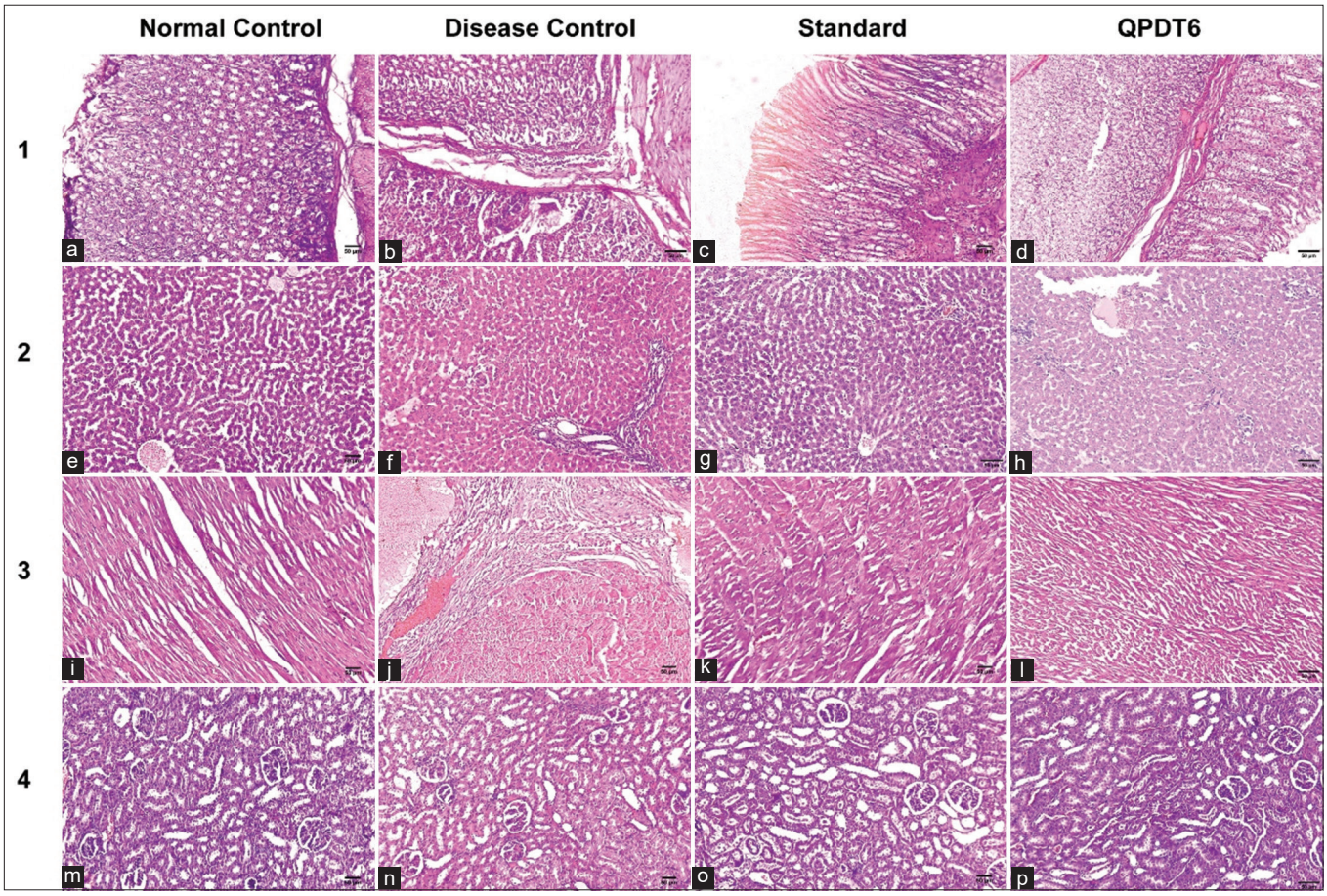

Figure 3: Hematoxylin and eosin-stained sections of rat stomach, liver, heart, and kidney tissues from different experimental groups in the cotton pellet-induced granuloma model. Tissue sections are arranged row-wise as stomach (a-d), liver (e-h), heart (i-l), and kidney (m-p), whereas the experimental groups from left to right represent normal control, disease control, standard-treated (celecoxib), and QPDT6-treated groups, respectively. Magnification: 100×, Scale bar: 50 μm

their capability to stabilize proteins under inflammatory conditions. Among the tested derivatives, QPDT6 showed comparatively better inhibitory activity.

COX enzymes play a crucial role in prostaglandin-mediated inflammatory responses. Selective COX-2 inhibition is generally associated with reduced gastrointestinal side

Table 4: Effect of QPDT6 and celecoxib on COX-2 and COX-1 activity in paw tissue of rats with carrageenan-induced paw edema

Sample	COX activity (U/mL)			% Activity	
	Total	DUP-677	SC-560	COX-1	COX-2
Disease control	71.5±1.04	27.01±0.78	42.8±0.79	-	-
QPDT6	28.92±0.52	21.93±0.26	6.57±0.65	75.83±0.46	22.76±2.66
Celecoxib	25.11±0.69	20.02±0.52	4.56±0.54	79.75±0.72	18.15±2.2

Values are given as mean±standard deviation, *n*=3 in each group. COX: Cyclooxygenase, QPDT: Quinoline-linked pyrimidines derived from thiourea

effects.[20] The synthesized compounds showed greater COX-2 inhibition than COX-1, indicating preferential COX-2 suppression. QPDT6 showed enhanced COX-2 inhibition, which may be attributed to favorable interactions of substituted quinoline and pyrimidine moieties within the binding pocket of COX-2, which in agreement with our previously reported molecular docking studies.[6]

Among these 9 synthesized compounds, we have selected QPDT6, which showed top results in *in vitro* assays, for further detailed study in animal models. Carrageenan-induced paw edema is a well-known acute inflammatory model involving the formation of mediators of inflammation such as serotonin, histamine, and prostaglandins.[21] Significant reduction of paw edema volume after treatment with QPDT6 indicates anti-inflammatory potential. The observed activity may be associated with suppression of prostaglandin synthesis through COX-2 inhibition, which is supported by preferential COX-2 inhibition in paw tissues. The cotton pellet-induced granuloma model is commonly used to evaluate the proliferative phase of chronic inflammation.[22] Reduction in granuloma weight in QPDT6-treated groups suggests the ability of the compound to suppress the chronic inflammatory process and granulomatous tissue formation. Histopathological evaluation further supported that QPDT6 did not cause pathological alterations in the major organs under the study conditions. Absence of gastric mucosal damage or ulcerative lesions suggests that QPDT6 lacks ulcerogenic potential. Reduced gastric toxicity may be associated with preferential COX-2 inhibition over COX-1.

Overall, the present findings suggest that the synthesized quinoline derivatives possess promising anti-inflammatory activity with preferential COX-2 inhibition and reduced ulcerogenic liability. These quinoline-linked pyrimidine derivatives may serve as a potential lead for further optimization and development of safer anti-inflammatory agents.

CONCLUSION

The study demonstrated the synthesis and evaluation of novel quinoline-linked pyrimidine derivatives with promising anti-inflammatory potential. The synthesized compounds showed moderate to significant activity in protein denaturation and COX inhibition assays. The one selected compound demonstrated effective reduction of acute and chronic inflammation in experimental animal models. Preferential inhibition of COX-2 over COX-1, together with the absence of significant ulcerogenic effects and favorable histopathological observations, suggests a safer anti-inflammatory profile. The findings obtained in this study support the capability of these derivatives as reliable lead molecules for the development of more effective and safer anti-inflammatory agents.

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AUTHORS' CONTRIBUTIONS

Deepthi K: Methodology, data curation, formal analysis, writing-original draft; Manjunath S. Katagi: Conceptualization, investigation, validation, writing-review and editing; Jennifer Fernandes: Investigation, project administration, supervision, writing-review and editing. All authors have read and approved the final manuscript.

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