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Synthesis, characterization and biological activity evaluation of novel thiazole derivatives containing acetic acid residue as selective COX-1 inhibitors

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Abstract

The fact that the side effect profiles of the COX inhibitors available in the market is very high and most of these side effects are caused by non-selective inhibitors increases the need for new selective COX-1 inhibitors. In this study, carried out to develop a new COX-1 inhibitor, the thiazole ring system was preferred because of its known activity in the vary different field. Additionally, The acid residue, which is in the structure of the most commonly used COX inhibitors such as ıbuprofen and flurbiprofen, was synthesized. 2-(4-((4-(Substituted phenyl)thiazol-2-yl)amino)phenyl)acetic acid (3a-3c) series consisting of 3 new compounds was synthesized. The structures of the obtained compounds were elucidated using ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy data. The *in vitro* COX inhibitory activity of the compounds was determined using fluorimetric methods.Among the synthesized compounds, the compound **3c** showed similar activity with the reference drug against the COX-1 enzyme. When the selective COX-1 inhibitory potentials of the synthesized compounds are examined, compound **3c** comes to the fore. According to the results of this study, it is recommended to investigate the selective COX-1 inhibitory activities of new compounds to be synthesized with modifications to be made on the active derivative in the project.

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1. Introduction

Cyclooxygenase (COX) enzymes; are integral membrane proteins containing heme as cofactors. There are two isoforms of the COX enzyme called COX-1-2. COX-2 is restricted in the nuclear membrane and endoplasmic reticulum (ER), whereas COX-1 is found in the ER. Both enzymes convert arachidonic acid to prostaglandins, but they differ from each other in their distribution and physiological roles in the organism. The amino acid sequences are 60-65% identical. COX-1 covers 576 amino acids; COX-2 covers 587 amino acids. The COX-1 enzyme is encoded by gene located on the ninth chromosome. It is found in almost all tissues under physiological conditions. COX-1 produced in platelets is involved in the formation of thromboxane responsible for platelet aggregation. It is commonly found in the gastric mucosa. It is responsible for the formation of cytoprotective prostaglandins. By stimulating the synthesis of vasodilator prostaglandins (PGI2, PGE2 and PGD2) in the kidney, it plays a role in regulating blood flow, reducing vascular resistance, expanding renal blood vessels and increasing organ perfusion [1-3].

The selectivity to COX-1 has benefits, such as inhibition of platelet TXA2 produc-tion and absence of gastrointestinal toxicity, while non-selective COXs inhibitors have opposing cardiovascular side effectsdue to their action as reducers of prostacyclin (PGI2) biosynthesis, which has cardio protective influence presence a vasodilator and a potentplatelet aggregation inhibitor [4-8]. Therefore, within the scope of this study, it is planned to evaluate the synthesis and biological activities of new selective COX-1 inhibitors.

Thiazoles have diverse applications in drug improvement for treatment inflammation, allergies, HIV infections, hypertension, bacterial infections, hypnotics, schizophrenia, and pain as fibrinogen receptor antagonists with antithrombotic activity [9-19]. It was thought that such an active ring would be chosen as the main structure and a positive contribution

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to the activity would be made. In addition, acetic acid residue was added to the structure to increase COX-1 selectivity. Thus, it is thought that many side effects caused by non-selective COX inhibitors can be combated.

It is important to develop studies to develop new drugs in the pharmaceutical industry of our country. The discovery of a new drug may become a reality as a result of academic studies. As a matter of fact, projects to find an effective new compound are intensively carried out by pharmaceutical chemists in our country. In this study, considering the potential analgesic effect of the thiazole ring system, 3 new compounds were synthesized. In the design of the target compounds in the study, a biologically active thiazole ring was considered as a constant, and aromatic systems were placed on both sides. One of these aromatic systems has been substituted with acetic acid. The aim here is to provide the acidic structure found in most COX-1 inhibitors. Synthesis compounds were subjected to efficacy tests for COX inhibition. % inhibition values and IC_{50} values were recorded. A halogen-substituted benzene ring is placed on the other side. Here, the contribution of the fluorine atom in flurbiprofen to the activity was tried to be caught. At the same time, a compound containing chlorine atom instead of fluorine atom was also synthesized and it was desired to examine how halogens would affect the activity among themselves.

2. Materials and Methods

2.1. Chemistry

All reagents were purchased from commercial suppliers and used as such.^{1}H-NMR and ^{13}C-NMR, (nuclear magnetic resonance) were performed using Bruker DPX 300 FT-NMR spectrometer and Bruker DPX 75 MHz spectrometer, respectively (Bruker Bioscience, Billerica, MA, USA). Coupling constants (*J*) were stated in Hertz (Hz). Mass spectra were verified on an APCI-MS (Advion, New York, USA) using the APCI method.

2.1.1. Synthesis of 2-(4-Aminophenyl)acetic acid hydrochloride (1)

2-(4-aminophenyl)acetic acid (0.03 mol) was dissolved in water (20 ml). A solution of HCl in water was added in 2-(4-aminophenyl)acetic acid solution in water as portions. It was decided that the reaction was complete by applying TLC. Before this reaction was terminated, the next step was passed.

2.1.2. Synthesis of [4-(carbamothioylamino) phenyl]acetic acid (2)

KSCN (0.036 mol) was added to the reaction content obtained in the synthesis of 2-(4-Aminophenyl)acetic acid hydrochloride. The reaction mixture was refluxed for 10 hours. After achievement of the reaction, the solvent was evaporated, and the hastened compound was filtered.

2.1.3. Synthesis of target compounds (3a-3c)

Compound **2** (0.001 mol) and appropriate 2-bromoacetophenone (0.001 mol) were refluxed in EtOH for 8 hours. After completion of the reaction, the precipitated product was filtered, dried recrystallized from EtOH.

2-(4-((4-(4-Fluorophenyl) thiazol-2-yl)amino)phenyl) acetic acid (3a)

Harvest: 83 %, ^{1}H-NMR (DMSO-d_6, 300 MHz): δ = 3.33 ppm (s, 2H, –CH_2–), 7.20 ppm (d, 2H, *J*=8.7 Hz, phenyl), 7.22-7.28 ppm (m, 3H, Thiazole+phenyl), 7.57 ppm (d, 2H, *J*=8.7 Hz, phenyl), 7.95 ppm (dd, 2H, J_1=5.6 Hz, J_2=8.9 Hz, phenyl), 10.23 ppm (s, 1H, -NH). ^{13}C-NMR (DMSO-d_6, 75 MHz): δ =45.53 ppm, 102.70 ppm, 115.91 ppm (*J*=21.6 Hz), 117.19 ppm, 128.09 ppm (*J*=7.9 Hz), 130.20 ppm, 131.35 ppm, 131.69 ppm (*J*=3.4 Hz), 139.46 ppm, 149.45 ppm, 162.05 ppm (*J*=244.3 Hz), 163.95 ppm, 174.51 ppm. APCI-MS $[M+H]^+$: $C_{17}H_{13}FN_2O_2S$; calculated:329.1; found:329.7.

2-(4-((4-(2,4-Difluorophenyl) thiazol-2-yl)amino) phenyl)acetic acid (3b)

Harvest: 79 %, ^{1}H-NMR (DMSO-d_6, 300 MHz): δ = 3.26 ppm (s, 2H, –CH_2–), 7.16 ppm (d, 1H, *J*=2.6 Hz, phenyl), 7.18-7.21 ppm (m, 3H, Thiazole+ phenyl), 7.34 ppm (td, 1H, J_1=2.6 Hz, J_2=9.2 Hz, phenyl), 7.54 ppm (d, 2H, *J*=8.7 Hz, phenyl), 8.13 ppm (td, 2H, J_1=6.9 Hz, J_2=8.9 Hz, phenyl), 10.36 ppm (bronsted singlet, 1H, -NH). ^{13}C-NMR (DMSO-d_6, 75 MHz): δ =44.83 ppm, 104.96 ppm (t, *J*=26.7 Hz), 106.9 ppm (d, *J*=13.8 Hz), 112.39 ppm (d, *J*=23.7 Hz), 117.20 ppm, 119.55 ppm, 130.11 ppm, 131.22 ppm, 132.70 ppm, 139.08 ppm, 143.32 ppm, 159.97 ppm (dd, J_1=12.8 Hz, J_2=250.2 Hz), 161.61 ppm (dd, J_1=12.7 Hz, J_2=245.1 Hz), 163.37 ppm, 175.09 ppm. APCI-MS $[M+H]^+$: $C_{17}H_{12}F_2N_2O_2S$; calculated:347.1; found:347.7.

2-(4-((4-(2,4-Dichlorophenyl)thiazol-2-yl)amino) phenyl) acetic acid (3c)

Harvest: 80 %, ^{1}H-NMR (DMSO-d_6, 300 MHz): δ = 3.21 ppm (s, 2H, –CH_2–), 7.17 ppm (d, 2H, *J*=8.5 Hz, phenyl), 7.35 ppm (s, 1H, Thiazole), 7.49-7.54 ppm (m, 3H, phenyl), 7.68 ppm (d, 1H, *J*=2.1 Hz, phenyl),

7.97 ppm (d, 1H, *J*=8.7 Hzr, phenyl), 10.37 ppm (s, 1H, -NH). ^{13}C-NMR (DMSO-d_6, 75 MHz): δ =47.35 ppm, 110.63 ppm, 119.38 ppm, 130.27 ppm, 132.29 ppm, 132.30 ppm, 132.45 ppm, 134.16 ppm, 134.77 ppm, 135.06 ppm, 135.20 ppm, 141.20 ppm, 148.14 ppm, 165.39 ppm, 177.01 ppm. APCI-MS $[M+H]^+$: $C_{17}H_{12}Cl_2N_2O_2S$; calculated:379.0; found:379.6.

2.2. Biological activity

The in vitro inhibition power of the synthesized compounds against COX-1/COX-2 isoenzymes was restrained by means of fluorometric COX-1 and COX-2 inhibitor screening kits (Biovision, Switzerland) according to the builder's orders [20,21]. The assay was founded on the fluorometric discovery of prostaglandin G2, the middle product made by the COX enzymes. The *in vitro* COX-1 and COX-2 inhibition assay procedure was carried out as previously declared by our research group [22, 23].

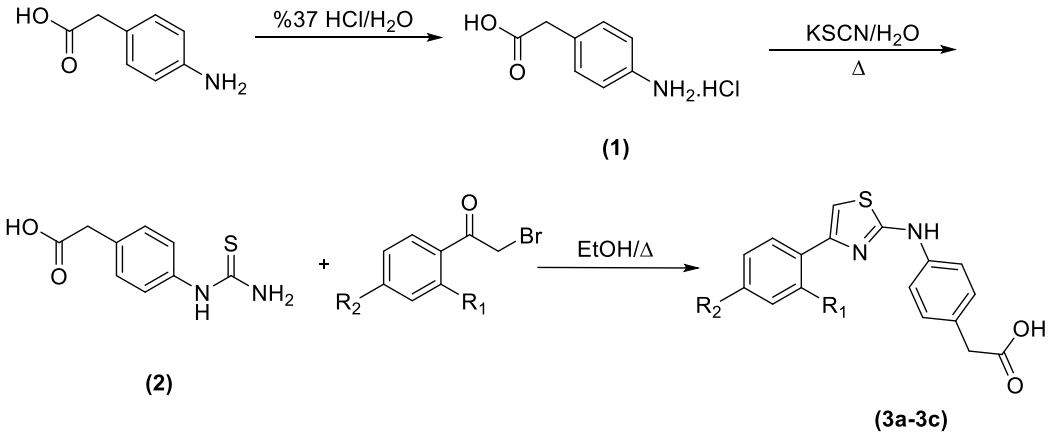
2.3. Molecular docking

Molecular docking studies were carried out using a structure-based protocol to reveal the binding mechanisms of compound 3c to the active site of the COX-1 enzyme. For this purpose, the crystal structure of COX-1 crystallized with flurbiprofen (PDB ID: 1EQH) [24] was extracted from the Protein Data Bank database [25]. Docking studies were performed as reported in previous studies [26-29].

3. Results and Discussion

3.1. Chemistry

The compounds 3a-3c were obtained as presented in Scheme 1. Firstly, 2-(4-Aminophenyl)acetic acid hydrochloride (1) was obtained by means of reaction between 2-(4-aminophenyl)acetic acid and HCl solution. This salt-forming reaction was proceeded by very careful dropwise addition. An ice bath was used while adding the HCl solution. Secondly, [4-(carbamothioylamino)phenyl]acetic acid (2) was synthesized using potassium thiocyanate. Continuing this reaction without terminating the first reaction medium significantly shortens the experiment time. The purpose of this event is to eliminate the possibility of decomposition of the substance we obtain in the form of salt in the time it takes until the water end. Finally, the resulting compound (2) and the appropriate 2-bromoacetophenone were reacted to synthesize the target compounds. The structures of the gained compounds were demonstrated by means of spectroscopic methods, such as, ^{1}H-NMR, ^{13}C-NMR and APCI-MS (Supplementary Data).



Compounds	R_1	R_2
3a	-H	-F
3b	-F	-F
3c	-Cl	-Cl

Scheme 1: Synthesis pathway of obtained compounds (3a-3c)

3.2. Biological activity

The fluorometric COX enzymes inhibition assay was applied to determine the inhibition power of all the synthesized thiazole derivatives [20-22]. The enzyme activity procedure was performed conferring to the inhibition fractions and concentrations of the derivatives as two steps. The first step of the enzyme inhibition assay was performed by means of the applications of 10^{-3} and 10^{-4} M of the produced compounds and reference drugs. The results of this

step were given in Table 1. The compounds which presented more than 50% inhibitory activity at 10^{-4} M concentration were designated for the another step of inhibition procedure and this step was performed using their additional concentrations by serialized dilutions (extending from 10^{-5} M to 10^{-9} M). The percent inhibition rates (at 10^{-3} M to 10^{-9} M) and the semi highest inhibitory concentration (IC_{50}) principles of the designated compounds were given in Table 1.

Firstly, it could be said by looking Table 1 that all compounds showed higher inhibition power against COX-1 enzyme. None of the compounds displayed more than 50% inhibition at 10^{-4} M concentration on COX-2 enzyme. On the other hand, compound 3c demonstrated more than 50% inhibitory activity on COX-1 enzyme and the second step of enzyme inhibition assay was carried out with further concentrations of this compound to calculate the IC_{50} value Among the synthesized derivatives, compound 3c was found to be the most active agent with an IC_{50} value of 2.518±0.120 μM. When this value compared to that of reference drugs, it was seen that compound 3c displayed similar potent inhibition profile with ibuprofen (IC_{50}=2.450±0.135μM).

Compound 3c has chlorine substituent unlike other compounds. According to the activity results, it is seen that the chlorine substituent contributes positively to the activity compared to the fluorine substituent. Docking studies with COX-1 enzyme active site and compound 3c were performed to explain how this contribution might be.

Table 1. %Inhibition of the synthesized compounds, ibuprofen, celexocib and nimesulide against COX-1 and COX-2 enzymes

Compounds	COX-1 % Inhibition		COX-1 IC_{50} (μM)	COX-2 % Inhibition		COX-2 IC_{50} (μM)	Selectivity	Selectivity index (SI)
	10^{-3} M	10^{-4} M		10^{-3} M	10^{-4} M			
3a	97.796 ±1.450	62.750 ±1.108	>10	92.345 ±1.702	40.643 ±0.932	>100	COX-1	>10
3b	94.247 ±1.632	67.159 ±1.023	>10	93.473 ±1.465	40.943 ±0.815	>100	COX-1	>10
3c	97.458 ±1.957	89.365 ±1.234	2.518 ±0.120	95.216 ±1.258	49.108 ±0.902	>100	COX-1	>39.714
Ibuprofen	98.152 ±1.058	89.361 ±1.245	2.450 ±0.135	98.234 ±1.208	88.155 ±1.348	5.326 ±0.218	COX-1	2.174
Celecoxib	-	-	-	92.327 ±1.425	85.485 ±1.303	0.132 ±0.005	COX-2	-
Nimesulide	-	-	-	97.821 ±1.214	89.575 ±1.049	1.684 ±0.079	COX-2	-

3.3. Molecular docking

As mentioned in the COX enzymes inhibition power assay, compound 3c was found to be the most active derivative in the series on COX-1 enzyme. Therefore, docking studies were performed to prove its inhibition power by using *in silico* method. Through insertion studies, further information on the binding mode of compound 3c and evaluation of the effect of structural modifications on inhibitory activity against COX-1 enzyme could be sought. The X-ray crystal structure of COX-1 (COX-1 PDB Code: 1EQH) [24] retrieved from Protein Data Bank database [25] was used in the docking procedure. The rendered docking poses of compound 3c were presented in Figures 1-2. With the COX-1 enzyme active site of compound 3c; its 2-dimensional interaction is presented in Figure-1, and its 3-dimensional interaction is presented in Figure-2.

When Figure 1 is examined, the OH group of the acetic acid group formed a hydrogen bond with Tyr385 in the active site. It explains the selective effect of these compounds. The nitrogen of the thiazole ring formed a hydrogen bond with Arg120. Again, this ring exhibits π-π interaction with both Arg120 and Tyr355.

When examined compound 3c from the point of chemical structure, it was understood that compound 3c had the 2,4-dichlorophenyl ring differ from the additional obtained compounds. Furthermore, this studies demonstrated that the chlorine atom particularly at the 4nd situation of the phenyl ring was very crucial aimed at binding to the active site of the enzyme and so giving high inhibitory activity on COX-1. For the reason that, it was detected that there was a halogen bond between the chlorine atom at the 4nd location of the phenyl ring and the Arg83 (Figure 2). All these detected connections explained why compound 3c exhibited a good profile.

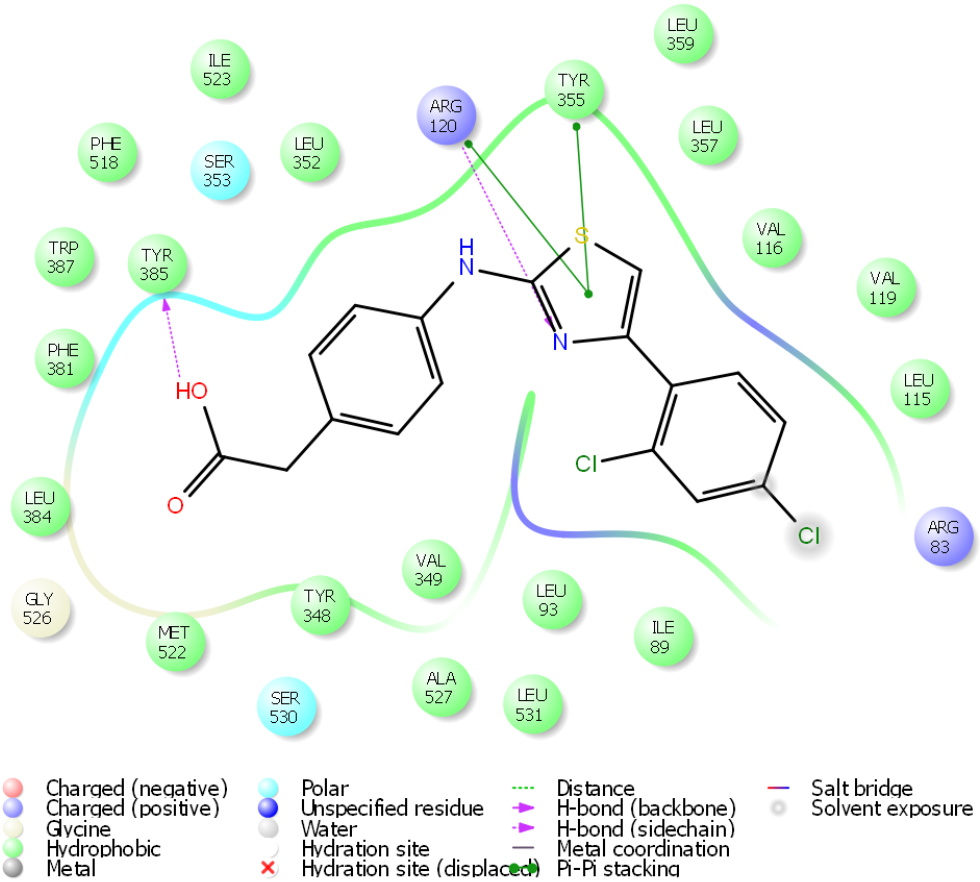


Figure 1. The 2D interacting mode of compound **3c** in the active region of ovis COX-1 enzyme (PDB ID:1EQH)

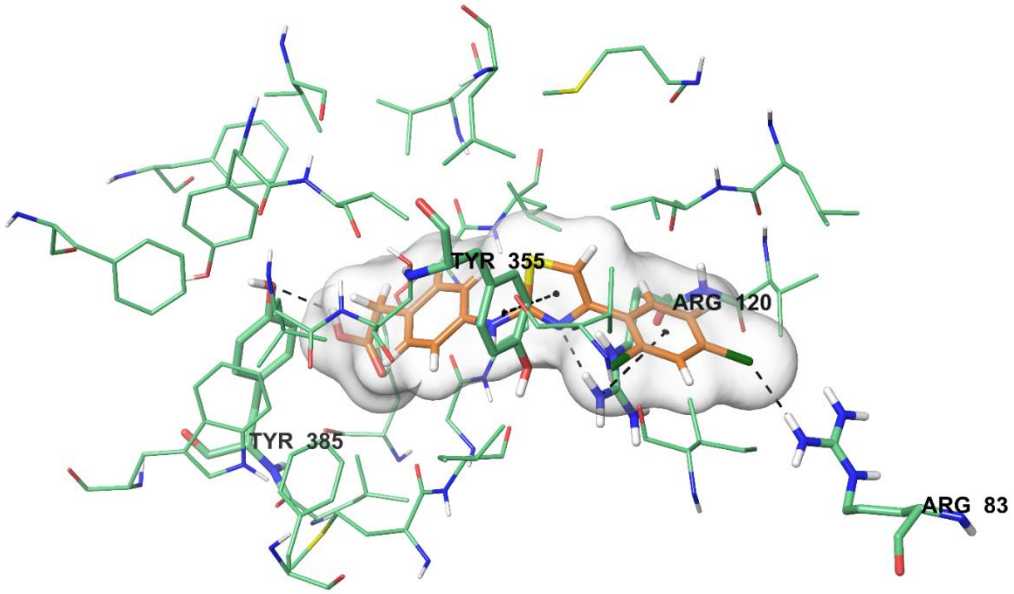


Figure 2. The 3D interacting mode of compound **3c** in the active region of ovis COX-1 enzyme (PDB ID:1EQH). The inhibitor colored with orange, and the important residue, colored with green, in the active site of the enzyme are presented by tube model.

4. Conclusion

Putting a new treatment into the service of humanity requires a long and meticulous work process lasting 12-15 years. Thanks to the new treatments put into service after these studies, people's lives are extended, their quality of life increases and important ways are covered in the fight against deadly diseases. While the average human lifespan in the world was at the age of 40 at the beginning of the 20th century, one of the important factors in reaching the age of 70 today is the development of new drugs and putting them at the service of humanity. The main purpose of drug development is to make a change in people's lives for the better. With each drug developed, it is necessary to obtain a preventive, curative or reducing effect on the signs and symptoms of the disease.

It is important to develop studies to develop new drugs in the pharmaceutical industry of our country. The discovery of a new drug may become a reality as a result of academic studies. As a matter of fact, projects to find an effective new compound are intensively carried out by pharmaceutical chemists in our country.

In this proposed study, the chain will form the first link in the long process for the discovery of a new drug. The promising results of the study give time to reach more active compounds.

Found in 1899, Aspirin® (Acetylsalicylic acid) is the first example of NSAID drugs. In the following years, many anti-inflammatory compounds such as ibuprofen, indomethacin, diclofenac and naproxen were developed and offered for treatment. The mechanism of action of NSAIDs is inhibition of prostaglandin (PG) synthesis by inhibition of cyclooxygenase enzymes and lipoxygenase. There is an urgent need to use new and safe anti-inflammatory drugs for common chronic inflammatory disorders such as rheumatoid arthritis.

In this study, considering the potential analgesic effect of the thiazole ring system, 3 new compounds were synthesized. The structures of the obtained compounds were elucidated by ^{1}H-NMR, ^{13}C-NMR and mass spectroscopic methods. Synthesis compounds were subjected to efficacy tests for COX inhibition. % inhibition values and IC_{50} values were recorded. The results showed that all compounds exhibited selective COX-1 inhibition. In addition, compound 3c exhibited similar inhibitory potential to a drug with widespread clinical use, such as ibuprofen. Therefore, according to the results of this study, it is recommended to investigate the analgesic activities of the new compounds to be synthesized in future studies by adhering to the synthesis methods used within the scope of the project.

When looking at the data obtained, the fact that the compounds show selective COX-1 inhibition. Compound 3c is very important for this activity areas. In summary, structural modifications can be further made on the basis of the new thiazoles to look for compounds with higher inhibitory activity against the human COX-1 enzyme.

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Conflicts of interest

The authors declare no conflict of interest, financial or otherwise.

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