

Article

Comparative Anti-Inflammatory Profile of Some Cytisine and Tetrahydroisoquinoline Derivatives: Interactions with COX-2 and Histamine H₁ Receptors

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Abstract

Accumulating evidence indicates extensive crosstalk between prostaglandin- and histamine-mediated signaling pathways. Histamine has been shown to induce COX-2 (Cyclooxygenase-2) expression and stimulate prostaglandin synthesis, whereas prostaglandins potentiate the vascular and inflammatory effects of histamine, creating a positive feedback loop that sustains inflammation. Based on these considerations, *in silico* molecular docking study of interaction of new cytisine and TGIQ (tetrahydroisoquinoline) derivatives with COX-2 and the histamine H₁ receptor was performed. Among the synthesized compounds, compound **1** exhibited the highest binding affinity toward Cyclooxygenase-2 (COX-2). Within the binding pocket of the human H₁ histamine receptor, the binding affinity profile differed markedly from that observed for COX-2, with compound **4** exhibiting the highest therapeutic potential and the strongest binding affinity, surpassing all reference compounds. The high anti-inflammatory efficacy of the studied compounds was confirmed in formalin- and histamine rat models. THIQ derivatives were shown to possess a higher anti-inflammatory index than cytisine derivatives. Comparative evaluation of the anti-inflammatory activity of isoquinoline and quinolizidine derivatives revealed that the isoquinoline derivatives possess marked anti-inflammatory properties combined with low toxicity, making them promising candidates for the development of novel, highly effective and safe anti-inflammatory agents for both oral and topical administration.

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Keywords: 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline derivatives; cytosine derivatives; COX-2; H₁-receptors

1. Introduction

Excessive or chronic inflammation underlies the pathogenesis of a wide range of diseases, including rheumatoid arthritis, bronchial asthma, allergic disorders, atherosclerosis, neurodegenerative diseases, and cancer. Therefore, the discovery of novel compounds capable of modulating key inflammatory mediators with improved efficacy and safety remains one of the major challenges in modern pharmacology and medicinal chemistry [1,2].

Cyclooxygenase-2 (COX-2) is one of the key enzymes involved in the inflammatory cascade. Unlike the constitutively expressed COX-1 isoform, COX-2 is induced by pro-inflammatory cytokines and catalyzes the conversion of arachidonic acid into prostaglandins, particularly prostaglandin *E*₂ (PGE₂). Overexpression of COX-2 has been implicated in numerous inflammatory disorders, making this enzyme an important therapeutic target. Indeed, selective inhibition of COX-2 represents the principal mechanism of action of many nonsteroidal anti-inflammatory drugs (NSAIDs) [3–5]. Histamine is another pivotal inflammatory mediator that is rapidly released from mast cells and basophils during tissue injury and allergic reactions. Most of its pro-inflammatory effects are mediated through the histamine H₁ receptor (H₁R), a member of the G protein-coupled receptor (GPCR) family. H₁R signaling promotes the recruitment and activation of immune cells at sites of inflammation, thereby amplifying the inflammatory response [6–8].

Accumulating evidence indicates extensive crosstalk between prostaglandin- and histamine-mediated signaling pathways. Histamine has been shown to induce COX-2 expression and stimulate prostaglandin synthesis, whereas prostaglandins potentiate the vascular and inflammatory effects of histamine, creating a positive feedback loop that sustains inflammation. Consequently, compounds capable of simultaneously modulating multiple inflammatory targets may be promising candidates for the development of next-generation anti-inflammatory agents [9,10].

Based on these considerations, investigating the interactions of newly synthesized compounds with COX-2 and the histamine H₁ receptor represents a rational strategy for predicting their anti-inflammatory potential and elucidating the molecular mechanisms underlying their biological activity.

Modification of nitrogen-containing heterocycles represents one of the most promising strategies in the design of biologically active compounds. In particular, the rigid tricyclic scaffold of the alkaloid cytosine, incorporating a piperidine moiety, offers a versatile platform for rational structural modification. Whereas the introduction of fluorinated and ethynyl substituents into an isolated piperidine ring has been exploited to develop potent membrane-stabilizing agents with different properties [11], structural modification of the cytosine scaffold itself has been shown to redirect its pharmacological profile toward pronounced anti-inflammatory activity. The 3-amino derivatives of 12-N-methylcytosine exhibit mnemonic activity comparable to, or even exceeding, that of the reference nootropic agent piracetam. An *in silico* screening of these derivatives against cyclooxygenase isoforms COX-1 and COX-2, followed by *in vivo* and *in vitro* validation, demonstrated that some 3-amino-12-N-methylcytosine derivatives possess significant anti-inflammatory activity and may be considered potential selective COX-2 inhibitors [12].

Tetrahydroisoquinolines (THIQ) contain a piperidine-like ring fused to a benzene ring, forming a privileged bicyclic nitrogen-containing scaffold. THIQ derivatives have been shown to possess a broad spectrum of pharmacological activities, while exhibiting

low toxicity [13–15]. The anti-inflammatory activity of **33** derivatives of 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline was investigated in animal experiments. A relationship between the activity of the compounds and the nature of their substituents, as well as their positional and mutual arrangement in the C ring (1-Ar-), was established. In silico modeling of these 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline derivatives' interaction with the COX-2 (PDB ID: 1PXX) active site revealed that the nitro-derivatives exhibited the highest stability owing to their superior capacity for electrostatic and hydrogen bond formation compared to brominated compounds [16].

A new selective tetrahydroisoquinoline-7-carboxamide-based DDR1 inhibitor was discovered to tightly bind the DDR1 protein and potently inhibit its kinase function with a K_d value of 2.2 nM and an IC_{50} value of 6.6 nM, respectively. The compound dose-dependently inhibited lipopolysaccharide (LPS)-induced interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) release in mouse primary peritoneal macrophages (MPMs). In addition, it also exhibited promising in vivo anti-inflammatory effects in an LPS-induced mouse ALI model [17].

A series of 1,3,4-oxadiazole tetrahydroisoquinoline hybrids was designed and synthesized aiming to search for new and safe anti-inflammatory agents. The compounds were assessed in vitro for their inhibitory effects on the COX-1 and COX-2 isoenzymes. Notably, the tested 3 derivatives exhibited high potency on COX-2 with IC_{50} values 1.72, 0.89 and 1.05 μ M, respectively, comparable to that of the reference drug celecoxib (IC_{50} = 0.82 μ M) [18].

Thus, published studies indicate that structural modification of THIQ and cytosine derivatives can result in compounds exhibiting high anti-inflammatory activity.

The aim of this study was to comparatively evaluate the anti-inflammatory properties of some new cytosine and tetrahydroisoquinoline derivatives by investigating their interactions with cyclooxygenase-2 (COX-2) and histamine H_1 receptors using in silico and in vivo models, and select lead substances for further investigation.

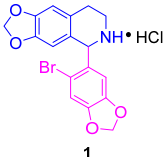
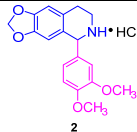
2. Materials and Methods

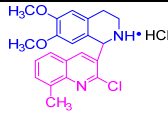
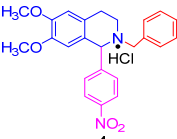
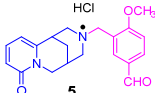
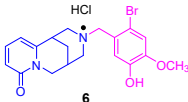
Tetrahydroisoquinoline derivatives were prepared according to the method described in [13]. As quinolizidine derivatives, N-(3-formyl-6-methoxybenzyl)cytosine hydrochloride and N-(6-bromo-3-hydroxy-4-methoxybenzyl)cytosine hydrochloride were studied [19].

2.1. Investigated Substances

Newly synthesized alkaloid derivatives, obtained by scientists V. I. Vinogradova and Sh. N. Zhurakulov at the Laboratory of Chemistry of Alkaloids, Institute of Chemistry of Plant Substances of the Academy of Sciences of the Republic of Uzbekistan, were selected as the objects of research (Table 1).

Table 1. Chemical structures of the newly studied isoquinoline and quinolizidine derivatives.

Compound 1		1-(2'-Bromo-4',5'-methylenedioxyphenyl)-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline hydrochloride
Compound 2		1-(3',4'-Dimethoxyphenyl)-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline hydrochloride

Compound 3		2-Chloro-3'-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-1-yl)-8-methylquinoline hydrochloride
Compound 4		1-(4'-Nitrophenyl)-N-benzyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride
Compound 5		N-3-Formyl-6-methoxybenzyl cytosine hydrochloride
Compound 6		N-6-Bromo-3-oxy-4-methoxybenzyl cytosine hydrochloride

2.2. Chemistry

2.2.1. General Information

Solvents and reagents were purchased from Sigma-Aldrich (Sigma-Aldrich GmbH, Taufkirchen Germany). Starting materials, including some brominated benzaldehydes, were synthesized from the corresponding benzaldehyde in the laboratory; the purity of the reagents was 95–98%. Mass spectra were recorded using an Agilent 1200 Infinity high-performance liquid chromatograph and a 6420 Triple Quad LC-MS mass detector (Agilent Technologies, Santa Clara, CA, USA; electrospray ionization, +ESI TIC scan). IR spectra were recorded on an FTIR System 2000 spectrometer (PerkinElmer, Inc., Waltham, MA, USA) in KBr tablets. NMR spectra were recorded on JNM-ECX400 and JNM-ECZ600R spectrometers (Jeol, Akishima, Japan) at 400 and 600 MHz for ^1H and 100/150 MHz for ^{13}C ; CDCl_3 and $\text{DMSO}-d_6$ were used as solvents, with TMS (0 ppm) as the internal standard for ^1H NMR and the residual solvent signal (CDCl_3 , 77.16 ppm) as the internal reference for ^{13}C NMR. NMR spectra were processed using MestReNova 14.2.0 software (Mestrelab Research S.L., Santiago de Compostela, Spain). Reaction progress and the purity of the obtained compounds were monitored by thin-layer chromatography (TLC) on Silufol UV-254 plates (10 × 20 cm, Sigma-Aldrich GmbH, Steinheim, Germany) using the solvent systems CHCl_3 –MeOH 6:1 (System I) and 8:1 (System II), with visualization under 254 nm UV light. Melting points were determined on a Boetius microheating-stage apparatus (VEB Analytik, Dresden, Germany). Carbon, hydrogen, and nitrogen contents were determined using a Thermo Scientific FlashSmart elemental analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Prior to analysis, all hydrochloride salts were dried under high vacuum (≈ 0.1 mbar) at 50 °C for 24 h over P_2O_5 . Halogen contents were determined after combustion of the samples by the Schöniger flask method using an alkaline aqueous solution containing hydrogen peroxide. For compounds 2–5, the resulting chloride ions were quantified by potentiometric titration with a standard AgNO_3 solution. For compounds 1 and 6, which contain both chlorine and bromine, chloride and bromide ions were quantified separately by ion chromatography (Metrohm 930 Compact IC system equipped with a Metrosep A Supp 5 column, using a 3.2 mM Na_2CO_3 /1.0 mM NaHCO_3 aqueous eluent). For compound 3, the chlorine value represents total chlorine (covalently bound chlorine and chloride ion), since the combustion method does not distinguish between them. The found values agree with the calculated ones within $\pm 0.4\%$. The slight deviations observed in C and H values may be attributed to residual moisture, as hydrochloride salts are often hygroscopic.

2.2.2. General Procedure for the Synthesis of 1-Aryl-6,7-Methylenedioxy-1,2,3,4-Tetrahydroisoquinolines (Compounds 1, 2) and 2-Chloro-3-(6,7-Dimethoxy-1,2,3,4-Tetrahydroisoquinolin-1-yl)-8-Methylquinoline (Compound 3)

A solution of 3,4-methylenedioxyphenethylamine (0.84 g, 5 mmol) and the corresponding substituted benzaldehyde (5 mmol), or 2-chloro-8-methylquinoline-3-carbaldehyde, in benzene (30 mL) was refluxed using a Dean–Stark apparatus until no further water evolution was observed (approximately 2 h). The solvent was removed under reduced pressure, and trifluoroacetic acid (10 mL) was added to the resulting imine. The mixture was heated on a water bath for approximately 4 h. After completion of the reaction, the mixture was cooled and basified with aqueous ammonia to pH 9–10. The resulting amine was exhaustively extracted with chloroform. After removal of the solvent, the crude product was dissolved in acetone and acidified with concentrated hydrochloric acid to pH 5–6. The precipitated hydrochloride salt was collected by filtration and purified by recrystallization from acetone.

2.2.3. 1-(2'-Bromo-4',5'-Methylenedioxyphenyl)-6,7-Methylenedioxy-1,2,3,4-Tetrahydroisoquinoline (Compound 1)

Compound 1 was obtained from 3,4-methylenedioxyphenethylamine (0.84 g, 5 mmol) and 2-bromo-4,5-methylenedioxybenzaldehyde (0.76 g, 5 mmol) according to the general procedure. Yield: 1.64 g (79%). Melting point of the hydrochloride: 258–261 °C (acetone). R_f = 0.78 (System II). IR (KBr, ν_{max} , cm^{-1}): 3424, 2967, 2840, 2562, 1674, 1516, 1479, 1445, 1393, 1374, 1306, 1285, 1215, 1129, 1034, 998, 927, 861, 841, 801, 762, 719. Analysis calculated for $C_{17}H_{15}BrClNO_4$: C, 49.48; H, 3.66; N, 3.39; Cl, 8.59; Br, 19.36. Found: C, 49.25; H, 3.78; N, 3.28; Cl, 8.74; Br, 19.18. ^1H NMR (400 MHz, DMSO- d_6 , δ , ppm, J, Hz): 2.93 (2H, dt, J = 6.0, 12.0 Hz, H-4), 3.21 (2H, m, H-3), 5.75 (1H, s, H-1), 5.96 (2H, d, J = 6.0 Hz, 3'-OCH₂O-4'), 6.09 (1H, s, H-8), 6.10 (2H, d, J = 6.0 Hz, 6-OCH₂O-7), 6.86 (1H, s, H-5), 6.93 (1H, s, H-5'), 7.37 (1H, s, H-2'), 9.60 and 10.20 (0.5H each, br s, NH). Reaction progress and purity were confirmed by TLC (single spot).

2.2.4. 1-(3',4'-Dimethoxyphenyl)-6,7-Methylenedioxy-1,2,3,4-Tetrahydroisoquinoline (Compound 2)

Compound 2 was obtained from 3,4-methylenedioxyphenethylamine (0.84 g, 5 mmol) and veratraldehyde (0.85 g, 5 mmol) according to the general procedure. Yield: 1.10 g (62%). Melting point of the hydrochloride: 282–284 °C (acetone). R_f = 0.55 (System I). IR (KBr, ν_{max} , cm^{-1}): 3437, 2929, 2911, 1610, 1517, 1495, 1471, 1243, 1223, 1130, 1101, 1027, 955, 859. Analysis calculated for $C_{18}H_{20}ClNO_4$: C, 61.80; H, 5.76; N, 4.00; Cl, 10.13. Found: C, 61.49; H, 5.94; N, 4.07; Cl, 9.91. ^1H NMR (400 MHz, DMSO- d_6 , δ , ppm, J, Hz): 2.93 (2H, dt, J = 5.2, 17.2 Hz, H-4), 3.20 (2H, m, H-3), 3.74 (3H, s, 3'-OCH₃), 3.76 (3H, s, 4'-OCH₃), 5.55 (1H, s, H-1), 5.96 (2H, s, OCH₂O), 6.23 (1H, s, H-8), 6.77 (1H, d, J = 8.0 Hz, H-5'), 6.84 (1H, s, H-5), 6.99 (1H, d, J = 8.0 Hz, H-6'), 7.07 (1H, s, H-2'), 9.31 and 9.82 (0.5H each, br s, NH).

2.2.5. 2-Chloro-3-(6,7-Dimethoxy-1,2,3,4-Tetrahydroisoquinolin-1-yl)-8-Methylquinoline (Compound 3)

Compound 3 was synthesized according to the general procedure from 3,4-dimethoxyphenethylamine (1.37 g, 7.5 mmol) and 2-chloro-8-methylquinoline-3-carbaldehyde (1.55 g, 7.5 mmol). Yield: 1.90 g (73%). Melting point of the hydrochloride: 262–264 °C (MeOH). R_f = 0.22 (System I). IR (KBr, ν_{max} , cm^{-1}): 3165, 3006, 1641, 1613, 1516, 1257, 1223, 1102, 1035, 812, 754, 745, 539. Analysis calculated for $C_{21}H_{22}Cl_2N_2O_2$: C, 62.23; H, 5.47; N, 6.91; Total Cl, 17.49. Found: C, 62.05; H, 5.56; N, 6.76; Total Cl, 17.77. ^1H NMR (600 MHz, CDCl₃, δ , ppm, J, Hz): 10.86 (1H, NH-2'), 7.58 (1H, d, J = 7.2 Hz, H-5), 7.27 (1H, d, J

= 7.8 Hz, H-7), 7.16 (1H, s, H-4), 7.07 (1H, t, J = 7.6 Hz, H-6), 6.68 (1H, s, H-5'), 6.43 (1H, s, H-8'), 5.45 (1H, s, H-1'), 3.89 (3H, s, 6'-OCH₃), 3.72 (3H, s, 7'-OCH₃), 2.89–3.00 (3H, m, H-3' and H_a-4'), 2.70–2.75 (1H, m, H_b-4'). ¹³C NMR (150 MHz, CDCl₃, δ , ppm): 163.64 (C-2), 148.01 (C-6'), 147.28 (C-7'), 139.50 (C-4), 136.31 (C-8a), 134.80 (C-8), 131.53 (C-7), 128.57 (C-4a), 127.24 (C-8'a), 126.34 (C-5), 123.38 (C-3), 122.43 (C-6), 119.64 (C-4'a), 111.77 (C-5'), 110.98 (C-8'), 56.02 (6'-OCH₃), 55.94 (7'-OCH₃), 54.80 (C-1'), 39.25 (C-3'), 29.18 (C-4'), 17.18 (8-CH₃).

2.2.6. 1-(4-Nitrophenyl)-2-Benzyl-6,7-Dimethoxy-1,2,3,4-Tetrahydroisoquinoline (Compound 4)

A mixture of 1-(4-nitrophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (2.00 g, 6.37 mmol), potassium carbonate (1.76 g, 12.7 mmol), and benzyl chloride (1.61 g, 12.7 mmol) was reacted, and the product was isolated and purified to afford compound 4. Yield: 2.29 g (89%). Melting point: 134–136 °C (acetone). R_f = 0.61 (System V). Analysis calculated for C₂₄H₂₅ClN₂O₄: C, 65.38; H, 5.72; N, 6.35; Cl, 8.04. Found: C, 65.11; H, 5.87; N, 6.54; Cl, 7.90. ¹H NMR (600 MHz, CDCl₃, δ , ppm, J , Hz): 8.18 (2H, d, J = 8.9 Hz, H-3',5'), 7.54 (2H, d, J = 8.9 Hz, H-2',6'), 7.32–7.27 (4H, m, Ar-H), 7.26–7.23 (1H, m, Ar-H), 6.64 (1H, s, H-8), 6.14 (1H, s, H-5), 4.68 (1H, s, H-1), 3.86 (3H, s, 7-OCH₃), 3.72 (1H, d, J = 13.5 Hz, H_a-9), 3.62 (3H, s, 6-OCH₃), 3.37 (1H, d, J = 13.5 Hz, H_b-9), 3.06 (1H, dt, J = 11.9, 5.0 Hz, H-3), 2.97–2.92 (1H, m, H-3), 2.74 (1H, dt, J = 16.0, 4.4 Hz, H-4), 2.57 (1H, ddd, J = 11.9, 8.9, 4.1 Hz, H-4). ¹³C NMR (150 MHz, CDCl₃, δ , ppm): 152.54 (C-1'), 147.99 (C-6), 147.44 (C-7), 147.29 (C-4'), 138.90 (C-1''), 130.28 (C-2',6'), 128.75 (C-3'',5''), 128.43 (C-2'',6''), 128.22 (C-8a), 127.26 (C-4a, 4''), 123.64 (C-3',5'), 111.46 (C-5), 111.34 (C-8), 67.15 (C-1), 58.91 (C-9), 55.99 (6-OCH₃), 55.94 (7-OCH₃), 46.82 (C-3), 28.10 (C-4).

2.2.7. N-(5'-Formyl-2'-Methoxybenzyl)Cytisine (Compound 5)

A mixture of cytisine (1.0 g, 5.26 mmol), 3-chloromethyl-4-methoxybenzaldehyde (0.97 g, 5.25 mmol), and Na₂CO₃ (0.2 g, 1.89 mmol) in ethanol (10 mL) was stirred at room temperature for 3 days; reaction progress was monitored by TLC. The inorganic precipitate was filtered off, and the filtrate was concentrated by evaporation of the solvent. Upon standing, crystals formed, which were collected by filtration and dried at room temperature to afford compound 5. Yield: 1.3 g (73%); R_f = 0.2 (System I); Analysis calculated for C, 64.08; H, 6.18; N, 7.47; Cl, 9.46. Found: C, 63.94; H, 6.29; N, 7.26; Cl, 9.63. ¹H NMR (600 MHz, CDCl₃, δ , ppm, J , Hz) 9.59 (1H, s, CHO), 7.70 (1H, dd, J = 8.48, 2.10 Hz, H-4'), 7.25 (2H, m, H-4, H-6'), 6.84 (1H, d, J = 8.5 Hz, H-3'), 6.46 (1H, dd, J = 9.1, 1.6 Hz, H-3), 5.89 (1H, dd, J = 6.8, 1.6 Hz, H-5), 4.09 (1H, d, J = 15.2 Hz, H_{eq}-10), 3.85 (1H, dd, J = 15.2, 6.5 Hz, H_{ax}-10), 3.76 (3H, s, OCH₃), 3.47 (1H, d, J = 14.9 Hz, H_{eq}-14), 3.40 (1H, d, J = 14.9 Hz, H_{ax}-14), 2.93 (1H, d, J = 10.9 Hz, H_{eq}-11), 2.90 (1H, s, H-7), 2.82 (1H, dd, J = 10.7, 1.67 Hz, H_{eq}-13), 2.52 (1H, d, J = 10.8 Hz, H_{ax}-11), 2.42 (1H, d, J = 2.8 Hz, H-9), 2.29 (1H, dd, J = 10.7, 1.8 Hz, H_{ax}-13), 1.83 (2H, m, H-8).

2.2.8. N-(3'-Hydroxy-4'-Methoxy-6'-Bromobenzyl)Cytisine (Compound 6)

A mixture of cytisine (1.90 g, 0.010 mol) and 3-hydroxy-4-methoxy-6-bromobenzaldehyde (2.60 g, 0.012 mol) was refluxed in benzene (30 mL) for 6 h; reaction progress was monitored by TLC. After completion, the solvent was evaporated under reduced pressure, methanol (20 mL) was added to the residue, and the mixture was cooled in an ice bath for 1 h before NaBH₄ (5.0 g) was added. The solvent was removed to dryness, and the resulting precipitate was dissolved in water (20 mL) and exhaustively extracted with chloroform. The product was purified by crystallization or column chromatography on silica gel (chloroform–methanol gradient, 100:0 to 100:10, v/v) and crystallized from methanol to

afford compound **6**. Yield: 3.0 g (74%); Melting point 180–181 °C (MeOH); R_f = 0.63. Analysis calculated for $C_{19}H_{22}BrClN_2O_3$: C, 51.66; H, 5.02; N, 6.34; Cl, 8.02; Br, 18.09. Found: C, 51.32; H, 5.19; N, 6.38; Cl, 7.77; Br, 18.30. ^1H NMR (400 MHz, DMSO- d_6 , δ , ppm, J, Hz): 8.94 (1H, s, 3'-OH), 7.22 (1H, dd, J = 9.0, 7.0, H-4), 6.92 (1H, s, H-5'), 6.44 (1H, s, H-2'), 6.14 (1H, dd, J = 9.0, 1.4, H-3), 5.94 (1H, dd, J = 1.4, 7.0, H-5), 3.67 (4H, br s, 2H-10, 2H-14), 2.38 (3H, 4'-OCH₃), 2.92, 2.77–2.69 (3H, H-7, H_{eq}-11, H_{eq}-13), 2.20–2.28 (3H, H-9, H_{ax}-11, H_{ax}-13), 1.68 (2H, m, H-8).

For compounds **1–6**, chemical identity was confirmed by ^1H (and, where noted, ^{13}C) NMR and IR spectroscopy, and for compound **5**, the structural identity was additionally supported by an X-ray crystallographic image. Reaction progress and homogeneity were monitored by TLC in two independent solvent systems, with a single spot observed for the purified compounds. The original spectral images for newly synthesized compounds (**1–4**) and the X-ray structure image for compound **5** are available in the Supplementary Materials. Detailed textual analytical data for all compounds are provided in this experimental section.

2.3. Molecular Docking Protocol

Cyclooxygenase-2 and the human H₁ histamine receptor were selected as the molecular targets for evaluating the newly synthesized compounds. Molecular docking studies were performed for the six investigated compounds against the crystal structures of COX-2 (PDB ID: 1PXX) and the human H₁ histamine receptor (PDB ID: 8X64). The corresponding three-dimensional protein structures were retrieved from the Worldwide Protein Data Bank (PDB). For the COX-2 structure (1PXX), only Chain A was used in the docking simulations to ensure consistency with previous computational studies [20–22]. During protein preparation, all crystallographic water molecules and non-essential heteroatoms were removed using BIOVIA Discovery Studio Visualizer [23].

Prior to docking, to accurately simulate physiological conditions and address potential conformational variations, the tautomeric and protonation states of all investigated ligands and reference drugs were explicitly evaluated. The basic nitrogen atoms of the molecules were protonated at a physiological pH of 7.4. Subsequently, the 3D geometries of the protonated ligands were subjected to rigorous energy minimization using Avogadro software to obtain their most thermodynamically stable conformations. The optimized ligands and prepared receptor structures were then imported into PyRx v0.8, where polar hydrogen atoms were appropriately added, and Gasteiger partial atomic charges were assigned prior to their conversion into the required PDBQT format [24].

Ligand–receptor interactions were modeled using the AutoDock Vina v1.1.2 docking engine (Wizard Vina) implemented in PyRx v0.8 [25,26]. To ensure precise and complete coverage of the specific ligand-binding pockets, the docking search exhaustiveness parameter was set to 16 for both target proteins. For COX-2 (1PXX), the grid box was specifically centered at $x = 28.11$, $y = 23.42$, and $z = 16.33$ Å, with spatial dimensions of $20 \times 20 \times 20$ Å [21]. For the human H₁ histamine receptor (8X64), the search space was precisely defined to encompass the transmembrane binding cavity, with grid center coordinates of $x = 136.80$, $y = 130.01$, and $z = 173.41$ Å, and box dimensions of $15 \times 15 \times 15$ Å [22]. The resulting binding poses and predicted binding affinities were analyzed and visualized using BIOVIA Discovery Studio Visualizer v21.1.0.20298 and PyMOL v3.1.8, allowing a comprehensive comparative evaluation of the binding modes of the synthesized compounds relative to the reference drugs.

To assess the reliability and predictive performance of the docking protocol, a re-docking validation procedure was carried out using the PyRx/AutoDock Vina workflow [27]. The native co-crystallized ligands were extracted from their respective crystal structures and rigorously re-docked into the defined active sites. The accuracy of the docking

protocol was evaluated by calculating the root-mean-square deviation (RMSD) between the experimentally determined X-ray crystallographic conformations and the top-ranked predicted docking poses.

2.4. Biological Evaluation

The study was conducted on outbred white male rats weighing 200–220 g. The animals were maintained under standard laboratory conditions: temperature at 23 ± 2 °C, relative humidity of 48%, 12/12 h light/dark cycle, and ad libitum access to food and water. Dose selection and administration methods were carried out in accordance with the requirements of current guidelines for preclinical studies [28–30]. The study was performed in compliance with bioethical standards, including the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes and EU Directive 2010/63/EU [31–33].

Acute toxicity was evaluated as described in [13] in male albino laboratory mice weighing 18–21 g. Solutions of the test compounds at concentrations of 1%, 5%, and 10% were prepared and administered once orally to groups of six mice at each tested concentration. Following administration, the animals were maintained under standard vivarium conditions and observed for 14 days. During the observation period, the general condition and behavior of the animals were systematically monitored, including locomotor activity, respiratory pattern, response to external stimuli, the presence and severity of clinical signs of toxicity, and mortality. The onset, severity, and duration of any signs of intoxication were also recorded.

The median lethal dose (LD₅₀) of each test compound was determined using the Litchfield–Wilcoxon method. The calculated LD₅₀ values ranged from 320.0 to 1950.0 mg/kg. According to the toxicity classification proposed by Stefanov, A. V. the test compounds were assigned to toxicity Classes III–IV, corresponding to moderately toxic and low-toxicity substances, respectively [29].

The effects of the tested compounds on the inflammatory response development and cytokine levels were investigated using a formalin rat model as described before [16]. An acute inflammatory response was induced by the subplantar injection of 0.1 mL of a 2.5% formalin solution into the right hind paw of the experimental animals. The formalin solution was administered beneath the plantar aponeurosis under aseptic conditions. The animals were allocated to the following experimental groups:

Group I (intact group): healthy animals in which inflammation was not induced and which were used to establish baseline physiological values;

Group II (control group): animals in which acute inflammation was induced by formalin and which received no pharmacological treatment;

Group III (reference drug groups): animals that received diclofenac sodium orally at doses of 8.0 or 10.0 mg/kg prior to the induction of acute inflammation with formalin;

Group IV (experimental groups): animals that received the candidate compounds orally at doses of 0.1, 0.5, 1.0, 5.0, or 10.0 mg/kg prior to the induction of acute inflammation with formalin.

For oral administration, diclofenac sodium and all investigated compounds were freshly dissolved in distilled water immediately before use. As all test compounds were studied in their hydrochloride salt form, they exhibited high aqueous solubility, readily forming clear, transparent solutions; no organic co-solvents or surfactants were required. Solutions were administered by oral gavage in a volume adjusted to the body weight of each animal.

To evaluate changes in serum cytokine levels during the acute inflammatory response, blood samples were collected at two time points: before induction of inflamma-

tion, to determine baseline cytokine concentrations, and 180 min after formalin administration. Serum was separated from the blood samples, and cytokine concentrations were determined on the day of sample collection. The obtained results were used for a comparative assessment of the effects of the candidate compounds on serum cytokine levels in comparison with the untreated inflammatory control and reference drug groups.

Serum concentrations of interleukin-4 (IL-4), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α), which are key cytokines involved in the regulation of the inflammatory and immune responses, were determined using an enzyme-linked immunosorbent assay (ELISA). The analyses were performed using a *Mindray MR-96A* automated microplate reader (Mindray, China) and certified diagnostic ELISA kits manufactured by Vector-Best LLC (Russia), in accordance with the manufacturer's instructions.

Data are presented as means $\pm$ SEMs for different groups. Statistical analysis was performed using GraphPad Prism 10 software (GraphPad Software, San Diego, CA, USA), and significant differences between groups were assessed by one-way ANOVA followed by Dunnett's multiple comparisons test versus the control group.

In addition to oral administration, the compounds that showed the highest anti-inflammatory activity in the oral formalin-induced paw edema model were selected for topical evaluation. Ointment formulations of these compounds, at concentrations of 1%, 2%, or 5% (*w/w*), were prepared by pharmaceutical formulation specialists using standard semisolid dosage-form technology; 1% diclofenac sodium ointment served as the reference formulation. Animals were allocated to an intact group (no formalin, no treatment), a formalin-control group (formalin-induced inflammation, untreated), a reference group receiving 1% diclofenac sodium ointment, and experimental groups receiving the 1%, 2%, or 5% ointment formulations of the investigated compounds. Formulations were applied topically to the right hind paw immediately after the subplantar injection of formalin. Paw volume was measured at baseline, 3 h after formalin administration, and on days 2, 4, 6, 8, and 10 thereafter, using a plethysmometer (Ugo Basile, Gemonio, Italy).

The anti-inflammatory activity of the investigated compounds in the histamine-induced inflammation model was evaluated using two separate routes of administration: oral and topical.

For the oral protocol, animals were allocated to an intact group (no histamine injection, no treatment), a histamine-control group (histamine-induced edema, untreated), reference groups receiving desloratadine (0.01, 0.05, or 0.1 mg/kg) or diclofenac sodium (8.0 or 10.0 mg/kg) orally, and experimental groups receiving the investigated compounds (0.1, 0.5, 1.0, 5.0, or 10.0 mg/kg) orally, prior to the subplantar injection of 0.1 mL of a 0.1% histamine solution into the right hind paw. As in the formalin model, all solutions for oral administration were freshly prepared in distilled water immediately before use, taking advantage of the high aqueous solubility of the hydrochloride salt forms of the test compounds. Paw volume was recorded before histamine injection (initial) and 180 min thereafter using a plethysmometer (Ugo Basile, Gemonio, Italy).

For the topical protocol, 0.1 mL of a 0.1% histamine solution was administered subcutaneously into the rat's right hind paw. Following histamine administration, the reference formulations, namely 1.0% diclofenac gel and Fenistil® Gel (0.1% dimethindene maleate), as well as the test formulations containing the investigated compounds at concentrations of 1%, 2%, and 5%, were applied topically to the right hind paw of the experimental animals. The topical test formulations were prepared as ointments by pharmaceutical formulation specialists, in the same manner as the ointment formulations used in the formalin-induced paw edema model, at the indicated concentrations of 1%, 2%, and 5% (*w/w*). To evaluate the progression of the inflammatory response, paw volume was measured again 180 min after histamine injection using a plethysmometer (Ugo Basile, Gemonio, Italy).

3. Results

3.1. Molecular Docking

To elucidate the molecular mechanism underlying the anti-inflammatory activity of the newly synthesized compounds and to evaluate their binding affinity toward selected biological targets, an *in silico* molecular docking study was performed. Prior to docking, all ligands were prepared and protonated at a physiological pH of 7.4. The interactions of the synthesized compounds with cyclooxygenase-2 (COX-2; PDB ID: 1PXX) and the human H₁ histamine receptor (PDB ID: 8X64) were investigated. The docking results, including binding energies and the amino acid residues involved in stabilizing the ligand–protein complexes, were comparatively analyzed with those of the reference drugs diclofenac, desloratadine, and dimethindene (Tables 2 and 3).

The docking analysis demonstrated that all synthesized compounds exhibited favorable conformational fitting within the binding pockets of both target proteins. In the active site of COX-2, the calculated binding energies ranged from −8.2 to −9.2 kcal/mol, indicating favorable ligand–enzyme interactions. Likewise, the compounds showed high binding affinity toward the H₁ histamine receptor, with binding energies ranging from −7.9 to −10.0 kcal/mol, suggesting the formation of stable ligand–receptor complexes.

The reference compounds were subsequently analyzed to provide a comparative assessment of the binding modes. Diclofenac, a well-established nonsteroidal anti-inflammatory drug (NSAID), exhibited a binding energy of −8.9 kcal/mol. Molecular docking revealed the formation of hydrogen bonds with SER530 and TYR385, as well as a π –sulfur interaction with MET522. In addition, the ligand was stabilized by a π – π T-shaped interaction with TRP387, together with π –alkyl hydrophobic interactions involving VAL349, VAL523, and ALA527.

Desloratadine demonstrated a binding energy of −8.5 kcal/mol and formed hydrogen bonds with TYR385 and SER530. Additional stabilization of the ligand within the binding cavity was achieved through π –sigma and alkyl interactions with VAL349, ALA527, and VAL523, along with π –alkyl interactions involving LEU352, PHE518, and LEU531.

Among the reference compounds, dimetindene exhibited the highest binding affinity toward COX-2, with a binding energy of −9.0 kcal/mol. Its binding mode was characterized by the formation of a carbon–hydrogen bond with TYR385, a π –sigma interaction with VAL523, and an extensive network of hydrophobic interactions involving VAL349, LEU531, ALA527, LEU352, TYR355, VAL116, and LEU359. Collectively, these interactions contributed to the high stability of the ligand–protein complex (Table 2).

Dimethindene exhibited a binding affinity toward COX-2 with a binding energy of −8.8 kcal/mol. Its binding mode was characterized by the formation of a carbon–hydrogen bond and a π –sulfur interaction with MET522, alongside an amide– π -stacked interaction with GLY526. Furthermore, the complex was stabilized by π –sigma interactions with VAL523, as well as alkyl and π –alkyl interactions involving VAL349 and LEU352. Collectively, these interactions contributed to the high stability of the ligand–protein complex (Table 2).

Table 2. Binding affinities (kcal/mol) and molecular interactions of the synthesized compounds (1–6) and reference drugs within the active site of COX-2 (PDB ID: 1PXX).

Compounds	Binding Affinity (kcal/mol)	Molecular Interactions
Diclofenac	−8.9	1 HBD with SER530, TYR385, 1 pi-sulfur with MET522, 1 pi-pi t-shaped with TRP387, 1 pi-alkyl with VAL349, VAL523, ALA527
Desloratadine	−8.5	1 HBA with TYR385 and SER530, 1 p-sigma and 1 alkyl with VAL349, ALA527, 2 pi-sigma bonds and 2 alkyls with VAL523, 1 pi-alkyl with LEU352, PHE518, LEU531
Dimethindene	−8.8	1 carbon–hydrogen bond and 1 pi-sulfur with MET522, 1 amide-pi-stacked with GLY526, 2 pi-sigma bonds with VAL523, 1 alkyl with VAL349, 1 pi-alkyl with LEU352
Compound 1	−9.2	1 HBD with ARG120, 1 HBA with SER530, 1 carbon hydrogen bond with SER353, 1 pi-sigma with VAL349 and LEU531, 1 alkyl with VAL116, LEU359, MET522, 1 pi-alkyl with VAL523, TYR355, PHE518, TRP387, 2 pi-alkyls with ALA527
Compound 2	−8.2	1 pi-sigma with SER353, 2 pi-sigma bonds and 2 alkyls with VAL523, 1 alkyl with ALA516, 1 pi-alkyl with ALA527, VAL349, TYR355, 2 pi-alkyl with HIS90
Compound 3	−8.3	1 HBD with ARG120, 1 HBA with SER530, 1 pi-sigma with PHE518, 1 pi-sigma and 1 pi-alkyl with VAL349, 1 pi-sulfur and 1 alkyl with MET522, 1 alkyl with VAL116, 2 alkyls with VAL523, 1 pi-alkyl with LEU531, LEU352, 2 pi-alkyls with TYR355, 1 alkyl and 2 pi-alkyls with ALA527
Compound 4	−9.0	1 HBD with ARG513, 1 pi-donor hydrogen bond with HIS90, 1 pi-sigma with VAL349, 2 pi-sigma bonds with VAL523, 1 pi-sulfur with MET522, 1 pi-pi t-shaped with TRP387, 1 amide-pi stacked with GLY526, 1 alkyl with MET113, LEU359, ILE345, 2 alkyls with VAL116, 1 pi-alkyl with LEU384, TYR355, ALA527, 1 alkyl and 1 pi-alkyl with LEU531
Compound 5	−9.2	1 alkyl with ILE345, LEU352, 1 alkyl and 1 pi-alkyl with LEU531, LEU359, 2 alkyls and 1 pi-alkyl with VAL349, 1 pi-alkyl with VAL116, ALA527
Compound 6	−8.7	1 HBD and 1 pi-alkyl with TYR355, 1 carbon hydrogen bond with SER530, 1 alkyl with LEU352, ILE345, VAL116, 1 alkyl and 1 pi-alkyl with LEU531, 2 alkyls and 1 pi-alkyl with LEU359, VAL349, 1 pi-alkyl with ALA527

Analysis of the interactions between the H₁ histamine receptor and the reference ligands revealed distinct binding modes for each compound. Diclofenac exhibited a binding energy of −7.9 kcal/mol and formed hydrogen bond donor interactions with TYR431 and TRP428. The stability of the ligand within the receptor binding pocket was further enhanced by π – π T-shaped and π – π stacked interactions with TRP428 and TYR108, respectively, as well as π –anion contacts involving ASP107 and TYR458.

The tricyclic reference compounds, dimetindene (−9.3 kcal/mol) and desloratadine (−9.1 kcal/mol), penetrated more deeply into the receptor binding cavity than diclofenac. Both compounds established strong hydrogen bonds with TYR458, ASP107, and ASN198, together with extensive π – π hydrophobic interactions involving the aromatic residues TRP428, PHE432, and PHE435, resulting in the formation of highly stable ligand–receptor complexes (Table 3).

The tricyclic reference compounds, dimethindene (−8.4 kcal/mol) and desloratadine (−8.2 kcal/mol), penetrated deeply into the receptor binding cavity and established crucial electrostatic anchoring. Most notably, both compounds successfully formed the essential salt bridge interaction with ASP107, a critical requirement for H₁ receptor antagonism. In addition to this electrostatic bond, dimethindene was stabilized by a hydrogen bond with SER111, a carbon–hydrogen bond with ASP107, multiple π – π stacked and T-shaped interactions with TYR431, TYR458, PHE435, and TYR108, and a π –alkyl interaction with

ILE454. Similarly, desloratadine formed hydrogen bonds with TYR458, TYR431, and SER111, alongside a π -donor hydrogen bond with TYR108 and extensive π -alkyl hydrophobic interactions involving TYR87, ILE454, and TRP103, resulting in the formation of highly stable ligand–receptor complexes (Table 3).

Table 3. Binding affinities (kcal/mol) and molecular interactions of the synthesized compounds (1–6) and reference drugs within the active site of the human H₁ histamine receptor (PDB ID: 8X64).

Compounds	Binding Affinity (kcal/mol)	Molecular Interactions
Diclofenac	−7.9	1 HBD with TYR431, 1 HBD and 2 pi-pi t-shaped bonds with TRP428, 1 pi-pi stacked with TYR108, 1 pi-anion with ASP107, TYR458
Desloratadine	−8.2	1 HBD with TYR458, TYR431, SER111, 1 salt bridge with ASP107, 1 pi-donor hydrogen bond with TYR108, 1 pi-alkyl with TYR87, ILE454, TRP103
Dimethindene	−8.4	1 HBD with SER111, 1 pi-donor hydrogen bond, 1 pi-pi t-shaped with TYR431, 1 pi-donor hydrogen bond, 1 pi-pi stacked with TYR458, 1 pi-pi stacked with PHE435, 1 pi-pi t-shaped with TYR108, 1 carbon hydrogen bond and 1 salt bridge with ASP107, 1 pi-alkyl with ILE454
Compound 1	−8.9	1 HBD with TYR458, 1 pi-donor hydrogen bond, 1 pi-pi stacked, 1 pi-alkyl with TYR431, 1 salt bridge with ASP107, 1 alkyl with LEU104, 2 pi-alkyls with TRP428
Compound 2	−8.2	1 HBD and 1 pi-alkyl with TYR108, 1HBA, 1 pi-donor hydrogen bond and 1 pi-pi stacked with TYR458, 1 pi-donor hydrogen bond, 1 pi-pi stacked, 1 pi-alkyl with TYR431, 2 pi-sigma bonds with TRP428, 1 salt bridge with ASP107, 1 pi-alkyl with PHE432, ILE454
Compound 3	−10.0	1 HBD with TYR458, 1 HBD and 1 pi-pi t-shaped with TYR108, 1 pi-donor hydrogen bond and 2 pi-pi stacked bonds with TYR431, 2 pi-pi t-shaped bonds with TRP428, 1 salt bridge with ASP107, 1 pi-alkyl with LEU104
Compound 4	−7.9	1HBD with LYS191, 1HBA and 1 pi-pi t-shaped with TYR108, 1 pi-sigma and 1 pi-pi stacked with HIS450, 1 salt bridge and 1 pi-anion with ASP107, 1 pi-alkyl with TYR87
Compound 5	−8.7	1HBD with THR182, TRP428, 1 carbon–hydrogen bond, 1 pi-donor hydrogen bond, 1 pi-alkyl and 1 pi-pi stacked with TYR431, 1 carbon–hydrogen bond and 1 salt bridge with ASP107, 1 pi-cation with TYR108
Compound 6	−8.7	1 carbon–hydrogen bond with HIS450, 1 carbon–hydrogen bond, 1 pi-donor hydrogen bond, 1 pi-pi stacked with TYR431, 1 pi-sigma, 1 pi-alkyl with TRP428, 2 carbon–hydrogen bonds and 1 salt bridge with ASP107, 1 pi-alkyl with TYR108, ILE454, PHE432

Among the synthesized compounds, compounds **1** and **5** exhibited the highest binding affinities toward cyclooxygenase-2 (COX-2), both demonstrating a binding energy of −9.2 kcal/mol. The exceptional stability of compound **1** within the active site can be attributed to the formation of classical hydrogen bonds with the catalytically important residues ARG120 and SER530, as well as a carbon–hydrogen bond with SER353 (Figure 1A). Furthermore, the spatial structure of compound **1** established an extensive network of hydrophobic and π -interactions, including π -sigma contacts with VAL349 and LEU531, and π -alkyl interactions involving VAL523, TYR355, PHE518, TRP387, and ALA527. This comprehensive interaction profile resulted in a highly favorable conformational orientation within the binding pocket.

In contrast, compound **2** exhibited a slightly lower binding affinity (−8.2 kcal/mol). As depicted in Figure 1B, its stabilization within the binding cavity was predominantly driven by hydrophobic forces rather than conventional hydrogen bonds, establishing π -sigma interactions with SER353 and VAL523, alongside a network of alkyl and π -alkyl

contacts with ALA516, ALA527, VAL349, TYR355, and HIS90. For comparative assessment, the binding mode of the reference NSAID diclofenac (−8.9 kcal/mol) is illustrated in Figure 1C. Diclofenac firmly anchored to the active site via classical hydrogen bonds with SER530 and TYR385, complemented by a specific π -sulfur interaction with MET522 and a π - π T-shaped interaction with TRP387. Overall, the docking data indicates that derivatives capable of forming direct hydrogen bonds with key catalytic residues (such as compounds 1, 4, and 5) achieve binding affinities comparable to or exceeding those of the established reference drugs, while compounds relying primarily on hydrophobic contacts (like compounds 2 and 3) exhibit moderate, yet favorable, binding stability.

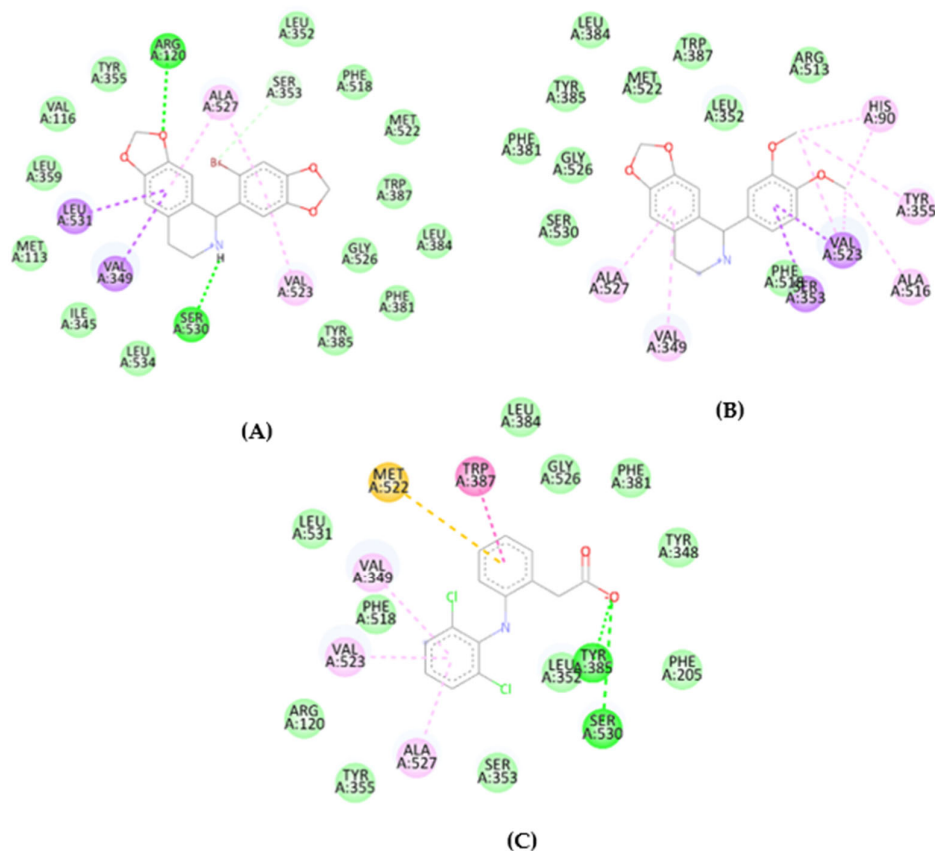


Figure 1. 2D interaction profiles of selected ligands with amino acid residues in the active site of COX-2 (PDB ID: 1PXX). (A) Compound 1 (−9.2 kcal/mol), (B) Compound 2 (−8.2 kcal/mol), and (C) Diclofenac (−8.9 kcal/mol). Color coding of intermolecular interactions: conventional hydrogen bond, green; carbon–hydrogen bond, light green; π -sigma interaction, dark purple; π - π T-shaped and stacked interactions, pink; alkyl and π -alkyl interactions, light pink; π -sulfur interaction, dark orange. Dashed lines indicate interactions between the ligand and amino acid residues.

Within the binding pocket of the human H_1 histamine receptor, simulating physiological conditions (pH 7.4) through explicit ligand protonation proved crucial for accurate interaction modeling. The docking results revealed that compound 3 exhibited the highest therapeutic potential and the strongest binding affinity (−10.0 kcal/mol), surpassing all reference compounds. The exceptional stability of this complex was primarily driven by its protonated basic nitrogen, which successfully established a critical electrostatic salt bridge with the conserved acidic residue ASP107. Furthermore, compound 3 was firmly accommodated within the receptor cavity through conventional hydrogen bonds with

TYR458 and TYR108, alongside a network of π - π stacked and T-shaped interactions involving TYR431 and TRP428, and a π -alkyl contact with LEU104 (Figure 2A).

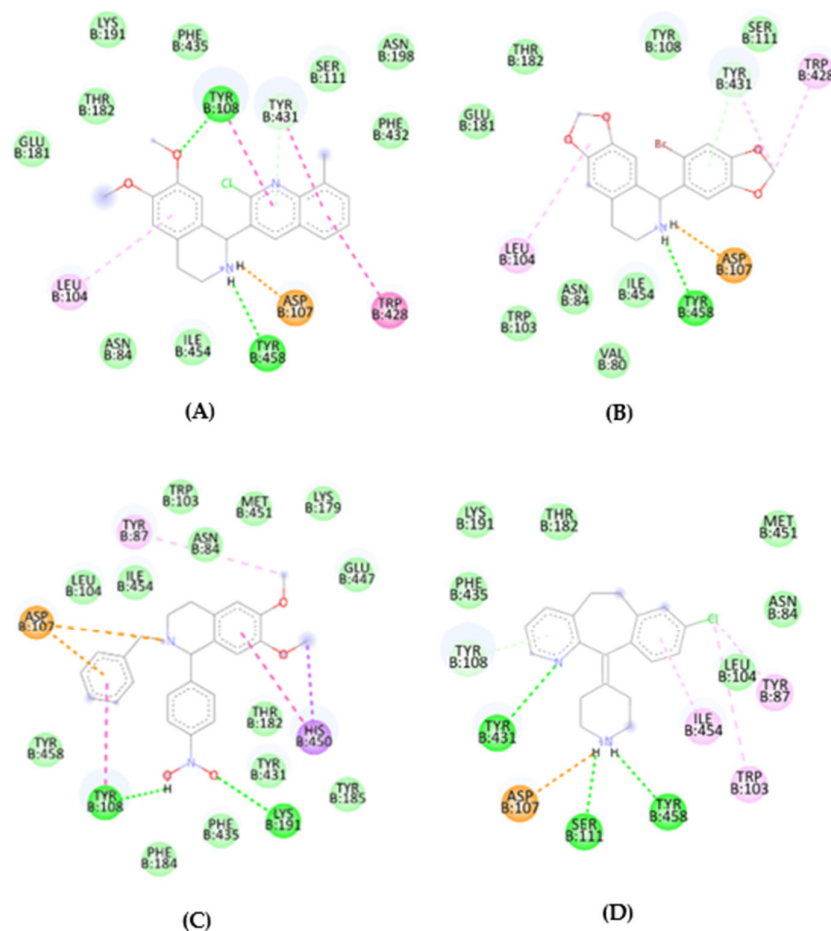


Figure 2. 2D interaction profiles of selected ligands with amino acid residues in the binding site of the human H₁ histamine receptor (PDB ID: 8X64). (A) Compound **3** (−10.0 kcal/mol), (B) Compound **1** (−8.9 kcal/mol), (C) Compound **4** (−7.9 kcal/mol), and (D) Desloratadine (−8.2 kcal/mol). Color coding of intermolecular interactions: conventional hydrogen bond, green; carbon–hydrogen bond, light green; salt bridge and π -anion interactions, dark orange; π -sigma interaction, dark purple; π - π stacked and π - π T-shaped interactions, pink; alkyl and π -alkyl interactions, light pink. Dashed lines indicate interactions between the ligand and amino acid residues.

Compound **1** (−8.9 kcal/mol) also demonstrated a highly favorable binding profile, surpassing the reference drug desloratadine (−8.2 kcal/mol). As depicted in Figure 2B, compound **1** maintained the essential salt bridge with ASP107, supplemented by a hydrogen bond with TYR458 and alkyl/ π -alkyl interactions with LEU104 and TRP428. In contrast, compound **4** exhibited the lowest binding affinity (−7.9 kcal/mol) among the selected series. Nevertheless, its protonated state enabled a dual electrostatic anchoring mechanism with ASP107, characterized by both a salt bridge and a π -anion interaction, complemented by hydrogen bonds with LYS191 and TYR108 (Figure 2C). For comparative evaluation, the binding mode of the reference antihistamine desloratadine is illustrated in Figure 2D, which similarly highlights the indispensable salt bridge with ASP107 alongside

hydrogen bonds with TYR458, TYR431, and SER111. Collectively, these findings underscore that ligand protonation and subsequent salt bridge formation with ASP107 are pivotal for stabilizing H₁ receptor–ligand complexes.

The three-dimensional representation of the globular structure of cyclooxygenase-2 (COX-2) illustrates that compound **5** penetrates deeply into the hydrophobic core of the enzyme (Figure 3A). Prior to the docking simulations, the structural preparation of the ligands included a rigorous evaluation of their potential tautomeric and protonation states, ensuring that the most thermodynamically stable tautomer at physiological pH (7.4) was selected for accurate 3D modeling. The enlarged 3D view demonstrates that the planar aromatic rings and functional groups of compound **5** fit into the active site without steric hindrance and are optimally oriented with respect to the surrounding amino acid residues. Specifically, the ligand is enveloped by a favorable hydrophobic microenvironment, establishing extensive alkyl and π -alkyl interactions with LEU359, VAL116, ILE345, LEU352, LEU531, and ALA527. Such a highly complementary binding mode accounts for the potent binding affinity (−9.2 kcal/mol) and the conformational stability of the ligand–protein complex [34].

Analysis of the binding mode of compound **3** within the seven-transmembrane (7TM) cavity of the human H₁ histamine receptor revealed that the ligand adopts a highly stable vertical orientation within the receptor channel (Figure 3B). Three-dimensional visualization of the binding microenvironment showed that the correctly protonated tautomeric state of the ligand forms an extensive and well-oriented network of electrostatic and hydrophobic interactions with key functional residues. Most notably, the protonated nitrogen atom is positioned in precise spatial proximity to ASP107, facilitating the critical salt bridge interaction. Furthermore, the molecular scaffold is securely anchored by strong contacts with surrounding aromatic and non-polar residues, including TYR108, LEU104, TRP428, TYR431, and TYR458. This precise spatial arrangement tightly restricts ligand displacement from the binding pocket, thereby contributing to the exceptional binding affinity (−10.0 kcal/mol) of the complex and strongly supporting its potential antihistamine activity [35].

Three-dimensional visualization of compound **3** within the active site of cyclooxygenase-2 (COX-2, PDB ID: 1PXX) revealed a highly favorable accommodation within the enzyme's binding pocket (Figure 4). Contrary to initial structural estimations, compound **3** successfully penetrated the hydrophobic core, achieving a solid binding affinity of −8.3 kcal/mol. This stability is driven by an optimal spatial geometry that permits key interactions with critical residues, including ARG120, SER530, MET522, and VAL349. Consequently, the ligand established a stable ligand–enzyme complex without significant steric hindrance.

However, the most remarkable binding profile for compound **3** was observed within the transmembrane cavity of the human H₁ histamine receptor (PDB ID: 8X64), where it achieved an outstanding binding affinity of −10.0 kcal/mol (Figure 4). The enlarged 3D visualization explicitly highlights the formation of a quintessential biological anchor: a highly stable salt bridge between the protonated basic nitrogen of the ligand and the highly conserved acidic residue ASP107. As a fundamental prerequisite for H₁ receptor antagonism, this specific electrostatic interaction restricts the conformational flexibility of the ligand within the pocket. The structural rigidity of this complex is further magnified by a dense network of supportive interactions, including π – π stacked and π – π T-shaped contacts with TRP428 and TYR431, alongside strong hydrogen bonding with TYR458 and TYR108, and a π -alkyl contact with LEU104. Together, this comprehensive interaction profile provides a compelling structural basis for the compound's potent therapeutic potential [36,37].

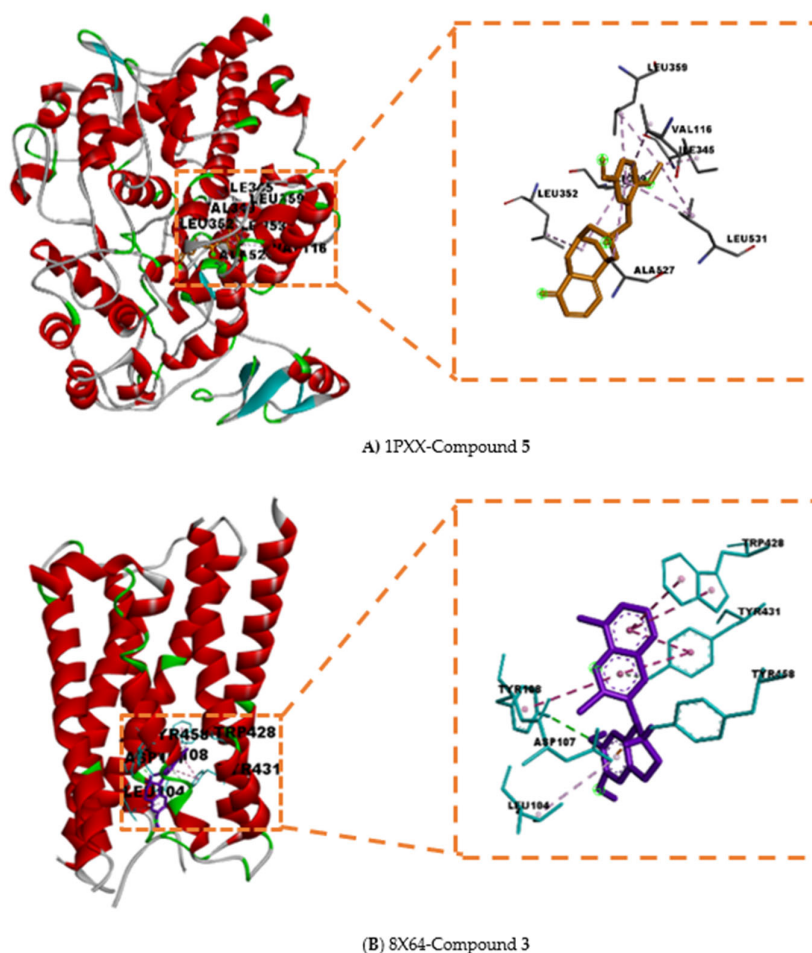


Figure 3. Three-dimensional docking conformations and binding interactions of the lead compounds within the binding sites of the target macromolecules. (A) Binding mode of Compound 5 in the active site of the COX-2 protein (PDB ID: 1PXX), showing the surrounding amino acid microenvironment. (B) Three-dimensional binding orientation and intermolecular interactions of Compound 3 within the binding cavity of the human H₁ histamine receptor (PDB ID: 8X64). The enlarged 3D views of the ligand–protein complexes, highlighted within the dashed orange boxes, are shown against the background of the overall secondary structures of the respective proteins.

To validate the reliability of the docking protocol, the co-crystallized ligands were re-docked into the active sites of their respective target proteins. Within the active site of the human H₁ histamine receptor (PDB ID: 8X64), the re-docked pose of the co-crystallized ligand demonstrated an almost perfect superposition with its experimentally determined X-ray crystallographic conformation (Figure 5A). The calculated root-mean-square deviation (RMSD) was 1.027 Å. According to the literature, an RMSD value of < 2.0 Å is generally accepted as the gold standard for high docking accuracy and protocol reliability [38].

Similarly, for cyclooxygenase-2 (COX-2, PDB ID: 1PXX), the re-docking of the co-crystallized ligand yielded a highly accurate RMSD value of 1.075 Å (Figure 5B). This excellent result falls well below the rigorous validation threshold of 2.0 Å, confirming that the applied docking methodology flawlessly reproduced the native ligand binding mode and spatial orientation, even within the complex and deeply buried hydrophobic channel

of the COX-2 active site. Consequently, these findings validate the selected docking parameters and ensure the high precision of the predicted binding modes for the newly synthesized derivatives against both biological targets [39].

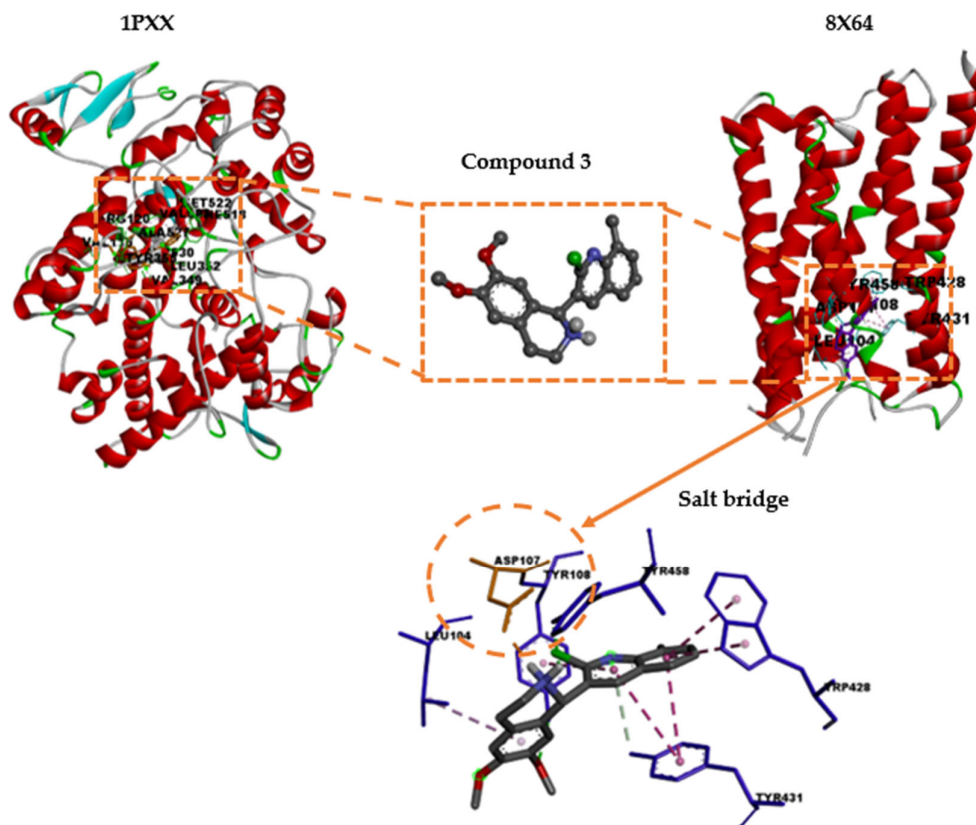


Figure 4. Three-dimensional docking poses and interaction conformations of Compound 3 within the active sites of COX-2 (PDB ID: 1PXX) and the human H₁ histamine receptor (PDB ID: 8X64). Top left: Overall binding orientation of Compound 3 within the active pocket of the COX-2 enzyme. Top right: Binding orientation of Compound 3 between the transmembrane α -helices of the H₁ receptor. Bottom: Enlarged three-dimensional view of the binding microenvironment, highlighted by dashed orange boxes, explicitly emphasizing the highly stable salt bridge formed between the protonated basic nitrogen of Compound 3 and the crucial ASP107 residue in the H₁ receptor.

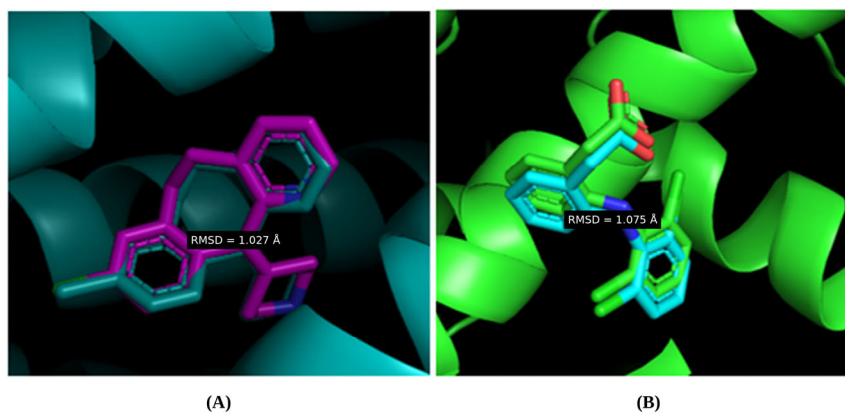


Figure 5. Superposition of the experimentally determined co-crystallized ligands (native X-ray structure) and their predicted re-docked conformations. (A) Re-docking validation in the human H₁

histamine receptor (PDB ID: 8X64) with an RMSD of 1.027 Å. **(B)** Re-docking validation in the COX-2 active site (PDB ID: 1PXX) with an RMSD of 1.075 Å.

3.2. Biological Experiments

Acute toxicity investigation revealed that all studied compounds may be classified as low-toxic and moderately toxic (Table 4).

Table 4. Acute Oral Toxicity of the Studied Compounds in Mice ($n = 6$, $p < 0.05$).

No	Compounds	LD ₅₀ , mg/kg
1	1-(2'-Bromo-4',5'-methylenedioxyphenyl)-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline hydrochloride	1107.9 (1017.3–1217.5)
2	1-(3',4'-Dimethoxyphenyl)-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline hydrochloride	299.1 (245.7–341.6)
3	2-Chloro-3'-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-1-yl)-8-methylquinoline hydrochloride	1300.0 (1198.7–1382.4)
4	1-(4'-Nitrophenyl)-N-benzyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride	1232.2 (1162.6–1291.2)
5	N-3-Formyl-6-methoxybenzyl cytosine hydrochloride	1116.9 (1082.3–1146.5)
6	N-6-Bromo-3-oxy-4-methoxybenzyl cytosine hydrochloride	1920.7 (1863.2–1960.9)

Formalin rat model demonstrated that in 3 h after subplantar administration of formalin, the rat paw volume increased 2.25-fold compared with the intact control. At a dose of 1 mg/kg orally, all tested compounds exhibited pronounced anti-inflammatory activity (70% and over), which was higher than those of diclofenac (62%) (Table 5).

Table 5. Comparative anti-inflammatory activity of investigated compounds in the rat formalin-induced inflammation model ($n = 6$, $p < 0.05$).

No	Compounds	Dose, mg/kg	Mean Paw Volume (mL)		Increase in Paw Volume From Initial		Anti-Inflammatory Effect, %
			Initial	In 3 h After Formalin Treatment	mL	%	
1	Control	0.2 mL	1.00 ± 0.02	1.80 ± 0.06	0.80	80.0	-
2	Diclofenac sodium	8.0	1.03 ± 0.21 ns	1.30 ± 0.21 ****	0.27	26.2	67.2
		10.0	1.07 ± 0.21 ns	1.31 ± 0.22 ***	0.24	22.4	72.0
		0.1	0.95 ± 0.03 ns	1.32 ± 0.05 ***	0.37	38.9	51.3
3	Compound 1	0.5	1.10 ± 0.03 ns	1.30 ± 0.05 ***	0.20	18.0	77.5
		1.0	1.10 ± 0.04 ns	1.32 ± 0.05 ***	0.22	20.0	75.0
		5.0	1.10 ± 0.04 ns	1.37 ± 0.05 **	0.27	24.5	69.3
		10.0	0.94 ± 0.03 ns	1.38 ± 0.05 **	0.44	46.8	41.5
		0.1	1.00 ± 0.03 ns	1.32 ± 0.05 ***	0.32	32.0	60.0
4	Compound 2	0.5	1.10 ± 0.04 ns	1.34 ± 0.05 ***	0.24	21.8	72.7
		1.0	1.10 ± 0.04 ns	1.30 ± 0.05 ***	0.20	18.0	77.5
		5.0	1.10 ± 0.05 ns	1.29 ± 0.05 ****	0.19	17.2	78.5
		10.0	0.96 ± 0.03 ns	1.30 ± 0.05 ***	0.34	35.4	55.7
		0.1	0.98 ± 0.03 ns	1.26 ± 0.05 ****	0.28	28.5	64.3
5	Compound 3	0.5	1.00 ± 0.04 ns	1.26 ± 0.05 ****	0.26	26.0	67.5
		1.0	1.10 ± 0.05 ns	1.33 ± 0.05 ***	0.23	20.9	73.8
		5.0	1.00 ± 0.03 ns	1.30 ± 0.05 ***	0.30 ^a	30.0	62.5
		10.0	1.10 ± 0.05 ns	1.44 ± 0.06 *	0.34	30.9	61.3
		0.1	0.98 ± 0.03 ns	1.26 ± 0.05 ****	0.28	28.5	64.3

6	Compound 4	0.1	0.90 ± 0.03 ns	1.40 ± 0.05 **	0.50	55.5	30.6
		0.5	0.92 ± 0.03 ns	1.31 ± 0.05 ***	0.39	42.3	47.1
		1.0	1.00 ± 0.04 ns	1.26 ± 0.06 ****	0.26	26.0	67.5
		5.0	0.90 ± 0.03 ns	1.18 ± 0.05 ****	0.28	31.1	61.1
		10.0	1.00 ± 0.04 ns	1.32 ± 0.05 ***	0.32	32.0	60.0
7	Compound 5	0.1	0.93 ± 0.04 ns	1.31 ± 0.05 ***	0.38	40.8	49.0
		0.5	0.94 ± 0.03 ns	1.22 ± 0.05 ****	0.28 ^a	29.7	62.8
		1.0	1.00 ± 0.04 ns	1.25 ± 0.05 ****	0.25	25.0	68.7
		5.0	0.98 ± 0.04 ns	1.20 ± 0.05 ****	0.22	22.4	72.0
		10.0	0.91 ± 0.04 ns	1.22 ± 0.06 ****	0.31	34.0	57.5
8	Compound 6	0.1	0.90 ± 0.04 ns	1.40 ± 0.06 **	0.50	55.5	30.6
		0.5	0.97 ± 0.04 ns	1.40 ± 0.06 **	0.43	44.3	44.6
		1.0	1.10 ± 0.05 ns	1.38 ± 0.06 **	0.28	25.4	68.2
		5.0	1.00 ± 0.05 ns	1.26 ± 0.05 ****	0.26	26.0	67.5
		10.0	0.90 ± 0.04 ns	1.24 ± 0.06 ****	0.34	37.7	52.8

Note: Values are presented as mean ± SEM ($n = 6$ animals per group). Group differences were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test vs. the control group (GraphPad Prism 10). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns = not significant ($p \geq 0.05$).

For a more rigorous assessment of the structure–activity relationship, the compounds were analyzed as two separate series: THIQ (1–4) and cytosine (5–6) derivatives. This distinction is methodologically appropriate because the two series differ substantially in their core pharmacophores and overall molecular scaffolds.

Within the THIQ series, compounds **1** and **2** exhibited the most pronounced anti-inflammatory activity, with maximal effects of 77.5% and 78.5%, respectively. Compound **1** contains a 2'-bromo-4',5'-methylenedioxyphenyl substituent, whereas compound **2** contains a 3',4'-dimethoxyphenyl group; both compounds share a 6,7-methylenedioxy-THIQ core. Despite the different aryl substituents, their maximal anti-inflammatory effects were very similar. However, the optimal doses differed markedly, being 0.5 mg/kg for compound **1** and 5 mg/kg for compound **2**. This finding may indicate that modification of the aryl substituent primarily affects pharmacological potency rather than the maximal attainable efficacy.

Compound **3**, containing a 2-chloro-8-methylquinoline moiety linked to a 6,7-dimethoxy-THIQ core, also demonstrated pronounced activity, reaching a maximum effect of 73.8% at 1 mg/kg. Thus, incorporation of a bulky heteroaromatic fragment did not abolish the anti-inflammatory activity of the THIQ scaffold, although the maximal effect was somewhat lower than those observed for compounds **1** and **2**.

Compound **4**, which contains a 4'-nitrophenyl group together with an N-benzyl substituent and a 6,7-dimethoxy-THIQ core, demonstrated the lowest maximal activity within the THIQ series (67.5% at 1 mg/kg). The lower activity may suggest that this combination of structural modifications is less favorable for the anti-inflammatory response. However, because compound **4** differs from compounds **1–3** in more than one structural feature, the contribution of an individual substituent cannot be established from the present data alone.

Overall, the maximal anti-inflammatory activity within the THIQ series followed the order: compound **2** (78.5%) $\approx$ compound **1** (77.5%) > compound **3** (73.8%) > compound **4** (67.5%).

Compounds **5** and **6** were analyzed separately because they possess a cytosine core. Both compounds share the same cytosine scaffold and differ primarily in the structure of the benzyl substituent attached to the nitrogen atom. Compound **5**, bearing a 3-formyl-6-methoxybenzyl substituent, exhibited a maximal anti-inflammatory effect of 72.0% at 5

mg/kg. Compound **6**, containing a 6-bromo-3-hydroxy-4-methoxybenzyl substituent, showed a somewhat lower maximal effect of 68.2%, but this effect was achieved at a lower dose of 1 mg/kg. Thus, replacement of the 3-formyl-6-methoxybenzyl substituent in compound **5** with the 6-bromo-3-hydroxy-4-methoxybenzyl substituent in compound **6** was associated with a modest reduction in maximal efficacy (72.0% vs. 68.2%) but an apparent shift toward a lower effective dose. This observation demonstrates the importance of distinguishing efficacy from potency when interpreting the structure–activity relationship.

Overall, within the cytosine series, compound **5** showed a higher maximal anti-inflammatory effect than compound **6**, whereas compound **6** achieved its maximal response at a lower dose. Therefore, modification of the N-benzyl substituent appears to influence both the magnitude of the maximal response and the dose–response profile.

To investigate the mechanism underlying the anti-inflammatory activity of the studied compounds, changes in serum cytokine levels were evaluated in experimental animals following oral administration during the acute and proliferative phases of inflammation. The obtained results (Table 6) demonstrated a decrease in the levels of the pro-inflammatory cytokines IL-6 and TNF- α , accompanied by restoration of IL-4 from the reduced level observed under formalin-induced inflammatory conditions toward values close to those of the intact control.

Our findings are consistent with the general anti-inflammatory effects of NSAIDs. In particular, a clinical study involving diclofenac, celecoxib, and ibuprofen demonstrated reductions in IL-6 and TNF- α in synovial fluid during NSAID treatment. In addition, a study of blood cells showed that diclofenac reduced IL-6 production, while a formalin-induced inflammation model demonstrated an increase in TNF- α associated with the inflammatory response [40]. Also, diclofenac has demonstrated pronounced activity against COX-2. The study on patients showed greater inhibitory activity of diclofenac against COX-2 than COX-1, supporting the possibility that suppression of the prostanoid pathway contributes to its anti-inflammatory effects [41]. In the research [42] celecoxib (5 mg/kg) was as potent as diclofenac in decreasing the elevated levels of IL-6, IL-1 β , TNF- α , LTB $_4$, and PGE $_2$.

Table 6. Serum Cytokine Profile (IL-4, IL-6, and TNF- α) in Experimental Animals with Acute Formalin-induced Inflammation ($n = 6$).

Compound	Dose, mg/kg	IL-4	IL-6	α -TNF
Intact		0.42 $\pm$ 0.03	0.31 $\pm$ 0.03	3.10 $\pm$ 0.10
Control (formalin)		0.11 $\pm$ 0.02	2.30 $\pm$ 0.08	4.31 $\pm$ 0.12
Diclofenac sodium	8.0	0.37 $\pm$ 0.03 ****	0.11 $\pm$ 0.02 ****	1.07 $\pm$ 0.05 ****
	10.0	0.41 $\pm$ 0.03 ****	0.09 $\pm$ 0.02 ****	1.02 $\pm$ 0.05 ****
Compound 1	0.5	0.35 $\pm$ 0.03 ****	0.12 $\pm$ 0.02 ****	1.11 $\pm$ 0.05 ****
	1.0	0.38 $\pm$ 0.03 ****	0.10 $\pm$ 0.02 ****	0.97 $\pm$ 0.05 ****
	5.0	0.40 $\pm$ 0.03 ****	0.09 $\pm$ 0.02 ****	0.95 $\pm$ 0.05 ****
Compound 2	0.5	0.47 $\pm$ 0.03 ****	0.08 $\pm$ 0.02 ****	0.94 $\pm$ 0.05 ****
	1.0	0.50 $\pm$ 0.04 ****	0.06 $\pm$ 0.01 ****	0.91 $\pm$ 0.05 ****
	5.0	0.44 $\pm$ 0.04 ****	0.10 $\pm$ 0.02 ****	0.99 $\pm$ 0.05 ****
Compound 3	0.5	0.49 $\pm$ 0.04 ****	0.07 $\pm$ 0.02 ****	0.96 $\pm$ 0.05 ****
	1.0	0.47 $\pm$ 0.04 ****	0.09 $\pm$ 0.02 ****	0.93 $\pm$ 0.05 ****
	5.0	0.52 $\pm$ 0.04 ****	0.05 $\pm$ 0.01 ****	0.89 $\pm$ 0.05 ****

Note: Values are presented as mean $\pm$ SEM ($n = 6$ animals per group). Group differences were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test vs. the control group (GraphPad Prism 10). **** $p < 0.0001$, ns = not significant ($p \geq 0.05$).

The results of the evaluation of the anti-inflammatory effects of ointments containing the test compounds at concentrations of 1%, 2%, and 5% in the formalin rat model are presented in Table 7. On day 10 after formalin administration, the ointment containing the reference drug diclofenac sodium (1%) reduced rat paw edema by 0.13 mL, whereas ointments containing compounds **1**, **2**, and **3** reduced paw edema by 0.12, 0.24, and 0.30 mL, respectively. These results indicate that the anti-inflammatory activity of the tested compounds is comparable to or markedly greater than that of 1% diclofenac sodium ointment. This suggests that further increasing the ointment concentration is unlikely to provide additional therapeutic benefit.

Table 7. Evaluation of the anti-inflammatory effects of ointments containing the test compounds in the rat formalin-induced inflammation model ($M \pm m$, $n = 6$, $p < 0.05$).

№	Compounds	Dose, %	Mean Paw Volume (mL)						
			Initial	In 3 h After Formalin Treatment	2-Day	4-Day	6-Day	8-Day	10-Day
1	Control		0.93 ± 0.05	1.38 ± 0.06	1.40 ± 0.07	1.42 ± 0.07	1.44 ± 0.07	1.42 ± 0.07	1.39 ± 0.08
2	Diclofenac sodium	1%	0.96 ± 0.05 ns	1.31 ± 0.06 ns	1.29 ± 0.06 ns	1.27 ± 0.07 ns	1.24 ± 0.07 ns	1.21 ± 0.07 ns	1.18 ± 0.07 ns
3	Compound 1	1%	0.92 ± 0.05 ns	1.24 ± 0.06 ns	1.21 ± 0.07 ns	1.17 ± 0.07 ns	1.15 ± 0.08 *	1.13 ± 0.08 ns	1.12 ± 0.08 ns
		2%	0.95 ± 0.05 ns	1.25 ± 0.06 ns	1.23 ± 0.07 ns	1.19 ± 0.07 ns	1.16 ± 0.07 ns	1.13 ± 0.08 ns	1.11 ± 0.08 ns
		5%	0.96 ± 0.05 ns	1.29 ± 0.06 ns	1.25 ± 0.06 ns	1.20 ± 0.07 ns	1.17 ± 0.07 ns	1.14 ± 0.07 ns	1.07 ± 0.08 *
4	Compound 2	1%	0.98 ± 0.05 ns	1.24 ± 0.06 ns	1.19 ± 0.07 ns	1.15 ± 0.07 ns	1.11 ± 0.07 *	1.06 ± 0.07 **	1.00 ± 0.07 **
		2%	0.93 ± 0.06 ns	1.19 ± 0.06 ns	1.16 ± 0.07 ns	1.13 ± 0.07 *	1.10 ± 0.07 *	1.04 ± 0.07 **	0.96 ± 0.07 **
		5%	0.96 ± 0.05 ns	1.21 ± 0.06 ns	1.17 ± 0.07 ns	1.14 ± 0.07 *	1.12 ± 0.07 *	1.05 ± 0.07 **	1.01 ± 0.07 **
5	Compound 3	1%	0.91 ± 0.06 ns	1.23 ± 0.06 ns	1.19 ± 0.07 ns	1.16 ± 0.07 ns	1.13 ± 0.07 *	1.08 ± 0.08 *	0.93 ± 0.07 ***
		2%	0.97 ± 0.05 ns	1.15 ± 0.06 ns	1.11 ± 0.06 *	1.08 ± 0.07 **	1.05 ± 0.07 **	1.03 ± 0.07 **	1.00 ± 0.08 **
		5%	0.95 ± 0.06 ns	1.17 ± 0.06 ns	1.14 ± 0.07 *	1.11 ± 0.07 *	1.08 ± 0.07 **	1.02 ± 0.08 **	0.96 ± 0.08 **

Note: Values are presented as mean ± SEM ($n = 6$ animals per group). Group differences were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test vs. the control group (GraphPad Prism 10). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = not significant ($p \geq 0.05$).

Desloratadine is a selective peripheral histamine H1-receptor antagonist, and its antihistaminic activity has been demonstrated in several experimental models. In particular, preclinical studies have shown that desloratadine significantly inhibits histamine-induced paw edema in mice, providing direct pharmacological evidence for the validity of this model for assessing histamine-mediated responses [43]. Thus, the significant inhibition of histamine-induced paw edema observed with desloratadine in our experiment provides an internal pharmacological validation of the model. The histamine rat model is primarily used to evaluate the exudative phase of acute inflammation, as histamine is one of the principal mediators responsible for the early increase in vascular permeability and edema formation. Therefore, inhibition of histamine-induced edema by tested compounds may interfere with histamine-mediated vascular responses.

Results are presented in Table 8. In the histamine-induced paw edema model, the H₁ receptor blocker desloratadine demonstrated the greatest activity. At a dose of 0.1 mg/kg orally, it produced an anti-inflammatory effect of 75.5%. Among the tested compounds, substance 2 exhibited the highest anti-inflammatory activity at a dose of 1 mg/kg (72.1%). Cytisine derivatives showed their greatest efficacy at a dose of 5 mg/kg.

Table 8. Comparative Anti-Inflammatory Effects of Investigated Compounds, and the Reference Preparations in Rats with Histamine-Induced Paw Edema ($M \pm m$, $n = 6$).

№	Compounds	Dose, mg/kg	Mean Paw Volume (mL)		Increase in Paw Volume from Initial		AIE, %
			Initial	In 3 h After Histamine Treatment	mL	%	
1	Control (histamine)	0.2ml	0.98 ± 0.05	1.46 ± 0.05	0.48	48.9	-
2	Diclofenac sodium	8.0	0.89 ± 0.04 ns	1.08 ± 0.05 ***	0.19	21.3	56.4
		10.0	0.93 ± 0.05 ns	1.10 ± 0.05 ***	0.17	18.2	62.7
3	Desloratadin	0.01	0.88 ± 0.04 ns	0.88 ± 0.05 ****	0.19	21.5	56.0
		0.05	0.95 ± 0.05 ns	1.12 ± 0.05 **	0.17	17.8	63.5
		0.1	0.92 ± 0.05 ns	1.03 ± 0.05 ****	0.11	11.9	75.6
4	Compound 3	0.1	0.98 ± 0.05 ns	1.22 ± 0.05 ns	0.24	24.4	50.1
		0.5	0.93 ± 0.05 ns	1.06 ± 0.05 ****	0.14	15.7	67.8
		1.0	0.89 ± 0.04 ns	1.03 ± 0.05 ****	0.13	13.9	71.5
		5.0	0.97 ± 0.05 ns	1.16 ± 0.05 **	0.19	19.5	60.1
		10.0	0.91 ± 0.05 ns	1.11 ± 0.05 ***	0.20	21.9	55.2
		0.1	0.96 ± 0.05 ns	1.14 ± 0.05 **	0.18	18.7	61.7
5	Compound 1	0.5	0.88 ± 0.05 ns	1.00 ± 0.05 ****	0.12	13.6	72.1
		1.0	0.94 ± 0.05 ns	1.06 ± 0.06 ****	0.12	12.7	74.0
		5.0	0.90 ± 0.05 ns	1.04 ± 0.05 ****	0.14	15.5	68.3
		10.0	0.96 ± 0.05 ns	1.15 ± 0.05 **	0.19	19.7	59.7
		0.1	0.92 ± 0.05 ns	1.10 ± 0.06 ***	0.18	19.5	60.1
6	Compound 2	0.5	0.87 ± 0.05 ns	1.02 ± 0.06 ****	0.15	17.2	64.8
		1.0	0.95 ± 0.05 ns	1.08 ± 0.06 ***	0.13	13.6	72.1
		5.0	0.91 ± 0.05 ns	1.03 ± 0.06 ****	0.12	13.1	73.2
		10.0	0.97 ± 0.05 ns	1.12 ± 0.06 **	0.15	15.4	68.5
		0.1	0.90 ± 0.05 ns	1.11 ± 0.06 ***	0.21	23.3	52.3
7	Compound 4	0.5	0.93 ± 0.05 ns	1.12 ± 0.06 **	0.19	20.4	58.2
		1.0	0.89 ± 0.05 ns	1.04 ± 0.06 ****	0.15	16.8	65.6
		5.0	0.94 ± 0.05 ns	1.08 ± 0.06 ***	0.17	18.0	63.1
		10.0	0.92 ± 0.05 ns	1.12 ± 0.06 **	0.20	21.7	55.6
		0.1	0.88 ± 0.05 ns	1.08 ± 0.06 ***	0.20	22.7	53.5
8	Compound 5	0.5	0.91 ± 0.05 ns	1.11 ± 0.06 ***	0.17	18.6	61.9
		1.0	0.96 ± 0.05 ns	1.11 ± 0.06 ***	0.15	15.6	68.0
		5.0	0.94 ± 0.06 ns	1.07 ± 0.06 ****	0.13	13.8	71.7
		10.0	0.99 ± 0.05 ns	1.15 ± 0.05 **	0.16	16.1	67.0
		0.1	0.92 ± 0.05 ns	1.15 ± 0.06 **	0.23	25.0	48.8
9	Compound 6	0.5	0.87 ± 0.05 ns	1.05 ± 0.06 ****	0.18	20.6	57.8
		1.0	0.96 ± 0.06 ns	1.12 ± 0.06 **	0.16	16.6	66.0
		5.0	0.91 ± 0.05 ns	1.06 ± 0.07 ****	0.15	16.4	66.4
		10.0	0.89 ± 0.05 ns	1.08 ± 0.06 ***	0.19	21.3	56.4

Note: Values are presented as mean ± SEM ($n = 6$ animals per group). Group differences were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test vs. the control group (GraphPad Prism 10). ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns = not significant ($p \geq 0.05$). Values in bold indicate the highest anti-inflammatory effect (AIE) for each compound.

Table 9 presents a comparative evaluation of the anti-inflammatory effects of ointments based on the studied THIQ derivatives and the reference drugs in the histamine-induced inflammation model. The obtained data demonstrate that substance 2 (1%) exhibited a high anti-inflammatory effect, comparable to that of dimetindene maleate.

Table 9. Comparative anti-inflammatory effects of ointments based on investigated compounds and reference drugs in histamine rat model (M ± SEM, *n* = 6).

№	Compounds	Dose, %	Mean Paw Volume (mL)		Increase in Paw Volume from Initial		AIE, %
			Initial	In 3 h After Histamine Treatment	mL	%	
1	Control (histamine)		0.78 ± 0.04	1.30 ± 0.05	0.52	66.6	-
2	Diclofenac sodium	1.0	0.83 ± 0.04 ns	1.12 ± 0.06 ns	0.29	34.9	47.5
3	Dimetindene maleate	0.1	0.76 ± 0.04 ns	0.88 ± 0.05 ****	0.12	15.7	76.4
4	Compound 3	1.0	0.80 ± 0.04 ns	1.09 ± 0.05 ns	0.29	36.2	45.6
		2.0	0.78 ± 0.05 ns	0.95 ± 0.05 ***	0.17	21.7	67.4
		5.0	0.82 ± 0.04 ns	0.96 ± 0.05 ***	0.14	17.0	74.4
5	Compound 2	1.0	0.79 ± 0.04 ns	0.94 ± 0.05 ****	0.15	18.9	71.6
		2.0	0.77 ± 0.04 ns	0.95 ± 0.05 ***	0.18	23.3	65.0
		5.0	0.83 ± 0.04 ns	1.09 ± 0.05 ns	0.26	31.3	53.0
6	Compound 1	1.0	0.78 ± 0.05 ns	1.09 ± 0.05 ns	0.31	39.7	40.3
		2.0	0.81 ± 0.04 ns	1.10 ± 0.05 ns	0.29	35.8	46.2
		5.0	0.79 ± 0.05 ns	1.05 ± 0.05 *	0.26	32.9	50.6

Note: Values are presented as mean ± SEM (*n* = 6 animals per group). Group differences were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test vs. the control group (GraphPad Prism 10). * *p* < 0.05, *** *p* < 0.001, **** *p* < 0.0001, ns = not significant (*p* ≥ 0.05).

4. Discussion

The literature contains numerous reports describing various aspects of the mechanisms underlying the anti-inflammatory activity of THIQ derivatives. Current research is focused on the development of moderately selective cyclooxygenase-2 (COX-2) inhibitors to minimize cardiovascular and gastrointestinal adverse effects.

In our investigation, all THIQ derivatives demonstrated high anti-inflammatory efficacy in the formalin-induced rat paw edema model at a dose of 1 mg/kg, with compound 1 exhibiting the greatest activity. Consistent with these *in vivo* findings, *in silico* molecular docking showed that compound 1 possessed the highest binding affinity for the COX-2 active site, supporting its superior biological activity. The cytosine derivative (compound 6) also demonstrated high efficacy in the formalin model at the same dose; however, its predicted binding affinity for COX-2 was lower than that of the THIQ derivatives.

In contrast, the binding affinity profile within the human H₁ histamine receptor differed substantially from that observed for COX-2. Compound 4 exhibited the strongest predicted binding affinity, exceeding that of all reference compounds. Nevertheless, this high *in silico* affinity was not fully reflected in the *in vivo* histamine-induced paw edema model, where compound 4 reduced paw edema by only 66% at a dose of 1 mg/kg. In comparison, the TGIQ derivatives produced greater anti-inflammatory effects at the same dose. Notably, compound 1 was the most active derivative in the histamine-induced inflammation model as well, despite not exhibiting the highest predicted affinity for the H₁ receptor. These findings suggest that the anti-inflammatory activity of compound 1 may involve additional pharmacological mechanisms beyond direct H₁ receptor antagonism.

Study [16] demonstrated that THIQ derivatives administered at a dose of 0.5 mg/kg in a formalin-induced arthritis model exhibited anti-inflammatory activity 3.3-fold greater than that of diclofenac sodium, while also showing a more favorable gastroprotective safety profile.

The results reported in study [38] indicate that the modified synthetic alkaloid 2-methoxy-4-(7-methoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)phenol (MHTP) exhibits anti-

inflammatory activity by inhibiting prostaglandin E₂ (PGE₂) function, suppressing the migration of inflammatory cells to the site of inflammation, and attenuating acute lung injury through inhibition of neutrophil infiltration into the lungs.

A patent application [44] describes a novel tetrahydroisoquinoline derivative with potent inhibitory activity against phosphodiesterase-4 (PDE4). This compound has been proposed for the prevention, treatment, or adjunctive therapy of a wide range of diseases associated with phosphodiesterase activity or expression, particularly PDE4-mediated immune and inflammatory disorders, such as psoriasis and arthritis.

It has also been demonstrated that (S)-1-(α -naphthylmethyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (CKD712) inhibits apoptosis during myocardial ischemia/reperfusion (I/R) injury *in vivo* by activating the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway and by reducing inflammation during ischemia/reperfusion [45].

In another study [46], (S)-1-(α -naphthylmethyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (CKD712) was investigated as a potential therapeutic agent for the treatment of sepsis. Numerous studies have demonstrated its marked anti-inflammatory properties. CKD712 was shown to inhibit lipopolysaccharide (LPS)-induced arachidonic acid release through suppression of the MAPK/NF- κ B signaling pathway, resulting in decreased expression of cytosolic phospholipase A₂ (cPLA₂) in RAW 264.7 macrophages.

There is evidence demonstrating the antioxidant activity of cytosine and 1,2,3,4-tetrahydroisoquinoline derivatives [47]. It can be hypothesized that, in addition to other mechanisms, the anti-exudative effect of these compounds is associated with enhanced activity of antioxidant defense enzymes.

Thus, the acute toxicity of all the investigated isoquinoline and quinolizidine derivatives was substantially lower than that of the reference drugs. In the visceral pain model, the studied compounds exhibited pronounced analgesic activity comparable to that of ketoprofen, while the 1,2,3,4-tetrahydroisoquinoline derivatives demonstrated greater efficacy than the cytosine derivatives. Comparative evaluation of the anti-inflammatory activity of isoquinoline and quinolizidine derivatives revealed that the isoquinoline derivatives possess marked anti-inflammatory properties combined with low toxicity, making them promising candidates for the development of novel, highly effective and safe anti-inflammatory agents for both oral and topical administration. Further in-depth studies are warranted to clarify the mechanisms of anti-inflammatory action and to comprehensively assess the safety of the investigated compounds.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/scipharm94040099/s1>, Figure S1: ¹H NMR spectrum of Compound 1; Figure S2. IR spectrum of Compound 1; Figure S3. ¹H NMR spectrum of Compound 2; Figure S4. IR spectrum of Compound 2; Figure S5. ¹H NMR spectrum of Compound 3; Figure S6. ¹³C NMR spectrum of Compound 3; Figure S7. +ESI-mass spectrum of Compound 3; Figure S8. ¹H NMR spectrum of Compound 4; Figure S9. ¹³C NMR spectrum of Compound 4; Figure S10. X-ray crystallographic structure image of Compound 5; Table S1. Elemental analysis data for the synthesized compounds.

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Abbreviations

The following abbreviations are used in this manuscript:

COX-2—Cyclooxygenase-2; PGE₂—Prostaglandin E₂; NSAIDs—Nonsteroidal anti-inflammatory drugs; TGIQ—Tetrahydroisoquinolines

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