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Research Article

COX inhibitory profiles of a series of thiadiazole-benzothiazole hybrids

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ABSTRACT

In an endeavour to identify small molecule COX-1 inhibitors, a colorimetric assay protocol was applied for the *in vitro* evaluation of COX-1 and 2 inhibitory potential of a series of thiadiazole-benzothiazole hybrids. The most potent and selective COX-1 inhibitor in this series was found as 2-[(5-amino-1,3,4-thiadiazol-2-yl)thio]-*N*-(6-chlorobenzothiazol-2-yl) acetamide (**7**) (51.36 ± 3.32% at 100 µM) compared to SC-560 (83.64 ± 3.76% at 1 µM). Compound **7** exerted weaker inhibitory effect on COX-2 (11.05 ± 1.69% at 100 µM). To explore its binding interactions at the active site of human COX-1 (PDB ID: 6Y3C), molecular docking studies were conducted. Compound **7** could establish hydrogen bonds with proper residues thanks to its amide C=O group. *In silico* studies were employed to shed light on their pharmacokinetic properties. Taken together, compound **7** can be considered as a potential lead compound for the generation of selective COX-1 inhibitors with enhanced efficacy.

Keywords: Benzothiazole, cyclooxygenase, molecular docking, thiadiazole

1. INTRODUCTION

Cyclooxgenases (COXs), which convert arachidonic acid (AA) into prostanoids in the AA cascade, are the key molecular targets for nonsteroidal anti-inflammatory drugs (NSAIDs) [1,2]. COX-1 and 2, two major isoforms of COX, are membrane-bound enzymes located in the endoplasmic reticulum and the nuclear envelope. COX-1 is the housekeeper isoform implicated in the maintenance of homeostasis, the protection of the gastric mucosa, the regulation of platelet aggregation, and renal perfusion [3]. On the other hand, COX-2 is the inducible isoform of COX that is associated with the disease-related inflammatory reactions [3,4].

Today, the paradigm of "good" housekeeping COX-1 and "bad" inducible COX-2 has lost its validity since it is known that COX-1 derived prostaglandins (PGs) also contribute to inflammation, COX-2 is also constitutively expressed in some tissues, and inhibiting COX-2 rather than COX-1 is not sufficient to prevent gastrointestinal (GI) toxicity [5-8].

Mounting evidence has demonstrated that COX-1 plays a key role in the pathogenesis of several diseases such as pain, neuroinflammation, cancer, and cardiovascular disorders. The inhibition of platelet COX-1 by low-dose aspirin is reported to be beneficial for preventing cardiovascular diseases. Furthermore, it is understood that the long-term use

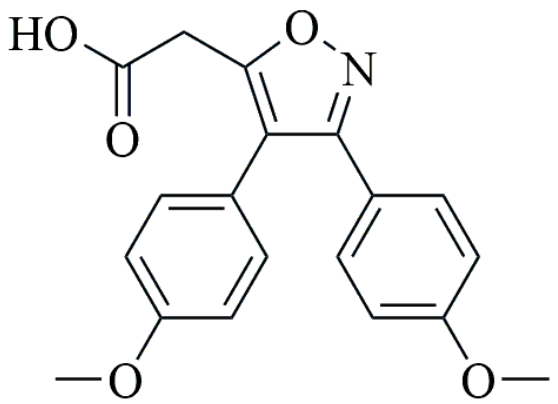


Figure 1. Mofezolac

of NSAIDs diminishes the incidence of some diseases including Alzheimer's Disease and some types of cancer. There is only one selective COX-1 inhibitor (mofezolac) (Figure 1) currently prescribed just in Japan for the treatment of pain and inflammation, and therefore the search for selective inhibitors of COX-1 has come into prominence [9-11].

The 1,3,4-thiadiazole has been emerged as a distinguished scaffold for the design of drug-like molecules due to its unique features (e.g. mesoionic nature and good liposolubility), which enable compounds with a thiadiazole motif to pass through cellular membranes and interact with biological targets properly [12-17]. In particular, 1,3,4-thiadiazoles have been reported to exhibit anti-inflammatory activity through several mechanisms including the inhibition of COXs [18-20].

The benzothiazole core serves as a privileged building block of small molecule ligands endowed with a broad range of biological activities such as anti-inflammatory activity targeting a plethora of crucial enzymes including COXs [21-28].

In the continuation of our recent studies on the discovery of selective COX-1 inhibitors [29,30], *in vitro* experiments were conducted to evaluate the COX inhibitory profiles of a series of thiadiazole-benzothiazole hybrids. Computational studies were also performed for the most active COX-1 inhibitor in this series.

2. MATERIALS AND METHODS

2.1. Chemistry

The reaction of 2-mercapto-5-methyl/amino-1,3,4-thiadiazole with appropriate 2-chloro-*N*-(benzothiazol-2-yl)acetamide in the presence of the base catalyst (potassium carbonate) afforded 2-[(5-methyl/amino-1,3,4-thiadiazol-2-yl)thio]-*N*-(benzothiazol-2-yl)acetamides (**1-10**). The synthetic procedure and the spectral data of these compounds were reported previously by Altintop *et al*. [31].

2.2. Biochemistry

2.2.1. Assessment of COX inhibition

The inhibitory effects of compounds **1-10** (at 100 µM) on COX-1 and 2 were examined using COX colorimetric inhibitor screening assay based on the instructions provided by the manufacturer (Cayman, Ann Arbor, MI, USA) as described earlier [32]. All experiments were performed in triplicate and the data were expressed as mean ± SD. The selective COX-1 inhibitor SC-560 (at 1 µM) and the selective COX-2 inhibitor rofecoxib (at 10 µM) were used in this study for comparison.

2.3. *In Silico* Studies

2.3.1. Molecular docking

Computational studies of compound **7** and mofezolac (selective COX-1 inhibitor) were performed on the X-ray crystallographic structure of the COX-1, which was retrieved from the Protein Data Bank (PDB) with the accession code 6Y3C [33,34]. The Protein Preparation Workflow of Schrödinger Release 2023-2 (Schrödinger, LLC, New York, NY, USA) was performed. The LigPrep module was used to prepare the ligands, which were drawn with 2D Sketcher. OPLS4 was selected as the force field. The Receptor Grid Generation was used to generate the grid box. Finally, the Ligand Docking was employed.

2.3.2. Prediction of pharmacokinetic profiles

The QikProp, the *in silico* absorption, distribution, metabolism, and excretion (ADME) module within

the Maestro suite (Schrödinger Release 2023-2, LLC, New York, USA), was used to estimate the pharmacokinetic profile of compound **7**.

3. RESULTS AND DISCUSSION

3.1. *In vitro* Studies

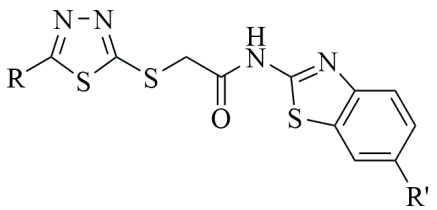
Among the tested compounds, compound **7** was found to be the most potent inhibitor of COX-1 (51.36 ± 3.32% at 100 µM) in comparison with SC-560 (83.64 ± 3.76% at 1 µM) (Table 1). The inhibitory effect of compound **7** on COX-2 (11.05 ± 1.69% at 100 µM) was found to be weaker than its inhibitory effect on COX-1.

Compound **1** inhibited COX-1 and 2 with the percentages of 25.50 ± 3.54 and 22.47 ± 2.67%, respectively. The COX inhibitory profile of compound **1** can be described as non-selective. Compound **2** inhibited only COX-1 (7.58 ± 1.16%), while compounds **4** (10.85 ± 2.05%) and **9** (8.82 ± 1.42%) caused only COX-2 inhibition. On the other hand, compounds **3**, **5**, **6**, **8** and **10** did not exert any inhibitory action against both COXs. Based on the *in vitro* data (Table 1), it can be concluded that the substitutions on the benzothiazole scaffold as well as on the thiadiazole ring are important for the inhibition of both COXs. The incorporation of the methyl and the ethoxy substituents into the sixth position of the benzothiazole scaffold caused the loss of COX inhibitory potency.

3.2. *In silico* Studies

To provide an insight into the mode of action, compound **7** was docked into the active site of COX-1 (PDB ID: 6Y3C). According to the *in silico* data, the compound showed good affinity to the active site of COX-1 as depicted in Figures 2 and 3. The interactions of the amide C=O moiety of compound **7** with Arg120 and Glu524 led to the formation of

Table 1. COX inhibitory effects of compounds **1-10**, SC-560 and rofecoxib



Compound (100 µM)	R	R'	Inhibition (%)	
			COX-1	COX-2
1	CH_3	H	25.50 ± 3.54	22.47 ± 2.67
2	CH_3	Cl	7.58 ± 1.16	----
3	CH_3	CH_3	----	----
4	CH_3	OCH_3	----	10.85 ± 2.05
5	CH_3	OC_2H_5	----	----
6	NH_2	H	----	----
7	NH_2	Cl	51.36 ± 3.32	11.05 ± 1.69
8	NH_2	CH_3	----	----
9	NH_2	OCH_3	----	8.82 ± 1.42
10	NH_2	OC_2H_5	----	----
SC-560 (1 µM)	-	-	83.64 ± 3.76	nt
Rofecoxib (10 µM)	-	-	nt	96.48 ± 3.54

---- : no inhibition; nt: not tested.

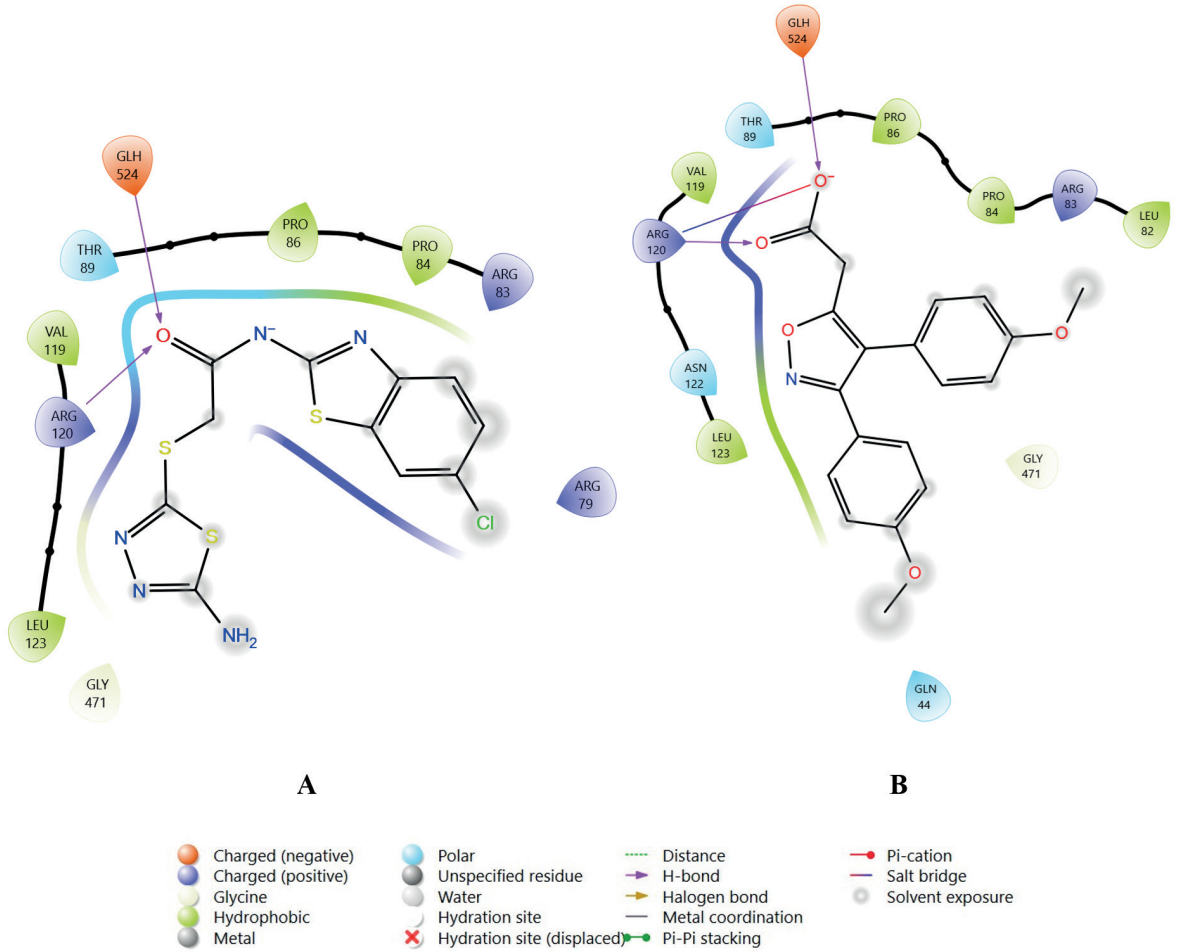


Figure 2. Docking interactions of compound **7 (A)** and mofezolac **(B)** in the active site of COX-1 (PDB ID: 6Y3C).

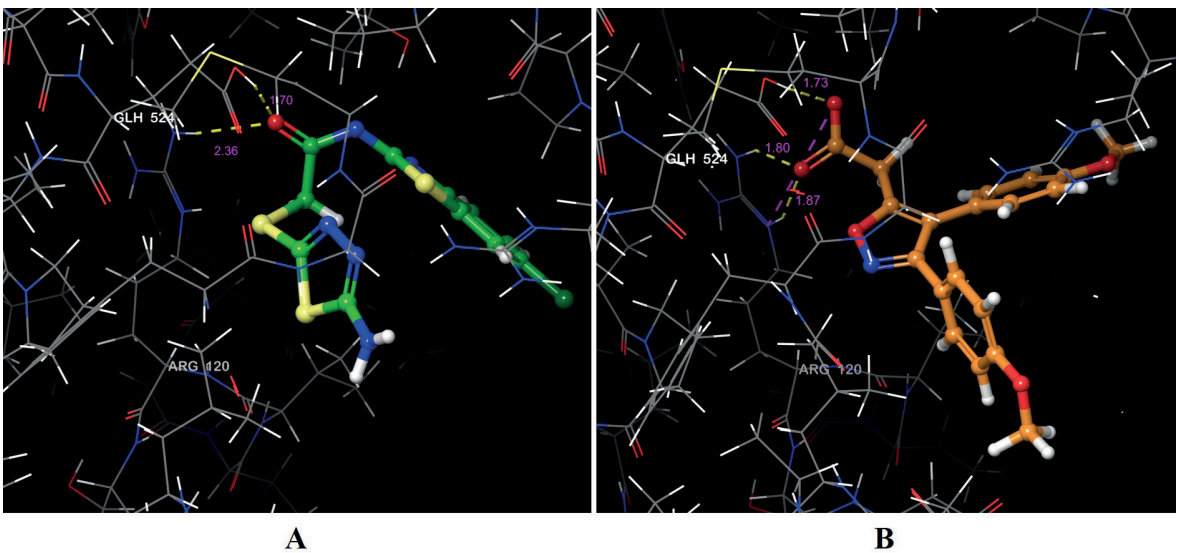


Figure 3. Docked poses of compound **7 (A)** and mofezolac **(B)** in the active site of COX-1 (PDB ID: 6Y3C) (Yellow and pink dashes represent the hydrogen bonds and the salt bridge, respectively).

Table 2. Docking scores (kcal/mol), glide gscores (kcal/mol) and glide emodel (kcal/mol) results of compound **7** and mofezolac for COX-1 enzyme (PDB ID: 6Y3C)

Compound	COX-1		
	Docking score	**Glide gscore**	**Glide emodel**
7	-3.696	-4.943	-53.325
Mofezolac	-5.047	-5.047	-50.495

hydrogen bonds. The selective COX-1 inhibitor mofezolac formed hydrogen bonds with Arg120 and Glu524. It can be concluded that these interactions (hydrogen bonds with proper residues) may be responsible for the observed COX-1 inhibitory activity of compound **7**. As illustrated in Figure 2, compound **7** does not form the salt bridge that mofezolac does with Arg120. This missed interaction may explain the lower COX-1 inhibitory activity of compound **7** compared to that of mofezolac [35]. The docking scores of compound **7** and mofezolac were found as -3.696 and -5.047 kcal/mol, respectively (Table 2).

ADME experiments require a large number of *in vivo* tests and, consequently, ethical procedures, and therefore these assays are expensive and time-consuming for a large number of chemical compounds. As a result, *in silico* procedures are frequently applied to evaluate the pharmacokinetic profiles of drug candidates [36]. In this context, compound **7** was subjected to a computational study for the prediction of its pharmacokinetic characteristics. As presented in Table 3, the SASA, CIQPlogS, QPlogBB, QPPMDCK values of compound **7** were predicted to fall within the range recommended by the QikProp. Moreover, this compound did not cause any violation of Lipinski's and Jorgensen's rules, making it a drug-like molecule with favorable oral bioavailability.

Table 3. Predicted pharmacokinetic features of compound **7**

Property or descriptor	Compound 7
SASA*	592.498
CIQPlogS*	-4.650
QPlogPo/w*	2.033
QPlogBB*	-1.271
QPPMDCK*	748.996
QPlogKhsa*	-0.236
Rule of Five*	0
Rule of Three*	0

* **SASA:** Total solvent accessible surface area in square angstroms using a probe with a 1.4 Å radius (Recommended range: 300.0 – 1000.0). **CIQPlogS:** Conformation-independent predicted aqueous solubility (Recommended range: –6.5 – 0.5). **QPlogPo/w:** Predicted octanol/water partition coefficient (Recommended range: -2.0 – 6.5). **QPlogBB:** Predicted brain/blood partition coefficient (Recommended range: –3.0 – 1.2). **QPPMDCK:** Predicted apparent MDCK cell permeability in nm/sec (< 25 poor, > 500 great). **QPlogKhsa:** Prediction of binding to human serum albumin (Recommended range: -1.5 – 1.5). **Rule of Five:** Number of violations of Lipinski's rule of five. The rules are molecular weight of the molecule < 500, QPlogPo/w < 5, hydrogen bond donor atoms ≤ 5, hydrogen bond acceptor atoms ≤ 10. Compounds that provide these rules are considered as drug-like molecules (maximum is 4). **Rule of Three:** Number of violations of Jorgensen's rule of three. The three rules are: predicted aqueous solubility (QPlogS) > -5.7, predicted apparent Caco-2 cell permeability (QPPCaco in nm/s) > 22 nm/s, # primary metabolites < 7 (maximum is 3). Compounds with fewer (and preferably no) violations of these rules are more likely to be orally available agents (Schrödinger Release 2023-2, LLC, New York, USA).

4. CONCLUSION

In conclusion, we described the *in vitro* inhibitory effects of a series of thiadiazole-benzothiazole hybrids on COX-1 and 2. Among the tested compounds, compound **7** was identified as the most potent and selective inhibitor of COX-1. The *in silico* studies suggest that the amide C=O moiety of compound **7** with Arg120 and Glu524 led to the formation of hydrogen bonds, which is considered to play a crucial role in COX-1 inhibitory activity. Based on the QikProp data, compound **7** was predicted to possess favorable drug-like characteristics and oral bioavailability. In the view of this research, a new class of selective COX-1 inhibitors could be designed *via* the molecular modification of compound **7** for the treatment of many illnesses in which selective inhibition of COX-1 is required.

Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Author contribution

Concept: AÖ; Design: AÖ; Materials: AÖ, HET; Data Collection and/or Processing: AÖ, HET; Analysis and/or Interpretation: AÖ, HET; Literature Search: AÖ; Writing: AÖ; Critical Reviews: AÖ, HET.

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Conflict of interest

The authors declared that there is no conflict of interest.

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