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Anti-Inflammatory Potential of 1-Aryl-6,7-Dimethoxy-1,2,3,4-Tetrahydroisoquinolines: Structure-Activity Relationship and COX-2 Binding

Azizbek A. Azamatov [1], Firuza M. Tursunkhodzhaeva [1,*], Sherzod N. Zhurakulov [2], Zufar D. Boboev [1], Kuvonchbek F. Kuchimov [2], Urkhiya K. Aytmuratova [1], Ilhomjon S. Ortikov [1], Robiya Sh. Abdurazakova [3] and Valentina I. Vinogradova [4]

[1] Department of Pharmacology and Toxicology, Yunusov Institute of the Chemistry of Plant Substances, Tashkent, Uzbekistan
[2] Laboratory of Alkaloids Chemistry, Yunusov Institute of the Chemistry of Plant Substances, Tashkent, Uzbekistan
[3] Samarkand State Medical Institute, Samarkand, Uzbekistan
[4] Yunusov Institute of the Chemistry of Plant Substances, Tashkent, Uzbekistan
* Correspondence: ftm40438@gmail.com

Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used around the world for their pain-relieving and fever-reducing properties. However, excessive intake of NSAIDs can lead to harmful effects on multiple body systems, including the cardiovascular, gastrointestinal, hepatic, renal, and nervous systems. The anti-inflammatory activity of 34 derivatives of 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline was investigated in vivo. A relationship between the activity of the compounds and the nature of the substituents, as well as their positional and mutual arrangement in the C ring (1-Ar-), was established. In silico modelling of 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline derivatives interaction with COX-2 (PDB ID: 1PXX) active site indicated that the nitro-derivatives exhibited the highest stability owing to their superior capacity for electrostatic and hydrogen bond formation compare to brominated compounds. The presented data on the effect of substituents $–NH_2$, –OH, and $–OCH_3$ in ring C (1-Ar-) of 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines on anti-inflammatory activity further stimulate the search for new highly effective derivatives within this series.

Keywords: 1-aryl-1,2,3,4-tetrahydroisoquinoline; anti-inflammatory activity; structure-activity; COX-2

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1. Introduction

Isoquinoline derivatives exhibit a wide spectrum of biological activity. Among them, compounds with antitumor, antidiabetic, antiviral, anti-inflammatory, immunosuppressive, antiparasitic, neuroprotective, hepatoprotective, and cardioprotective effects have been identified [1]. A number of 1,2,3,4-tetrahydroisoquinoline derivatives have demonstrated analgesic, antipyretic, anticholinesterase, antioxidant, and anticonvulsant activities [2,3]. The diisoquinoline alkaloid berberine exhibits pronounced anti-inflammatory activity, the mechanism of which is associated with the regulation of pro- and anti-inflammatory cytokines [4]. Demethyleneberberine, which differs from berberine by the presence of a 2,3-dioxy group instead of a 2,3-methylenedioxy group, reduces inflammatory responses by inhibiting the NF-κB pathway and regulating the Th-cell balance [5]. Among aporphine alkaloids, thalicmidine and others possess anti-inflammatory properties, while norisoboldine exerts anti-arthritic effects through anti-inflammatory and immunoregulatory mechanisms [6]. The bisbenzylisoquinoline alkaloid curine has been shown to inhibit the inflammatory phase II in the formalin test, reduce the number of pain responses in the acetic acid writhing test, and additionally decrease the production of prostaglandin E2 [7].

Current researches are focused on identifying moderately selective cyclooxygenase-2 (COX-2) inhibitors in order to overcome the adverse effects of highly selective COX-2 and non-selective COX-2 inhibitors. In [8,9], hybrids of 1,2,4-triazole and tetrahydroisoquinoline demonstrated anti-COX-2 activity comparable to that of celecoxib (IC_{50} from 0.58 to 1.27 μM) were synthesized. The compounds significantly reduced levels of key inflammatory mediators such as prostaglandin E2 (PGE-2), tumor necrosis factor (TNF-α), and interleukin-6 (IL-6).

The analgesic and anti-inflammatory activity of 1-(4'-dimethylaminophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride was studied under conditions of thermal (hot plate test) and chemical (acetic writhing test) irritation, anti-inflammatory activity - on the model of acute inflammatory arthritis. The compound showed a pronounced anti-inflammatory effect at a dose of 0.5 mg/kg, 3.3 times greater than the effect of diclofenac sodium [10]. In [11] authors evaluate the isoquinoline (N-substituted (E)-4-arylidene-isoquinoline-1,3-dione) derivatives for their anti-inflammatory, analgesic and antipyretic activity potentials in animal models using indomethacin and diclofenac sodium as the standard.

New hybrid structures exhibiting dual agonist/antagonist properties at κ- and μ-opioid receptors based on TGIQ-valine have been developed [12–14].

A current trend is the development of hybrid compounds (for example, with Quercetin) that combine analgesic activity with antioxidant and neuroprotective effects. Such compounds are considered promising agents for the management of pain associated with neurodegenerative diseases, for example, Alzheimer's disease [16].

The newly obtained hybrids of ibuprofen with 1,2,3,4-tetrahydroisoquinoline, and piperidine were screened for in vitro antioxidant, antitryptic, and inhibition of albumin denaturation activity. The lipophilicity was established using both reversed-phase thin layer chromatography and in silico calculations [17].

Thus, the search for compounds with anti-inflammatory activity among isoquinoline derivatives is a promising direction.

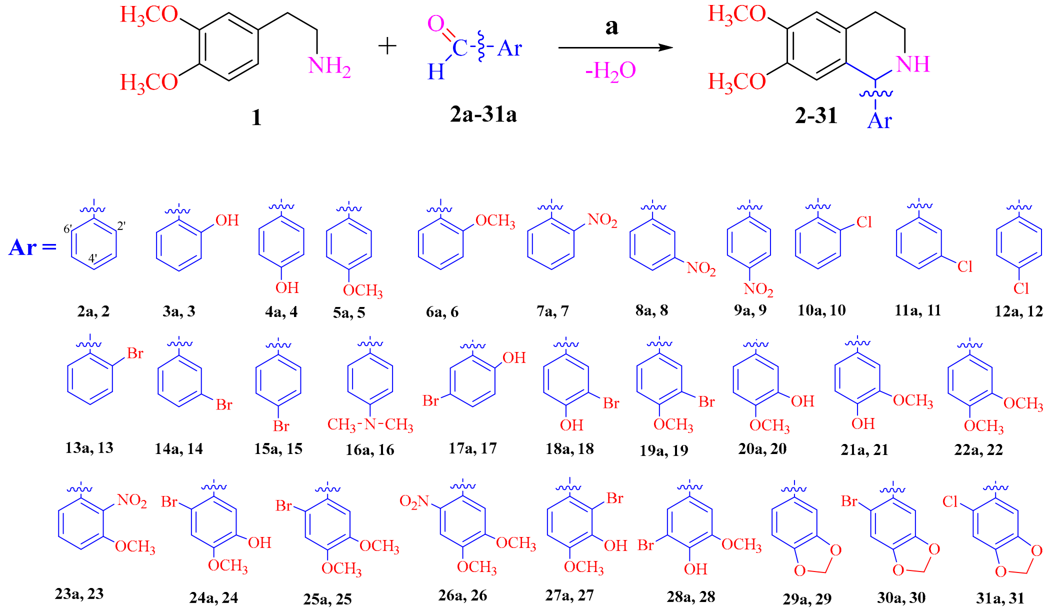
In the previous studies [10], we investigated the toxicity of 30 derivatives of 1-aryl-1,2,3,4-tetrahydroisoquinolines, their local anesthetic and anticonvulsant activity, and analyzed the structure-toxicity and structure-activity relationship. Also, we investigated analgesic and anti-inflammatory effects of individual TGIQ 1-(4'-Dimethylaminophenyl)-6,7-Dimethoxy-1,2,3,4-Tetrahydroisoquinoline Hydrochloride [3].

The presented research aims to investigate the in vivo anti-inflammatory effects and to evaluate structure-activity relation of thirty-three derivatives of 1-aryl-1,2,3,4-tetrahydroisoquinoline. These studies are essential for predicting the anti-inflammatory effects and potential side effects of the examined substances, which may serve as a basis for the development of new high effective non-steroid anti-inflammatory drugs. Additionally, in silico studies were conducted, applying molecular docking simulation to investigate the binding of the tested substances at the binding site of cyclooxygenase-2 (COX-2) enzyme (PDB ID: 1PXX). The structure-activity relationship of the compounds was studied to determine the essential structural features necessary for their anti-inflammatory activity. A predictive ADMET study was conducted using Discovery Studio 4.0 software to determine the pharmacokinetic profiles and evaluate the drug-likeness of the tested compounds. The results, based on their chemical structures, showed a strong correlation with various parameters, such as aqueous solubility, absorption level, blood-brain barrier permeability, hepatotoxicity probability, atom-based log P98 (A LogP98), plasma protein binding (PPB) level, cytochrome P450 2D6 (CYP2D6), and 2D polar surface area (ADMET 2D PSA).

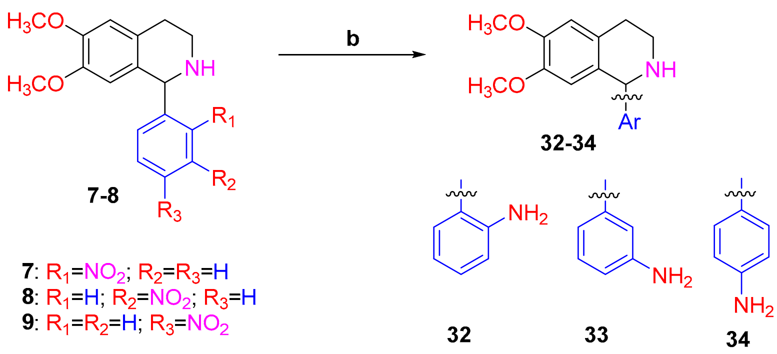
2. Results

2.1. Synthesis of the Compounds

Series of derivatives of 1-aryl-1,2,3,4-tetrahydroisoquinoline were obtained as described in [10,18].



Scheme 1. Synthesis of 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline derivatives **2-31,** (**a**: CF_3COOH, reflux 4–6 h).



Scheme 2. Reduction of 1-(2′-nitrophenyl)- **(7),** 1-(3′-nitrophenyl)- **(8),** 1-(3′-nitrophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines **(9),** resulting in the formation of derivatives **32-34, (b:** $SnCl_2 \times 2H_2O$, HCl, C_2H_5OH, reflux, 4 h**).**

2.2. Biological Evaluation

Anti-Inflammatory Effect on Formalin Rat Model

The animal study of the effects of 1-aryl-1,2,3,4-tetrahydroisoquinolines (Table 1) showed that at a dose of 1 mg/kg substances 33, 23, 15, 16 and 19 were the most effective and demonstrated greater anti-inflammatory effect (AIE) compared to the reference drugs ketoprofen and sodium diclofenac.

Table 1. Anti-inflammatory effect of 1-aryl-1,2,3,4-tetrahydroisoquinolines at a dose of 1 mg/kg (p.o.) in the formalin rat model.

Examined Compound (Substituent)	AIE, %	Examined Compound (Substituent)	AIE, %
Control (formalin)	-	Ketoprofen (5 mg/kg)	57.6
		Sodium diclofenac (8 mg/kg)	66.3
1-Phenyl- **(2)**	70.1	1-(3′-Bromo-4′-hydroxyphenyl)- (**18**)	52.0
1-(2′-Hydroxyphenyl)- **(3)**	60.6	**1-(3′-Bromo-4′-methoxyphenyl)- (19)**	**76.8**
1-(4′- Hydroxyphenyl)- **(4)**	23.1	1-(3′-Hydroxy-4′- methoxyphenyl)- (**20**)	54.1
1-(4′- Methoxyphenyl) - (**5**)	27.5	1-(4′-Hydroxy-3′- methoxyphenyl)- (**21**)	50.7
1-(2′- Methoxyphenyl)- (**6**)	61.3	1-(3′,4′-Dimethoxyphenyl)- (**22**)	57.6
1-(2′-Nitrophenyl)- (7)	**69.3**	**1-(3′-Methoxy-2′-nitrophenyl)- (23)**	**80.8**
1-(3′- Nitrophenyl) (**8**)	67.5	1-(2′-Bromo-5′-hydroxy-4′-methoxyphenyl)- (**24**)	76.8
1-(4′- Nitrophenyl)- (**9**)	44.8	1-(2′-Bromo-4′,5′-dimethoxy)- (**25**)	42.3
1-(2′-Chlorophenyl)- (**10**)	50.8	1-(4′,5′-Dimethoxy-2′-nitrophenyl)- (**26**)	52.7
1-(3′- Chlorophenyl)- (**11**)	52.7	1-(2′-Bromo-3′-hydroxy-4′-methoxy)- (**27**)	34.9
1-(4′- Chlorophenyl)- (**12**)	40.1	1-(5′-Bromo-4′-hydroxy-3′-methoxyphenyl)- (**28**)	29.8
1-(2′-Bromophenyl)- (**13**)	69.5	1-(3′,4′-Methylenedioxyphenyl)- (**29**)	71.1
1-(3′- Bromophenyl)- (**14**)	44.6	1-(2′-Bromo-4′,5′-Methylenedioxyphenyl)- (**30**)	46.8
1-(4′- Bromophenyl)- (15)	**77.1**	1-(4′,5′-Methylenedioxy-2′-chlorophenyl)- (**31**)	40.6
1-(4′-Dimethylaminophenyl)- (16)	**77.6**	1-(2′-Aminophenyl)- (**32**)	63.9
1-(5′-Bromo-2′-hydroxyphenyl)- (**17**)	53.5	**1-(3′-Aminophenyl)- (33)**	**81.2**
		1-(4′- Aminophenyl)- (**34**)	66.0

Data were shown as mean±SD of different groups (N=6). The $p<0.05$ was considered statistically significant.

The effects of the most active compounds 33, 23, 15, 16 and 19 on cytokine levels showed that, under the influence of these compounds, the elevated levels of interleukins IL-4, IL-6, and TNF observed in formalin-induced inflammation approached the levels found in intact animals (Table 2).

Table 2. Effect of the examined substances on the cytokine levels in blood serum of rats at a dose of 1 mg/kg (p.o.) in the formalin rat model.

Examined Compound	Dose, mg/kg	IL-4, pg/ml	IL-6, pg/ml	TNF, pg/ml
Control 1 (intact animals)		0.16 (0.145-0.175)	0.31 (0.29-0.33)	0.65 (0.32-0.68)
Control 2 (formalin)		2.0 (1.84-2.16)*	2.4 (1.37-3.43)*	1.275 (2.93-3.26)*
2	1.0	0.22 (0.150-0.178)**	0.09 (0.02-0.15)**	0.97 (0.88-1.06)**
7	1.0	0.40 (0.166-0.190)**	0.13 (0.10-0.17)**	1.15 (1.06-1.25)**

15	1.0	0.44 (0.141-0.148)**	0.07 (0.02-0.11)**	0.97 (0.90-1.04)**
16	1.0	0.41 (0.168-0.192)**	0.07 (0.03-0.11)**	0.97 (0.90-1.04)**
19	1.0	0.45 (0.171-0.192)**	0.09 (0.07-0.12)**	0.97 (0.93-1.00)**
23	1.0	0.42 (0.164-0.196)**	0.06 (0.03-0.08)**	0.92 (0.88-0.96)**
24	1.0	0.34 (0.165-0.190)**	0.08 (0.05-0.11)**	0.98 (0.94-1.02)**
29	1.0	0.35 (0.164-0.188)**	0.07 (0.04-0.09)**	0.96 (0.92-1.00)**
33	1.0	0.36 (0.169-0.190)**	0.07 (0.04-0.10)**	0.94 (0.90-0.98)**

*- reliability relatively to intact group, **- reliability relatively to control group ($p<0.05$).

2.3. Molecular Docking

The docking results of the 33 tested compounds targeting the cyclooxygenase-2 (COX-2) enzyme (PDB ID: 1PXX) [12] are presented in Table 3. The 2D interactions were compared to the downloaded ligand after re-docking with an RMSD value of 1.61 Å. All tested compounds exhibited a comparable binding mode, with C-Docker interaction energy ranging from $E = -7.83$ to -9.66 Kcal/mol, compared to the ligand ($E = -7.90$ Kcal/mol). Visualization revealed that the ligand formed one hydrogen bond donor (HBD) with SER530 and one pi-sulfur bond with MET522. Additionally, hydrophobic interactions were observed, including a pi-pi T-shaped bond with TRP387 and pi-alkyl bonds with each of VAL349, VAL523, and ALA527. Most of the tested compounds showed similar interactions with the surrounding amino acid residues and a comparable binding mode (Table 3, Figure 1).

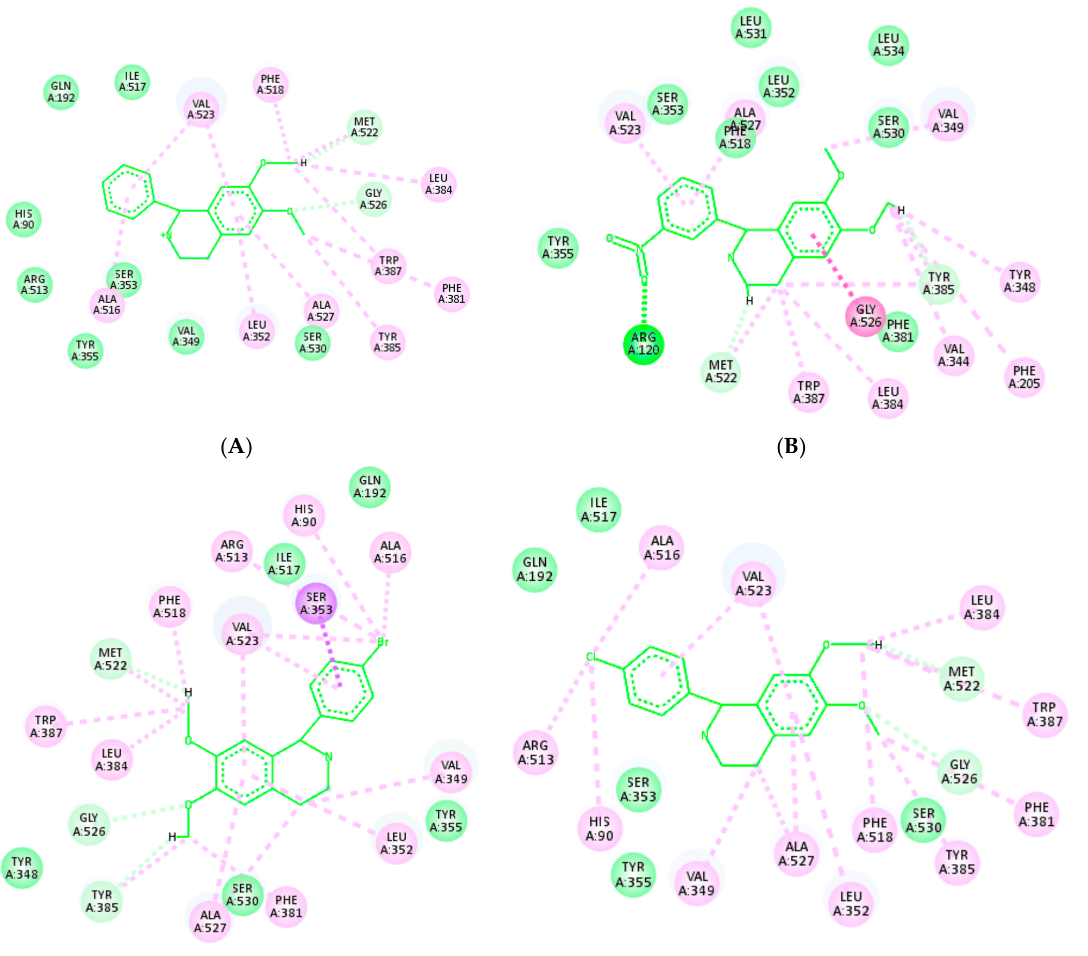
Table 3. Docking score (Kcal/mol) and interactions of compounds **2–34** in the active site of COX-2 (PDB ID: 1PXX).

Compound	Ligand/C-Docker Interaction Energy (Kcal/mol)	Amino Acid Residues Interaction
Ligand (2-[2-(2,6-dichloroanilino)-phenyl] acetic acid)	-7.90	1 HBD with **SER530**, 1 pi-sulfur with **MET522**, 1 pi-pi t-shaped with **TRP387**, 1 pi-alkyl with **VAL349**, **VAL523**, **ALA527**
2	-7.83	1 carbon-hydrogen bond with **GLY526**, 1 carbon-hydrogen bond and 1 alkyl bond with **MET522**, 1 alkyl bond with **LEU384**, 1 pi-alkyl with **PHE381**, **TYR385**, **PHE518**, **LEU352**, **ALA527** and **ALA516**, 2 pi-alkyls with **TRP387** and **VAL523**
3	-8.06	1 HBD and 1 pi-alkyl with **TYR355**, 1HBA and 1 pi-sulfur with **MET522**, 1 pi-pi t-shaped with **TRP387**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, **VAL523**, **VAL349**, 1 alkyl with **LEU531**
4	-7.94	1 HBD and 1 pi-alkyl with **PHE518**, 1HBA with **GLN192**, 1 carbon-hydrogen bond with **GLY526**, 1 carbon-hydrogen bond and 1 alkyl bond with **MET522**, 1 pi-long pair and 2 pi-alkyls with **LEU352**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, 1 alkyl bond with **VAL349**, **LEU384**,

		1 alkyl bond and 2 pi-alkyls with **VAL523**, 1 pi-alkyl with **PHE381**, **TYR385**, **TRP387**, **ALA516**
5	-7.87	1 HBA and 1 carbon-hydrogen bond with **MET522**, 3 carbon-hydrogen bonds with **SER353**, 1 carbon-hydrogen bond with **SER530**, 1 amide-pi stacked with **GLY526**, 1 alkyl bond with **LEU384**, **VAL349**, 1 alkyl bond and 1 pi-alkyl with **VAL523**, 1 pi-alkyl with **HIS90**, **TYR355**, **PHE205**, **PHE381**, **TRP387**, **ALA527**, 2 pi-alkyl with **TYR385**
6	-8.05	1 HBD with **ARG120**, 1HBD and 1 pi-alkyl with **TYR355**, 2 carbon-hydrogen bonds and 1 alkyl bond with **MET522**, 1 alkyl bond and 1 pi-alkyl with **VAL523**, **VAL349**, **ALA527**, 1 alkyl bond with **LEU384**, **LEU531**, 2 pi-alkyls with **TRP387**, 1 pi-alkyl with **PHE518**, **LEU352**
7	-9.16	1 HBD with **ARG120**, **SER530**, 1 HBD and 1 pi-alkyl with **TYR355**, 1 carbon-hydrogen bond with **LEU352**, 1 pi-sulfur with **MET522**, 1 pi-pi t-shaped with **TRP387**, 1 alkyl bond and 1 pi-alkyl with **VAL523**, **VAL349**, **ALA527**, 1 pi-alkyl with **LEU531**
8	-9.66	2 HBDs with **ARG120**, 1 carbon-hydrogen bond and 1 alkyl bond with **MET522**, 1 amide-pi stacked with **GLY526**, 1 carbon-hydrogen bond with **TYR385**, 1 alkyl bond with **LEU384**, **VAL349**, **VAL344**, 1 pi-alkyl with **PHE205**, **TYR348**, **TRP387**, **VAL523**, **ALA527**, 2 pi-alkyls with **TYR385**
9	-8.69	1 HBD with **HIS90**, **ILE517**, 1 HBD and 1 pi-alkyl with **PHE518**, 1 carbon-hydrogen with **ARG513**, **GLY526**, 1 carbon-hydrogen bond and 1 alkyl bond with **MET522**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, 1 alkyl bond with **VAL349**, **LEU384**, 1 alkyl bond and 2 pi-alkyls with **VAL523**, 1 pi-alkyl with **PHE381**, **TYR385**, **LEU352**, 2 pi-alkyls with **TRP387**
10	-8.37	1 HBD and 1 pi-alkyl with **TYR355**, 1 pi-sulfur with **MET522**, 1 pi-pi t-shaped with **TRP387**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, **VAL523**, **VAL349**, 1 alkyl with **LEU531**
11	-8.36	1 HBD with **SER530**, 1 carbon-hydrogen bond and 1 alkyl bond with **MET522**, 1 carbon-hydrogen bond and 2 pi-alkyls with **TYR385**, 1 amide-pi stacked with **GLY526**, 1 alkyl bond with **LEU384**, **VAL349**, 1 alkyl bond **(halogen bond)** and 1 pi-alkyl with **VAL523**, 1 pi-alkyl with **PHE205**, **TYR348**, **TYR355 (halogen bond)**, **LEU352**, 2 pi-alkyls with **TRP387**
12	-8.14	1 carbon-hydrogen bond with **GLY526**, 1 carbon-hydrogen bond and 1 alkyl bond with **MET522**, 1 alkyl bond with **ALA516 (halogen bond)**, **ARG513 (halogen bond)**, **VAL349**, **LEU384**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, 1 pi-alkyl with **HIS90 (halogen bond)**, **PHE381**, **TYR385**, **PHE518**, **LEU352**, 2 pi-alkyls with **TRP387**, **VAL523**
13	-8.38	1 HBD and 1 pi-alkyl with **TYR355**, pi-donor hydrogen bond with **SER530**, 1 alkyl bond with **LEU531**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, **VAL523**, 1 alkyl bond and 2 pi-alkyls with **VAL349**, 1 pi-alkyl with **PHE205**, **TYR385**, **LEU352**
14	-8.57	1 HBD and 1 pi-alkyl with **TYR355**, 1 pi-sulfur with **MET522**, 1 pi-pi t-shaped with **TRP387**, 1 alkyl bond with **LEU531**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, **VAL523**, **VAL349**, 1 pi-alkyl with **PHE381 (halogen bond)**, **TYR385 (halogen bond)**, **LEU352**
15	-8.26	1 carbon-hydrogen bond with **GLY526**, 1 carbon-hydrogen bond and 1 alkyl bond with **MET522**, 1 carbon-hydrogen bond and 1 pi-alkyl with **TYR385**, 1 pi-sigma with **SER353**, 1 alkyl bond with **ALA516 (halogen**

		bond), **VAL349**, **ARG513 (halogen bond)**, **LEU384**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, 1 alkyl bond **(halogen bond)** and 2 pi-alkyls with **VAL523**, 1 pi-alkyl with **HIS90 (halogen bond)**, **PHE381**, **TRP387**, **PHE518**, **LEU352**
16	-8.25	1 carbon-hydrogen bond with **GLY526**, **GLN192**, 1 carbon-hydrogen bond and 1 alkyl with **PHE518**, 1 carbon-hydrogen bond and 1 alkyl bond with **MET522**, 1 carbon-hydrogen bond and 1 pi-sigma with **SER353**, 1 alkyl bonds with **VAL349**, **ARG513**, **LEU384**, 2 alkyl bonds with **ALA516**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, 1 alkyl bond and 2 pi-alkyls with **VAL523**, 1 pi-alkyl with **PHE381**, **TYR385**, **TRP387**, **LEU352**, 2 pi-alkyls with **HIS90**
17	-8.54	1 HBA with **MET522**, 1 HBD and 1 pi-alkyl with **TYR355**, 1 pi-pi t-shaped with **TRP387**, 1 alkyl bond with **LEU531**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, **VAL523**, **VAL349**, 1 pi-alkyl **(halogen bond)** with **PHE381**, **TYR385**
18	-8.48	1 HBA and 1 pi-alkyl with **TYR385 (halogen bond)**, 1 HBD and 1 pi-alkyl with **TYR355**, 1 alkyl bond with **LEU384 (halogen bond)**, **LEU531**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, **VAL523**, 1 alkyl bond and 2 pi-alkyls with **VAL349**, 1 pi-alkyl with **PHE381 (halogen bond)**, **TRP387 (halogen bond)**, **LEU352**
19	-8.48	1 carbon-hydrogen bond with **SER353**, 1 amide-pi stacked with **GLY526**, 1 alkyl bond with **LEU384**, **VAL344**, 2 alkyl bonds with **VAL349**, 2 alkyl bonds and 1 pi-alkyl with **VAL523 (halogen bond)**, 1 pi-alkyl with **HIS90**, **PHE205**, **TYR348**, **TYR355**, **TYR385**, **PHE518 (halogen bond)**, **ALA527**, 2 pi-alkyls with **TRP387**
20	-8.35	1 HBA with **GLN192**, 1 HBD and 1 pi-alkyl with **HIS90**, 1 carbon-hydrogen bond with **GLY526**, 1 carbon-hydrogen bond and 1 alkyl bond with **MET522**, 1 alkyl bond with **ALA516**, **VAL349**, **ARG513**, **LEU384**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, 1 alkyl bond and 2 pi-alkyls with **VAL523**, 1 pi-alkyl with **PHE381**, **TYR385**, **PHE518**, **LEU352**, 2 pi-alkyls with **TRP387**
21	-7.84	1 HBD and 1 pi-alkyl with **TYR355**, pi-donor hydrogen bond with **SER530**, 1 alkyl bond with **VAL344**, **LEU534**, **LEU531**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, **VAL523**, 2 alkyl bonds and 2 pi-alkyls with **VAL349**, 1 pi-alkyl with **PHE205**, **TYR348**, **LEU352**
22	-8.33	1 HBD and 2 pi-alkyls with **TYR355**, 1 carbon-hydrogen bond with **SER353**, 1 amide-pi stacked with **GLY526**, 1 alkyl bond with **LEU384**, **VAL344**, 3 alkyl bonds with **VAL349**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, **VAL523**, 1 pi-alkyl with **HIS90**, **PHE205**, **TYR348**, **TYR385**, **PHE518**, 2 pi-alkyls with **TRP387**
23	-8.80	1 HBD and 1 carbon-hydrogen bond with **SER530**, 1 HBD and 1 pi-alkyl with **TYR355**, 1 carbon-hydrogen bond and 1 pi-alkyl with **TYR385**, 1 pi-sulfur with **MET522**, 1 pi-pi t-shaped with **TRP387**, 1 alkyl bond with **LEU531**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, **VAL523**, **VAL349**, 1 pi-alkyl with **PHE381**
24	-8.37	1 HBD and 1 pi-alkyl with **TYR355**, 1 pi-donor hydrogen bond with **SER530**, 1 alkyl bond with **LEU531**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, **VAL523**, 1 alkyl bond and 2 pi-alkyls with **VAL349**, 1 pi-alkyl with **PHE205**, **PHE381**, **TYR385**
25	-8.64	1 HBD and 1 pi-alkyl with **TYR355**, pi-donor hydrogen bond with **SER530**, 1 alkyl bond with **LEU531**, **LEU534**, **VAL344**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, **VAL523**, 2 alkyl bonds and 2 pi-alkyls

		with **VAL349**, 1 pi-alkyl with **PHE205**, **PHE381**, **TYR348**, **TYR385**, **LEU352**
26	-8.23	1 carbon-hydrogen bond with **LEU352**, 1 carbon-hydrogen bond and 1 pi-alkyl with **TYR355**, 1 alkyl bond with **LEU384**, **MET522**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, **VAL349**, 2 alkyl bonds and 1 pi-alkyl with **VAL523**, 1 pi-alkyl with **HIS90**, **PHE381**, **TYR385**, 2 pi-alkyls with **TRP387**, **PHE518**
27	-8.24	1 HBD and 1 pi-alkyl with **TYR355**, 1 halogen bond and 1 alkyl bond with **MET522**, 1 alkyl bond with **LEU531**, **VAL344**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, 1 alkyl bond and 2 pi-alkyls with **VAL349**, 1 pi-alkyl with **PHE205**, **TYR348**, **TYR385**, **VAL523**, **LEU352**
28	-8.42	1 HBD with **SER530**, 1 HBA, 1 carbon-hydrogen bond and 1 pi-alkyl **(halogen bond)** with **TYR385**, 1 amide-pi stacked with **GLY526**, 1 alkyl bond with **LEU384 (halogen bond)**, **VAL344**, **LEU531**, **VAL116**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, **VAL523**, 2 alkyl bonds and 2 pi-alkyls with **VAL349**, 1 pi-alkyl with **TYR348**, **TYR385**, **LEU352**, 2 pi-alkyls **(halogen bond)** with **TRP387**
29	-8.30	1 HBD with **HIS90**, 1 carbon-hydrogen bond with **GLY526**, **GLN192**, 1 carbon-hydrogen bond and 1 alkyl bond with **MET522**, pi-sigma bond with **SER353**, 1 alkyl bond with **ALA516**, **VAL349**, **LEU384**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, 1 pi-alkyl with **PHE381**, **TYR385**, **LEU352**, 2 pi-alkyls with **TRP387**, **PHE518**, **VAL523**
30	-8.83	1 HBD and 1 pi-alkyl with **TYR355**, pi-donor hydrogen bond with **SER530**, 1 alkyl bond with **LEU531**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, **VAL523**, 1 alkyl bond and 2 pi-alkyls with **VAL349**, 1 pi-alkyl with **PHE205**, **TYR348**, **TYR385**, **LEU352**
31	-8.47	1 HBD and 1 pi-alkyl with **TYR355**, 1 carbon-hydrogen bond and 1 pi-alkyl with **TYR385**, 1 alkyl bond with **LEU531**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, **VAL523**, 1 alkyl bond and 2 pi-alkyls with **VAL349**, 1 pi-alkyl with **PHE381**, **LEU352**
32	-8.17	1 HBD and 1 pi-alkyl with **TYR355**, 1 HBA with **MET522**, 1 pi-pi t-shaped with **TRP387**, 1 alkyl bond with **LEU531**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, **VAL523**, **VAL349**
33	-8.95	1 HBD and 1 pi-alkyl with **TYR355**, 1 pi-pi t-shaped with **TRP387**, 1 alkyl bond with **LEU531**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, **VAL523**, **VAL349**
34	-8.01	1 HBD and 1 pi-alkyl with **TYR355**, 1 pi-donor hydrogen bond with **SER530**, 1 alkyl bond with **LEU531**, 1 alkyl bond and 1 pi-alkyl with **ALA527**, **VAL523**, 1 alkyl bond and 2 pi-alkyls with **VAL349**



(**A**) (**B**)

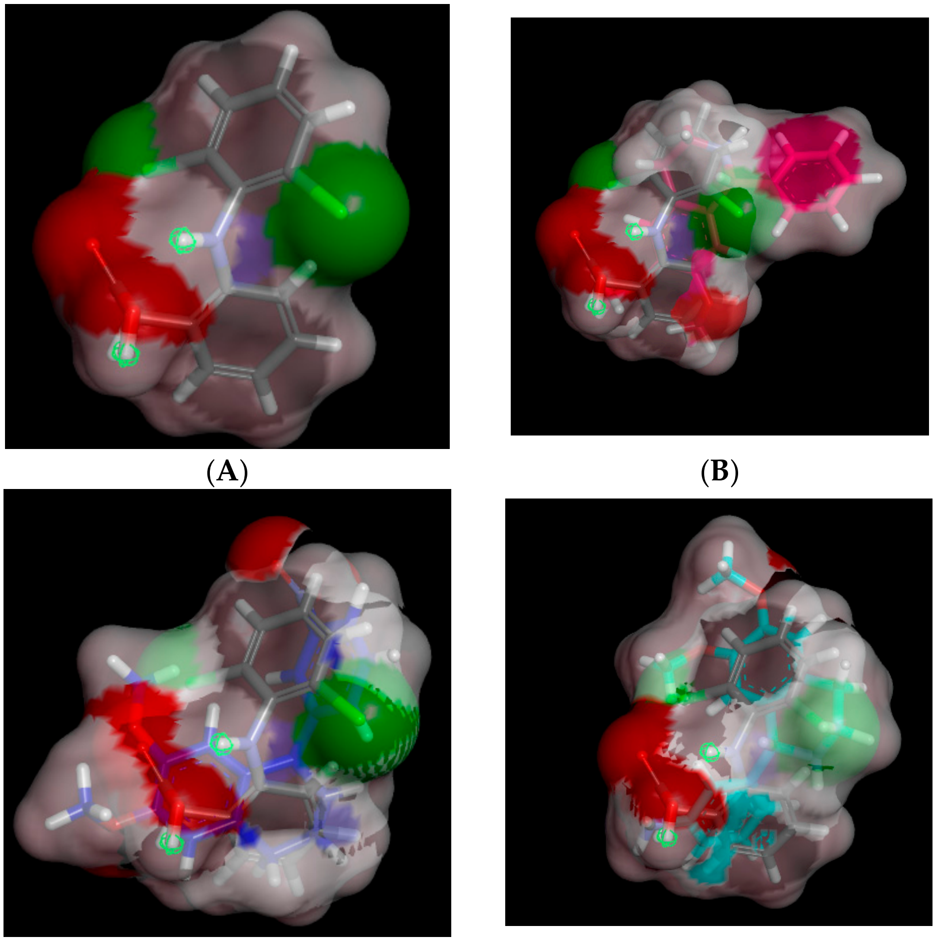
(**C**) (**D**)

Figure 1. The binding interactions with amino acid residues in the active site of COX-2 (PDB ID: 1PXX). **(A)**: Compound **8** (E = −9.66 Kcal/mol), **(B)**: compound **2** (E = −7.83 Kcal/mol), **(C)**: compound **15** (E = −8.26 Kcal/mol), **(D)**: compound **12** (E = −8.14 Kcal/mol) (Color code for interactions: H-bond: Green, carbon-H bond: light blue, Pi-Sigma bond: purple, Amide-pi Stacked bond: pink, Alkyl and Pi-alkyl bond: light pink).

2.4. Structure Activity Relationship

Molecular docking results were analyzed to study the essential pharmacophoric features in the studied scaffold represented as 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (1-ArTHIQ) derivatives. Compound **8** showed the best conformation with the most promising binding pose in the COX-2 binding pocket. Results were superior regarding both the binding interactions with the key amino acid residues and the C-Docker binding interaction energy (E= −9.66 Kcal/mol), confirming good fitting with stable binding interaction exceeding the ligand, where two conventional hydrogen bonds, one carbon-hydrogen bond, one amide-pi stacked interaction, along with several alkyl and pi-alkyl interactions within the binding site (Table 3). It was determined that the position of the nitro group on the phenyl ring significantly influences binding stability: while the meta-position (compound **8**) exhibited minimal energy, binding affinity slightly weakened in the ortho- (compound **7**, E=−9.16 Kcal/mol) and para- (compound **9**, E=−8.69 Kcal/mol) positions. The high activity of nitro derivatives is attributed to the formation of numerous hydrogen bonds with amino acid residues. Specifically, compound **8** forms two robust hydrogen bonds with ARG120, which serves as the primary stabilizing factor for its complex. In comparison, compound **2** showed a binding energy of E=−7.83 Kcal/mol and this lower affinity is due to the absence of donor-acceptor bonds, relying instead on weak carbon-hydrogen and hydrophobic interactions with MET522 and GLY526. The hydrogen bonds formed by compound **7** and compound **9** significantly enhance their activity compared to compound **2** [13,14].

Further modifications of the phenyl ring yielded specific results. In compound **29**, the presence of a 3,4-methylenedioxy group (E=−8.30 Kcal/mol) facilitated hydrogen bonding with HIS90 in the active site. The introduction of halogen atoms in compounds **30** and **31** further strengthened the binding. Notably, the 2-bromo substituent in compound **30** formed hydrogen and pi-alkyl bonds with TYR355, lowering the energy to E=−8.83 Kcal/mol. This value outperforms the E=−8.47 Kcal/mol recorded for the 2-chloro derivative in compound **31**. Analysis of the bromine position in compounds **13, 14,** and **15** revealed that the meta-bromo derivative, represented by compound **14**, provided the best results with an energy of E=−8.57 Kcal/mol. This indicates that, similar to nitro derivatives, meta-substituted compounds align most effectively with the active site geometry. The superiority of nitro-containing compounds over brominated derivatives is linked to their higher capacity for electrostatic and hydrogen bond formation [15]. An overlay between the ligand and compounds **2**, **8**, and **33** at the active site was presented (Figure 2).



(**A**) (**B**)

(**C**) (**D**)

Figure 2. Solvent-accessible surface (SAS) representations of the reference ligand and compounds. (**A**): ligand, (**B**): overlay of ligand and compound **2** (reddish), (**C**): overlay of ligand and compound **8** (dark blue), (**D**): overlay of ligand and compound **33** (cyan). Colored according to atom types (CPK coloring). Visualized using BIOVIA Discovery Studio.

Docking analysis of the studied 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline derivatives revealed essential pharmacophoric features for binding within the COX-2 active site. Compound **8** demonstrated the best results, forming a more stable complex compared to the ligand due to the formation of two robust hydrogen bonds with the ARG120 residue. The study indicated that the position of the nitro group on the phenyl ring significantly influences activity. Specifically, the meta-position provided the highest affinity, while binding energy slightly decreased in the ortho and para positions. The lower activity of compound **2** was attributed to the absence of donor-acceptor bonds. Furthermore, modifications of the phenyl ring showed that the 3,4-methylenedioxy group facilitated interactions with HIS90, and the 2-bromo substituent formed additional bonds with TYR355. Overall, nitro-derivatives exhibited the highest stability owing to their superior capacity for electrostatic and hydrogen bond formation compared to brominated compounds.

2.5. Predictive ADMET Study

A predictive ADMET study was conducted using Discovery Studio 4.0 software to determine the pharmacokinetic profiles and evaluate the drug-likeness of the tested compounds. The results, based on their chemical structures, showed a strong correlation with various parameters, such as aqueous solubility, absorption level, blood-brain barrier permeability, hepatotoxicity probability, atom-based log P98 (A LogP98), plasma protein binding (PPB) level, cytochrome P450 2D6 (CYP2D6), and 2D polar surface area (ADMET 2D PSA).

3. Discussion

The structure–activity relationship analysis showed that the highest anti-inflammatory activity was exhibited by monosubstituted derivatives in ring C containing amino, dimethylamino, nitro groups, or a bromine atom at the C-3′ or C-4′ carbon atoms (compounds 33 > 16 > 15 > 2 > 13 > 7 > 8). Their anti-inflammatory effect (AE) in both studied models ranged from 81.2% to 67.5%. The AE of these compounds exceeded that of the reference drug sodium diclofenac at doses of 8–10 mg/kg and was comparable to that of ketoprofen at a dose of 5 mg/kg. A positive influence of substituents (OH, OCH_3, Cl, NO_2) in the ortho position (C-2′) on AE was observed compared with substitution at C-4′ (3 > 4, 6 > 5, 7 > 9, 10 > 12), whereas for bromine-containing compounds the opposite trend was noted (15 > 13 > 14). The absence of substitutients at these positions or substitution at C-2′ or C-3′ with nitro, amino, or methoxy groups led to a decrease in anti-inflammatory effect to 67–61% (7 > 8 > 34 > 32 > 6). Replacement of the hydrogen atom at these positions with Cl, OH, Br, or a nitro group resulted in a further reduction of AE to 52.7–44.8% (11 > 10 > 3 > 14 > 9).

Among disubstituted derivatives, high anti-inflammatory activity (81–71%) was demonstrated by compounds bearing methoxy and nitro, bromine and methoxy, or methylenedioxy substituents at the C-2′ and C-3′ or C-3′ and C-4′ carbon atoms (23 > 19 > 29). Additional introduction of a methoxy group at the 3′ or 4′ position of monosubstituted compounds increased their activity (23 > 7, 19 > 14).

Compound 24, containing three substituents at the C-2′, C-3′, and C-4′ positions (2-Br, 3-OH, and 4-OCH_3), exhibited good activity (76.8%). Other derivatives simultaneously substituted at three carbon atoms, including the C-5′ atom, showed moderate anti-inflammatory effects in the range of 52.7–29.8% (26 > 30 > 25 > 27 > 28). Among these, the most active were derivatives containing methoxy or methylenedioxy groups at C-4′ and C-5′ and a nitro group or bromine atom at C-2′ (compounds 26 and 30).

The study of the effect of 1,2,3,4-tetrahydroisoquinoline (THIQ) derivatives on the levels of anti-inflammatory and pro-inflammatory cytokines in the blood serum of rats with experimental inflammation showed that many of the examined compounds exhibiting high anti-inflammatory activity (60% and above) modulated, to varying degrees, the levels of pro- and anti-inflammatory cytokines (Table 2), promoting their more rapid return to values close to those of intact animals. In contrast, these parameters in the control group 2 animals with inflammation remained relatively elevated on 7th day of the experiment.

The combined results obtained from the study of anti-inflammatory activity and acute toxicity [10] of synthesized derivatives indicate that compounds 16, 33, and 24 (acute toxicity LD_{50} of 1250, 750, and 550 mg/kg, respectively) can be considered promising candidates for the development of medicinal agents.

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In silico modelling of 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline derivatives interaction with COX-2 active site indicated that the nitro-derivatives exhibited the highest stability owing to their superior capacity for electrostatic and hydrogen bond formation compare to brominated compounds. The presented data on the effect of substituents $–NH_2$, –OH, and $–OCH_3$ in ring C (1-Ar-) of 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines on anti-inflammatory activity further stimulate the search for new highly effective derivatives within this series.

The data from this in vivo and in silico study indicate that the anti-inflammatory effects of the leading substances from the examined series of 1-aryl-1,2,3,4 -tetrahydroisoquinolines may be related to their action on COX-2 enzyme and regulation of cytokine levels. Some of the investigated compounds may serve as lead compounds for development of new non-steroid anti- inflammatory drugs.

4. Materials and Methods

4.1. Biological Evaluation

The animal study was conducted by the International Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes (Strasbourg, 1986), and the Institutional Scientific Board of the Institute of the Chemistry of Plant Substances, Academy of Sciences of Uzbekistan approved the protocol based on an annual working plan of the Department of Pharmacology and Toxicology (Protocol No. 1 from January 16 in 2025).

The anti-inflammatory effects of the examined substances were studied in white male rats weighing 180-220 g divided on the following groups: intact, control (formalin model), sodium diclofenac group, ketoprofen group, experimental groups (for each checking substance separately). A simple randomization (coin toss “heads of tails”) was used for animal group formation. Each group contained 6 animals.

The effect of the studied compounds on the inflammatory response development was investigated using the formalin rat model after injection of formalin into the hind paw pads.

The levels of anti-inflammatory and pro-inflammatory cytokines were determined after subcutaneous injection of 0.1 mL of a 2% formalin solution into the pelvic region of the rat. To evaluate the dynamics of major cytokine production during the experiment, blood samples were collected from the animals before the start of the experiment and on 7th day after its initiation. The concentrations of the principal cytokines interleukin-4 (IL-4), interleukin-6 (IL-6), and tumor necrosis factor (TNF) were measured in rat serum by ELISA method using a Mindray MR-96A microplate immunoassay analyzer (China).

Data were presented as mean ± SD for different groups. Statistical analysis of the results was performed using IBM® SPSS® Statistics v27.0.1.0 software, and the significance of the obtained data was assessed using Student’s t-test.

4.2. Molecular Modelling

Among the three known COX isoforms (COX-1, COX-2, and COX-3), the inducible enzyme COX-2 is recognized as the most active during inflammatory processes [18]. Molecular docking studies of the 34 tested compounds were conducted against the cyclooxygenase-2 (COX-2) enzyme (PDB ID: 1PXX), with the crystal structure obtained from the Worldwide PDB Protein Data Bank [18–20]. For the simulation, the A chain of the protein was specifically selected.

Ligand-receptor interactions were modeled using the AutoDock 4.0 software within the MGL Tools 1.5.6 suite, employing the Lamarckian Genetic Algorithm (LGA) [21]. Preliminary protein preparation involved the removal of all water molecules from the crystal structure. Receptor and ligand files were converted to PDBQT format, incorporating missing hydrogen atoms and assigning partial atomic charges via the Gasteiger method. The grid box dimensions were set at 40 × 40 × 40 Å with central coordinates of x = 29.51, y = 22.92, and z = 17.87, encompassing the active binding site.

Visual inspection of the docking results and analysis of the interaction energy (E) were further performed using Discovery Studio 4.0 [22]. This facilitated a comparative analysis of the binding modes of the tested compounds against reference ligands and reported literature. The best-scoring pose for each compound was selected to represent key interactions with essential amino acid residues, including hydrogen bonding, hydrophobic interactions (pi–pi, pi–alkyl), and Van der Waals forces. Additionally, a predictive in silico ADMET study was conducted using Discovery Studio 4.0 to evaluate the pharmacokinetic profiles and drug-likeness of the compounds, as well as to screen for potential toxicity [17].

Abbreviations

The following abbreviations are used in this manuscript:

1-ArTHIQ	1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline
COX-2	Cyclooxigenase-2

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