Bioorganic Chemistry 85 (2019) 577–584

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Novel aryl carboximidamide and 3-aryl-1,2,4-oxadiazole analogues of naproxen as dual selective COX-2/15-LOX inhibitors: Design, synthesis and docking studies

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ARTICLE INFO

Keywords:
Aryl carboximidamides
1, 2, 4-oxadiazoles
COX-1
COX-2
15- LOX
Naproxen
Anti-inflammatory

ABSTRACT

A series of novel naproxen analogues containing 3-aryl-1,2,4-oxadiazoles moiety **(4b-g)** and their reaction intermediates aryl carboximidamides moiety **(3b-g)** was synthesized and evaluated *in vitro* as dual COXs/15-LOX inhibitors. Compounds **3b-g** exhibited superior inhibitory activity than celecoxib as COX-2 inhibitors. Compounds **3b-d** and **3g** were the most potent COX-2 inhibitors with IC_{50} range of 6.4 – 8.13 nM and higher selectivity indexes (**3b**, SI = 26.19; **3c**, SI = 13.73; **3d**, SI = 29.27; **3g**, SI = 18.00) comparing to celecoxib (IC_{50} = 42.60 nM, SI = 8.05). Regarding 15-LOX inhibitory activity, compounds belonging to aryl carboximidamide backbone **3b-e** and **3g** were the most potent with IC_{50} range of 1.77–4.91 nM comparing to meclofenamate sodium (IC_{50} = 5.64 μM). Data revealed that The levels of NO released by aryl carboximidamides **3b-g** were more higher than 3-aryl-1,2,4-oxadiazole derivatives **4b-g**, which correlated well with their COX-2 inhibitory activities.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most prevalent pharmaceuticals in the world [1]. Because they are potent inhibitors of cyclooxygenase (COX) enzymes, they reduce the synthesis of pro-inflammatory prostaglandins (PGs) [2]. They are employed for their analgesic, antipyretic, and anti-inflammatory properties. Two related isozymes of the COX have been described, constitutive COX-1 and inducible COX-2 [3,4]. These two isoforms are genetically independent proteins located on different chromosomes. The binding sites are almost identical and the two COX isoforms share high sequence homology of 65%. COX-2 is an inducible isozyme implicated in different pathological processes such as several cancer types and inflammation. There are three main differences in the amino acids sequence between the COX-1 and COX-2 active sites. These structural differences produce main implications for the selectivity profile of NSAIDs [5]. For example, the substitution of valine 523 in the active site of the COX-2 for a relatively bulky isoleucine residue in COX-1 creates an additional side pocket. An access to this extra binding pocket is limited in the case of binding to COX-1 [6–8]. Prolonged administration of non-selective NSAIDs exhibit several undesired adverse drug reactions (ADRs) like gastrointestinal irritation, bleeding and ulceration. The main cause of such ADRs is the inhibitory effect on the gastroprotective prostanoids produced by COX-1 enzymes in the gastrointestinal tract [9–14]. Therefore, introduction of potent and selective COX-2 in-

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https://doi.org/10.1016/j.bioorg.2019.02.043
Received 21 November 2018; Received in revised form 9 February 2019; Accepted 19 February 2019
Available online 20 February 2019
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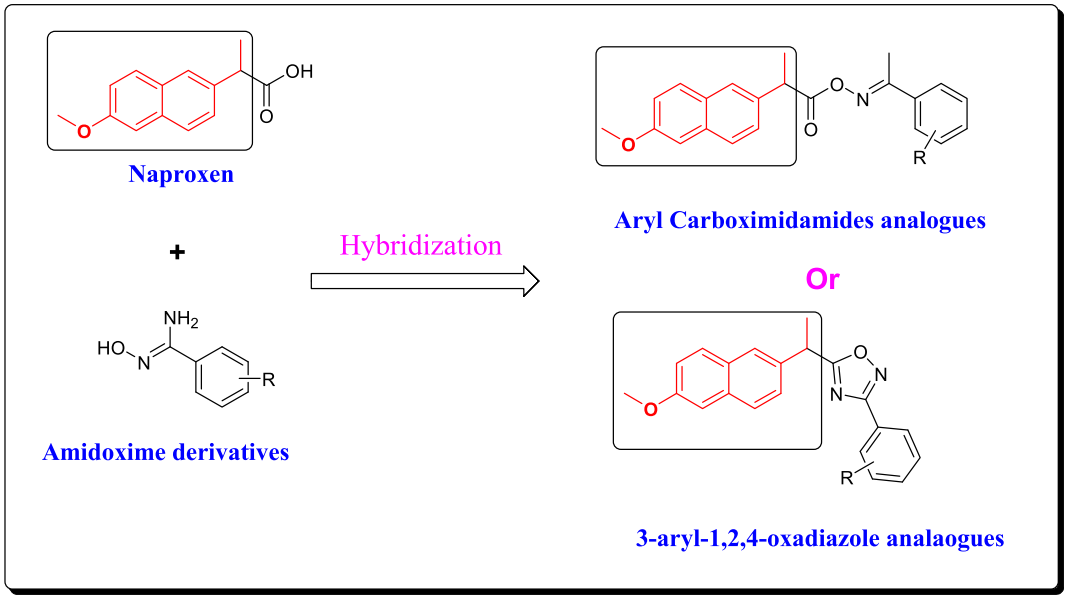


Fig. 1. Rational design of novel non-carboxylic analogues of naproxen.

hibitors with more favorable gastro-intestinal safety profile has become of great interest [15]. Moreover, the use of non-acidic prodrugs could decrease the direct ulcerogenic properties of acidic agents and improve the selectivity profile of NSAIDs toward COX-2 isoenzyme [16,17].

Leukotrienes themselves are implicated in the development of gastrointestinal ulcers, asthmas, and different inflammatory processes [18,19]. It is noteworthy that COX isozymes and LOX share the same substrate arachidonic acid; therefore, inhibition of prostaglandins production by cyclooxygenase pathway can lead to increased substrate availability and increased production of leukotrienes by lipoxygenase pathway [20–22]. Although, R. Mashima and T. Okuyama reported that 15-LOX performs multiple functions and that down regulation or general inhibition of 15-LOX delays the resolving of inflammation [23]. It is now clear that PGs and LTs have complementary effects regarding development and persistent inflammatory processes [24]. In view of these perceptions, it has been proposed that limiting the production of both leukotrienes and prostaglandins might have synergistic and wide spectrum anti-inflammatory effects, as well as, production of new entities with safe gastrointestinal and cardiovascular profiles [25]. Recently, enormous trials have been applied to develop dual COX and LOX inhibitors with moderate selectivity towards COX-2 at micromole level [26].

Amidoxime and 1,2,4-oxadiazole containing compounds showed significant pharmacological effects as antihyperglycemic activity, anti-inflammatory activity, antimycobacterial activity, muscarinic agonist activity and serotonergic inhibitory activity, peptide inhibitory activity [27–30]. Both moieties have been incorporated as carboxylic and ester group bioisosteres, respectively, with the aim of designing prodrugs with an improved PK and PD properties [30]. Moreover, for the purposes of synthesizing nitric oxide-releasing non-steroidal anti-inflammatory prodrugs, NO-NSAIDs, amidoxime moiety has shown its ability to release NO *in vivo* [31].

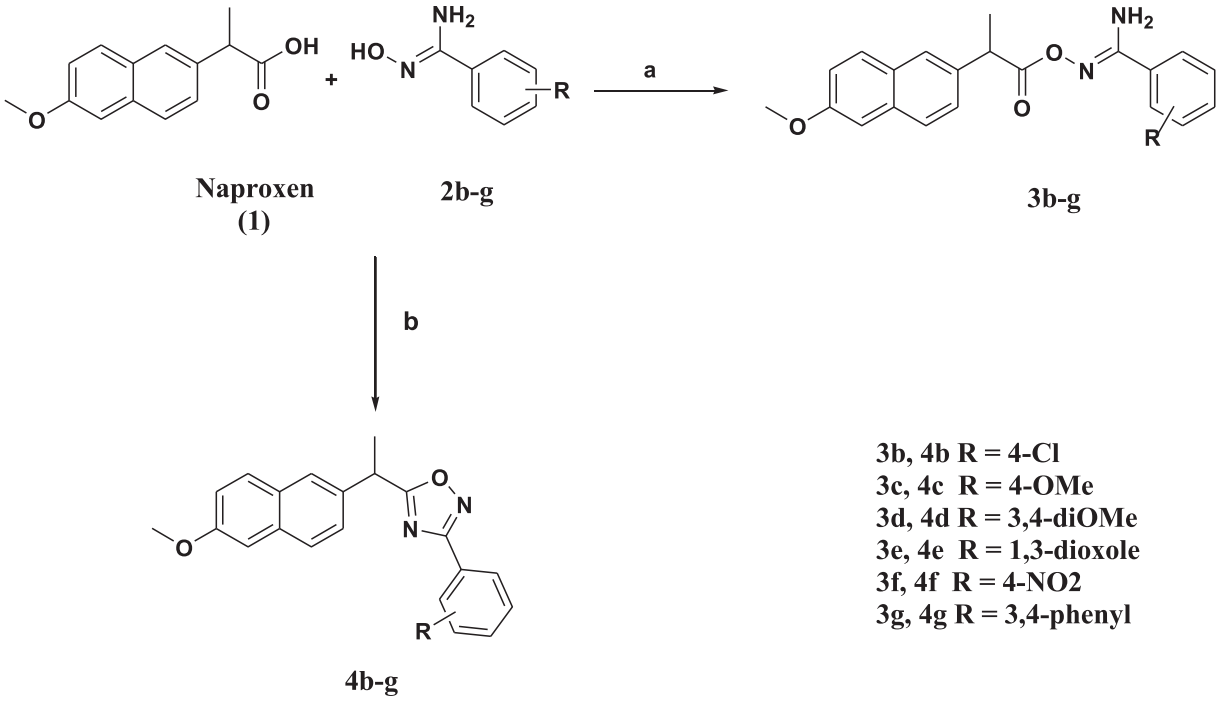
Naproxen is one of NSAIDs that has important medical applications in case of arthritis, ankylosing spondylitis, gout, menstrual cramps and others. However, its chronic use can adversely produce gastric ulceration and bleeding [32,33]. Therefore, synthetic approaches have being intensively applied to design naproxen derivatives with improved safety profile via derivatization of the carboxylate function of naproxen [34–36].

Encouraged by all these facts, we hereby report the synthesis of non-carboxylic analogues of naproxen by *O*-acylation of aryl amidoximes **(2b-g)** to the corresponding aryl carboximidamides **(3b-g)** and their cyclization to the corresponding 3-aryl-1,2,4-oxadiazoles **(4b-g)**. The rationally designed compounds have a typical molecular pattern to fulfill the pharmacophoric necessities for better COX binding recognition represented by naproxen moiety and an additional bulk volume represented by terminal phenyl ring to bind exclusively to COX-2 binding site. The current study has investigated the possibility of masking the naproxen carboxylic group to disguise its direct adverse reactions with potential *in vitro* selective COX-2 inhibitory activity (Fig. 1).

2. Results and discussion

2.1. Chemistry

The synthesis of aryl carboximidamides **3b-g** and 3-aryl-1,2,4-oxadiazole is described in Scheme 1. Amidoximes **2b-g** were prepared



Scheme 1. Synthesis of compounds **3b-g** and **4b-g. Reagent and reaction conditions**: (a) CDI, Acetonitrile, r.t. 3 h; (b) (a) CDI, Acetonitrile, r.t. 3 h, then reflux

according to reported procedures [37] were coupled with naproxen **1** using CDI at room temperature for 3 h to yield aryl carboximidamides **3b-g**. All the aryl carboximidamides **3b-g** were verified using **^{1}H NMR**, **^{13}C NMR** and elemental microanalyses. The **IR** spectrum of **3b** as a representative example of this series revealed the appearance of a Peaks at 3333 (NH_2), 1733 (C═O) and 1608 (C═N). The **^{1}H NMR** spectrum revealed the appearance of a broad signal at 6.86 assigned to NH_2, appearance of a doublet with three protons integration at 1.56 ppm assigned for (CH-$\underline{CH_3}$) and quartet at δ 4.18–426 (q, j = 7.0 Hz, 1H, $\underline{CH}$-CH_3) as well as aromatic protons. **^{13}C NMR** spectrum of **3b** showed the appearance of peaks at 18.70 and 43.66 which were assigned to CH-CH_3 carbon, and peaks at 171.99 and 157.70, which were assigned to C═O and C═N, respectively. Refluxing **2a-g** with naproxen **1** using CDI afforded 3-aryl-1,2,4-oxadiazole **4b-g**. All the final 3-aryl-1,2,4-oxadiazole structures **4b-g** were verified using **^{1}H NMR**, **^{13}C NMR** and elemental microanalyses. The **IR** spectrum of **4b** as a representative example of this series revealed the disappearance of Peaks at 3333 (NH_2) and 1733 (C═O). **^{1}H NMR** spectrum of **4b** revealed the disappearance of the broad signal at 6.86 assigned to NH_2, in addition, appearance of a doublet with three protons integration at 1.81 ppm assigned for (CH-$\underline{CH_3}$) and quartet at δ 4.76–4.81 (q, j = 7.2 Hz, 1H, $\underline{CH}$-CH_3) as well as aromatic protons.

2.2. Biology

2.2.1. In vitro cyclooxygenase (COX) inhibition assay

The *in vitro* biological activity assay was operated used to examine the ability of the newly synthesized compounds to inhibit both bovine COX-1 and COX-2 subtypes. A colorimetric enzyme immunoassay (EIA) kit was used to monitor the isozyme-specific inhibition [38]. The

Table 1
In vitro COX-1, COX-2 inhibition, 15-LOX and NO release of compounds **3b-g**, **4b-g** and the reference drug celecoxib for COX and meclofenamte sodium for 15-LOX.

Compd. No.	COX-1[a] (IC_{50} nM)	COX-2[a] (IC_{50} nM)	Selectivity ratio (SI[b])	15-LOX (IC_{50} nM)	NO (uM)
3b	187.52	7.15	26.19	1.77	19.65 ± 0.07
3c	102.78	7.48	13.73	4.91	18.23 ± 0.22
3d	187.52	6.40	29.27	2.07	24.40 ± 0.17
3e	52.18	11.15	4.67	4.58	18.97 ± 0.24
3f	190.48	27.11	7.02	11.53	20.6 ± 0.21
3g	146.41	8.13	18.00	3.34	20.1 ± 0.22
4b	380.09	95.72	3.97	24.16	17.02 ± 0.2
4c	283.84	100.45	2.82	34.74	16.10 ± 0.18
4d	69.17	45.19	1.53	11.68	17.97 ± 0.41
4e	131.76	163.94	0.80	60.50	12.41 ± 0.54
4f	107.68	11.25	9.56	10.84	18.12 ± 0.09
4g	218.04	173.01	1.26	12.52	19.070 ± 0.4
celecoxib	342.93	42.60	8.05	–	22.30 ± 0.17
Meclofenamte Sodium	–	–	–	5.64	
Control	–	–	–	–	4.892 ± 0.07

[a] The concentration of the test compound required to produce 50% inhibition of COX-1 or COX-2. The results (IC_{50}, nM) is the mean of two determinations acquired using an ovine COX-1/COX-2 assay Kits (Cayman Chemicals Inc., Ann Arbor, MI, USA) and the deviation from the mean is < 10% of the mean value.
[b] *In vitro* COX-2 selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).

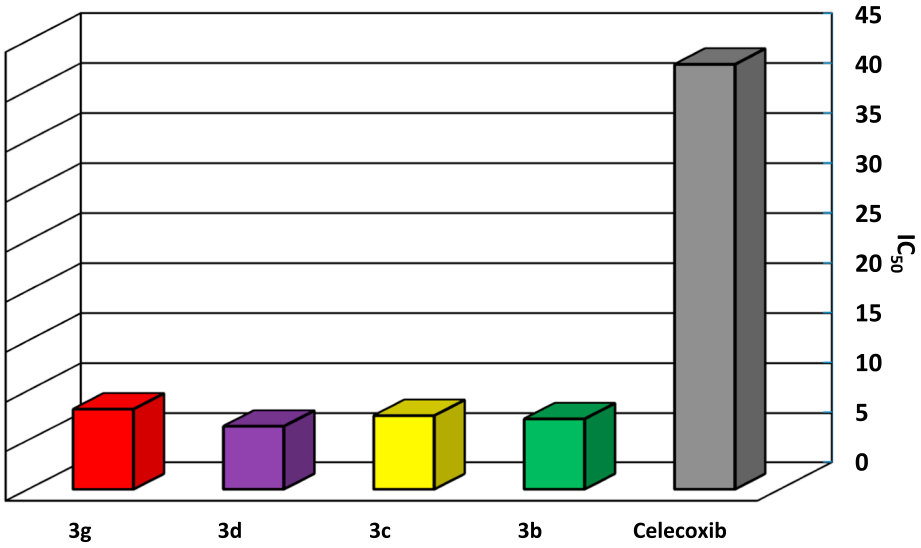


Fig. 2. IC_{50} of the most active compounds **3b**, **3c**, **3d**, **3g** and **celecoxib** on COX-2.

potency of the testing compounds was determined as the concentration causing 50% enzyme inhibition (IC_{50}). Also, the COX-2 selectivity indexes (SI values) were calculated, is defined as IC_{50} (COX-1)/IC_{50} (COX-2) and compared with that of the standard drug celecoxib. All compounds were tested and the data obtained were listed in Table 1.

As illustrated in Table 1, five most active compounds **3b**, **3c**, **3d**, **3g** and **4f** among both aryl carboximidamides and 3-aryl-1,2,4-oxadiazoles displayed good inhibitory activities against COX-2 and were more potent inhibitors of COX-2 (IC_{50} = 6.40–11.25 nM range) than COX-1 (IC_{50} = 102.78–187.52 nM range) with selectivity indexes (SI) in the range of 9.56–29.27 and that, in all cases, the measured activities were higher than that of celecoxib (SI = 8.05). Compound **3d** displayed the highest selectivity indexes (SI = 29.27) and it was bearing aryl carboximidamides backbone while on the other side compound **4d** was bearing same substitution pattern as compared to **3d** and difference was 3-aryl-1,2,4-oxadiazole moiety but it showed almost twenty folds less activity. Obviously, it can be concluded that all compounds (**except for 3f**) belonging to aryl carboximidamides backbone **3b-g** were better inhibitors of COX-2 as compared to other derivatives **4b-g** bearing same substitutions but different backbone of 3-aryl-1,2,4-oxadiazole.

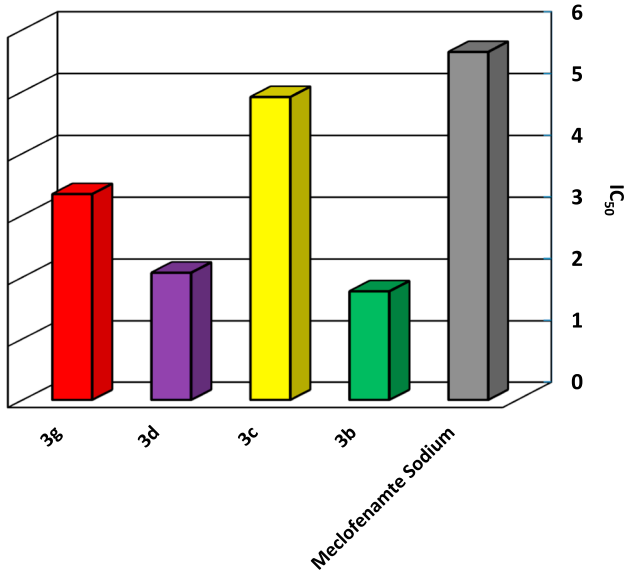


Fig. 3. IC_{50} of the most active compounds **3b**, **3c**, **3d**, **3g** and **meclofenamte sodium** on 15-LOX.

The effects of substituents introduced into both aryl carboximidamide and 3-aryl-1,2,4-oxadiazole core of compounds **3b-g** and **4b-g** demonstrated that 4-Cl, 4-OMe, 3,4-di-OMe and phenyl groups were the most appropriate substitutions and the corresponding compounds **3b**, **3c**, **3d** and **3g**, respectively were the most potent COX-2 inhibitors in this series (Fig. 2) (**3b**, IC_{50} = 7.15 nM, SI = 26.19; **3c**, IC_{50} = 7.48 nM, SI = 13.73; **3d**, IC_{50} = 6.40 nM, SI = 29.27; **3g**, IC_{50} = 8.13 nM, SI = 18.00) and that of celecoxib (IC_{50} = 42.60 nM, SI = 8.05). Compound **4g** (with a phenyl substituent in 3-aryl-1,2,4-oxadiazole core) was the least potent compound as COX-2 inhibitor (IC_{50} = 173.01 nM, SI = 1.26).

2.2.2. *In vitro lipoxygenase (15-LOX) inhibition assay*

Compounds **3b-g** and **4b-g** were evaluated for their ability to inhibit lipoxygenase (LOX) enzyme using the LOX enzyme assay kit [38]. The obtained data is listed in Table 1. Regarding 15-LOX inhibitory activity, compounds belonging to aryl carboximidamide backbone **3b**, **3c**, **3d**, **3e** and **3g** were the most active as 15-LOX inhibitors with (IC_{50} = 1.77, 4.91, 2.07, 4.58, and 3.43 nM, respectively) compared that of meclofenamate sodium (IC_{50} = 5.64 μM). Compounds belonging to 3-aryl-1,2,4-oxadiazole backbone **4b**, **4c**, **4d**, **4e** and **4g** showed moderate inhibitory activity against 15-LOX enzyme in the range of (IC_{50} = 11.68–60.50 nM) (Fig. 3).

2.2.3. *In vitro NO release detection*

To investigate whether the concentration of NO produced by these compounds was associated with their COX-2 inhibition activities, the levels of NO production in the cell lysates were determined [39]. Human lymphocytes cells were treated with 100 mM concentration of compounds and the amounts of nitrite, the principal oxidation product of NO in aqueous solution, were detected using the Griess assay. As shown in Table 1, variable levels of NO could release from all of these compounds, and compound **3d** generates the highest concentration of NO. Interestingly, it was observed that the levels of NO generated by compounds **3b-g** of aryl carboximidamides backbone were more higher than 3-aryl-1,2,4-oxadiazole derivatives **4b-g**, which correlated well with their selective COX-2 inhibitory activities *in vitro*. This study demonstrates that compound **3g** is promising lead compounds that had strong potential to be further developed as novel selective COX-2/15-LOX inhibitors with safe gastrointestinal and cardiovascular profiles.

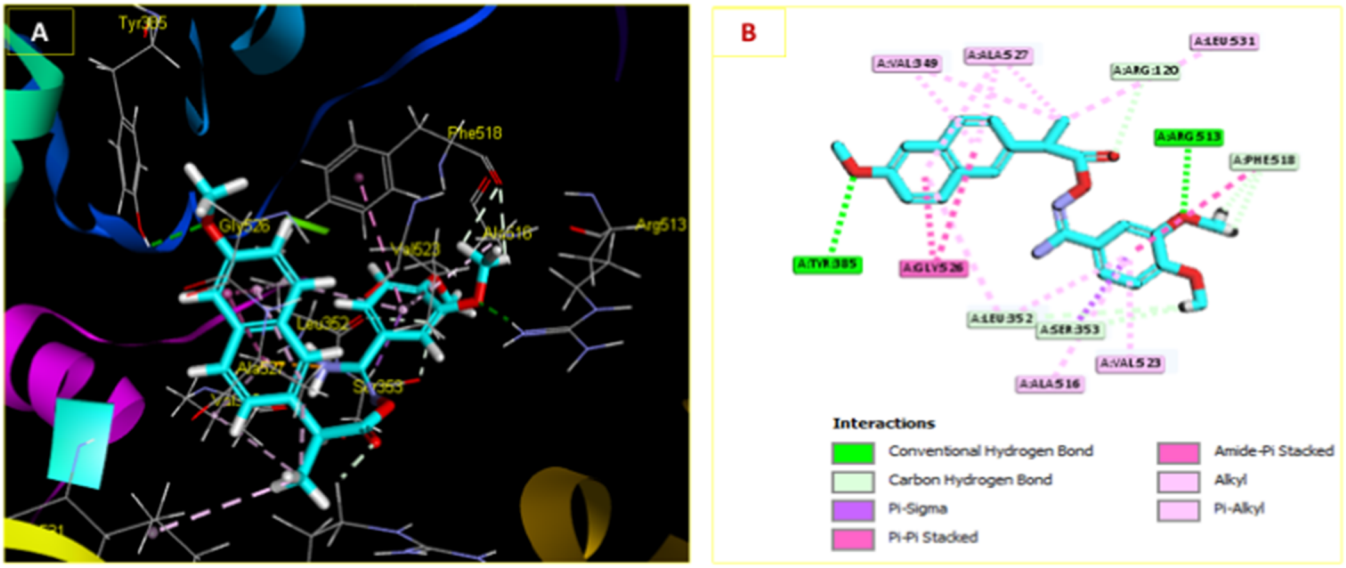


Fig. 4. (**A**) Docking and binding pattern of compound **3d** (cyan) into COX-2 active site (PDB code: 1CX2). (**B**) 2D interaction diagram of the top docking pose of the **3d** (cyan) within active site of COX-2(PDB code: 1CX2).

3. Molecular docking study

In order to elucidate the biological activity and the difference in selectivity profile of the newly synthesized naproxen derivatives toward COX subtypes based on their orientation and binding patterns, the most potent compound **3d** was selected for molecular docking studies into the active site of COX-2 using CDOCKER embedded in Discovery Studio software [40].

To validate our docking protocol settings, we have re-docked the co-crystallized ligand S-58 after being extracted from the used 3D protein structure of COX-2 (pdb code: 1CX2) using the same protocol settings utilized for docking simulation of our compound **3d**. It was clear that the used docking protocol closely reproduced the bound structure with RMSD value of1.03 Å confirming the confidence in our docking study.

The 3D crystal structures of COX-2 enzymes complexes with its co-crystallized ligand (PDB codes: 1CX2) was used for this study. The presence of an additional side pocket is one of the well-known features of COX-2 binding site. This side pocket was formed because of the replacement of the amino acid residue Ile523 in COX-1 with the less bulky Val523 in COX-2, which increases the volume of the COX-2 active site [41]. Moreover, it makes the COX-2 protein accommodate bulkier structures and allows other interactions with amino acid residues such as Arg513, substituted by a His513 in COX-1 [30]. It was reported that the traditional COX-2 inhibitors adopt binding with Arg513 in the COX-2 additional pocket, often via sulfonamide or sulfone groups, to attain their activity and selectivity over COX-1. Interestingly, analysis of the docking results of compound **3d** revealed that **3d** exhibited not only similar binding pattern and interactions to that of the co-crystallized bromocelecoxib, S-58 ligand, but also compound **3d** was engaged in hydrogen bond interaction with Arg513 (Fig. 4) which is essential for activity and selectivity toward COX-2 over COX-1. It was clear from (Fig. 4) that the orientation of the dimethoxy phenyl moiety in

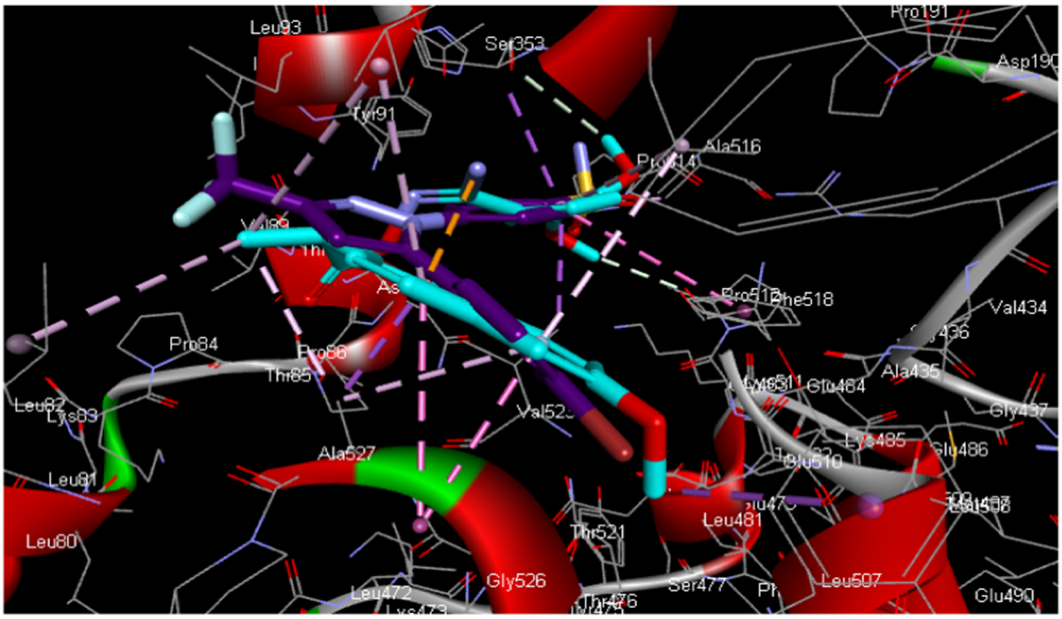


Fig. 5. The superimposition of the docked pose **3d** (cyan) and the co-crystallized S-58 (magenta) within active site of COX-2(PDB code: 1CX2). Dashed green lines represent hydrogen bonds.

compound **3d** within the additional side pocket of COX-2 was similar to fitting of sulfonamide moiety of S-58 into the same side pocket. In addition, compound **3d** formed hydrophobic interactions with Leu352, Ser353, Ala516, Phe518 and Val523 residues. Therefore and because of the larger volume of COX-2 active site, it could accommodate the dimethoxy phenyl moiety in compound **3d** with almost the same binding pattern within the entire COX-2 active site.

Furthermore, positioning the naphthalene nucleus in **3d** within the main active site and forming one hydrogen bond with Tyr385 in addition to many hydrophobic interactions with Val349, Leu352, Gly526, Ala527 and Leu531 residues, correlates well with the position and hydrophobic interactions of the trifluoromethyl pyrazole fragment of S-58 with the same residues (Fig. 5). Overall, these interactions and binding pattern of **3d** into COX-2 active site may reflect the high COX-2 inhibitory activity and selectivity of this compound.

4. Experimental

4.1. Chemistry

4.1.1. General procedure for the synthesis of N'-{[2-(6-Methoxy-2-naphthyl) propanoyl]oxy}aryl carboximidamides (3b-g)

To a solution of naproxen **1** (10 mmole, 1.2 g) in 30 ml acetonitrile, the *N,N'*-carbonyldiimidazole **CDI** (11 mmole, 1.2 g) was added and the mixture was stirred at room temperature for 30 min. The respective amidoximes **2b-g** (10 mmole) was then added and stirred for further 3 hrs. After completion of reaction (monitored with TLC), the formed precipitate **3b-g** was collected by filtration, washed several times with acetonitrile, dried and used without further purification.

4.1.1.1. 4-Chloro-N'-{[2-(6-methoxy-2-aphthyl)propanoyl]oxy}benzene carboximidamide (3b). Yield 92%; white solid; m.p.: 170–172 °C. IR (ATR)) ν_{max} 3481, 3333 (NH_2), 3065 (CH aromatic), 2985, 2957, 2839 (CH aliphatic), 1733 (C=O), 1608 (C=N) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.56–1.57 (d, j = 6.9 Hz, 3H, CH-$\underline{CH}$-CH_3), 3.87 (s, 3H, OCH_3), 4.18–4.23 (q, j = 7.0 Hz, 1H, $\underline{CH}$-CH_3), 6.86 (br. s, 2H, NH_2), 7.16–7.18 (d, j = 8.5 Hz, 1H, $CH_{arom.}$), 7.49 (s, 1H, $CH_{arom.}$), 7.51–7.55 (m, 3H, $CH_{arom.}$), 7.73–7.75 (d, j = 8.12 Hz, 2H, $CH_{arom.}$), 7.80–7.83 (m, 3H, $CH_{arom.}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 18.70, 43.66, 55.65, 106.30, 119.19, 119.40, 126.16, 126.93, 127.40, 128.85, 129.05, 129.62, 130.84, 133.84, 135.64, 136.25, 156.42, 157.70, 171.99. Elemental Analysis Calcd. (%) for $C_{21}H_{19}ClN_2O_3$ (382.84): C, 65.88; H, 5.00; N, 7.32. Found: C, 65.74; H, 4.91; N, 7.24.

4.1.1.2. 4-Methoxy-N'-{[2-(6-methoxy-2-naphthyl)propanoyl]oxy} benzene carboximidamide (3c). Yield 91%; white solid; m.p.: 178–179 °C. IR (ATR) ν_{max} 3502, 3377 (NH_2), 3011 (CH aromatic), 2991, 2960, 2898 (CH aliphatic), 1732 (C=O), 1602 (C=N) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.55–1.57 (d, j = 7.0 Hz, 3H, CH-$\underline{CH}$-CH_3), 3.79 (s, 3H, OCH_3 for phenyl), 3.88 (s, 3H, OCH_3 for naphthyl), 4.15–4.21 (q, j = 7.0 Hz, 1H, $\underline{CH}$-CH_3), 6.62 (br. s, 2H, NH_2), 6.97–6.99 (d, j = 8.4 Hz, 2H, $CH_{arom.}$), 7.16–7.18 (d, j = 8.9 Hz, 1H, $CH_{arom.}$), 7.31 (s, 1H, $CH_{arom.}$), 7.52–7.54 (d, j = 8.4 Hz, 1H, $CH_{arom.}$), 7.64–7.66 (d, j = 8.4 Hz, 2H, $CH_{arom.}$), 7.80–7.83 (m, 3H, $CH_{arom.}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 18.71, 43.72, 55.66, 55.74, 106.31, 114.14, 115.11, 119.18, 126.14, 126.94, 127.38, 128.63, 129.21, 129.62, 133.82, 136.36, 156.98, 157.69, 161.46, 172.05. Elemental Analysis Calcd. (%) for $C_{22}H_{22}N_2O_4$ (378.42): C, 69.83; H, 5.86; N, 7.40. Found: C, 69.66; H, 5.73; N, 7.36.

4.1.1.3. 3,4-Dimethoxy-N'-{[2-(6-methoxy-2-naphthyl)propanoyl]oxy} benzene carboximidamide (3d). Yield 87%; white solid; m.p.: 150–152 °C. IR (ATR) ν_{max} 3490, 3364 (NH_2), 3010 (CH aromatic), 2969, 2926, 2887 (CH aliphatic), 1733 (C=O), 1610 (C=N) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.55–1.57 (d, j = 7.4 Hz, 3H, CH-$\underline{CH}$-CH_3), 3.78 (s, 3H, OCH_3 for phenyl), 3.79 (s, 3H, OCH_3 for phenyl), 3.88 (s, 3H, OCH_3 for naphthyl), 4.16–4.22 (q, j = 7.1 Hz, 1H, $\underline{CH}$-CH_3), 6.64 (br. s, 2H, NH_2), 6.98–7.00 (d, j = 8.4 Hz, 1H, $CH_{arom.}$), 7.16–7.18 (d, j = 8.8 Hz, 1H, $CH_{arom.}$), 7.26–731 (m, 3H, $CH_{arom.}$), 7.52–7.54 (d, j = 8.6 Hz, 1H, $CH_{arom.}$), 7.80–7.82 (m, 3H, $CH_{arom.}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 18.71, 43.72, 55.66, 56.08 ($2OCH_3$), 106.30, 110.72, 111.77, 119.19, 120.06, 121.04, 126.12, 126.96, 127.38, 128.91, 129.61, 133.82, 136.37, 148.82, 151.17, 157.13, 157.69, 172.10. Elemental Analysis Calcd. (%) for $C_{23}H_{24}N_2O_5$ (408.44): C, 67.63; H, 5.92; N, 6.86. Found: C, 67.47; H, 5.89; N, 6.90.

4.1.1.4. N'-{[2-(6-Methoxy-2-naphthyl)propanoyl]oxy}-1,3-benzodioxole-5-carboximidamide (3e). Yield 85%; gray solid; m.p.: 172–173 °C. IR (ATR) ν_{max} 3508, 3393 (NH_2), 3065 (CH aromatic), 2986, 2932, 2889 (CH aliphatic), 1742 (C=O), 1597 (C=N) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.55–1.57 (d, j = 7.1 Hz, 3H, CH-$\underline{CH}$-CH_3), 3.87 (s, 3H, OCH_3), 4.15–4.20 (q, j = 7.1 Hz, 1H, $\underline{CH}$-CH_3), 6.07 (s, 2H, O-$\underline{CH_2}$-O), 6.65 (br. s, 2H, NH_2), 6.95–6.97 (d, j = 8.1 Hz, 1H, $CH_{arom.}$), 7.16–7.18 (d, j = 7.3 Hz, 1H, $CH_{arom.}$), 7.21 (s, 1H, $CH_{arom.}$), 7.24–7.26 (d, j = 8.2 Hz, 1H, $CH_{arom.}$), 7.31 (s, 1H, $CH_{arom.}$), 7.52–7.54 (d, j = 8.5 Hz, 1H, $CH_{arom.}$), 7.80–7.82 (m, 3H, $CH_{arom.}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 18.70, 43.69, 55.66, 101.97 (O-CH_2-O disappeared by Dept-153), 106.30, 107.33, 108.50, 121.55, 122.61, 126.14, 126.94, 127.38, 127.87, 128.91, 129.61, 133.82, 136.32, 147.70, 149.52, 156.92, 157.69, 172.02. Elemental Analysis Calcd. (%) for $C_{22}H_{20}N_2O_5$ (392.40): C, 67.34; H, 5.14; N, 7.14. Found: C, 67.28; H, 5.07; N, 7.20.

4.1.1.5. 4-Nitro-N'-{[2-(6-methoxy-2-naphthyl)propanoyl]oxy}benzene carboximidamide (3f). Yield 89%; beige solid; m.p.: 192–194 °C. IR (ATR) ν_{max} 3487, 3338 (NH_2), 3011 (CH aromatic), 2953, 2911, 2894 (CH aliphatic), 1733 (C=O), 1628 (C=N) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.57–1.59 (d, j = 7.1 Hz, 3H, CH-$\underline{CH}$-CH_3), 3.88 (s, 3H, OCH_3), 4.18–4.24 (q, j = 7.1 Hz, 1H, $\underline{CH}$-CH_3), 7.05 (br. s, 2H, NH_2), 7.16–7.18 (d, j = 8.8 Hz, 1H, $CH_{arom.}$), 7.30 (s, 1H, $CH_{arom.}$), 7.53–7.55 (d, j = 8.5 Hz, 1H, $CH_{arom.}$), 7.80–7.83 (m, 3H, $CH_{arom.}$), 7.97–7.99 (d, j = 8.4 Hz, 2H, $CH_{arom.}$), 8.27–8.29 (d, j = 8.4 Hz, 2H, $CH_{arom.}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 18.69, 43.63, 55.66, 106.30, 119.21, 123.92, 126.17, 126.91, 127.43, 128.66, 128.91, 129.63, 133.85, 136.13, 138.15, 149.12, 155.78, 157.71, 171.88. Elemental Analysis Calcd. (%) for $C_{21}H_{19}N_3O_5$ (393.39): C, 64.12; H, 4.87; N, 10.68. Found: C, 64.28; H, 4.50; N, 10.46.

4.1.1.6. N'-{[2-(6-Methoxy-2-naphthyl)propanoyl]oxy}naphthalene-2-carboximidamide (3g). Yield 86%; gray solid; m.p.: 188–189 °C. IR (ATR) ν_{max} 3495, 3361 (NH_2), 3065 (CH aromatic), 2985, 2934, 2856 (CH aliphatic), 1734 (C=O), 1608 (C=N) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.59–1.60 (d, j = 7.0 Hz, 3H, CH-$\underline{CH}$-CH_3), 3.88 (s, 3H, OCH_3), 4.21–4.26 (q, j = 7.0 Hz, 1H, $\underline{CH}$-CH_3), 6.89 (br. s, 2H, NH_2), 7.17–7.19 (d, j = 8.8 Hz, 1H, $CH_{arom.}$), 7.31 (s, 1H, $CH_{arom.}$), 7.55–7.59 (m, 3H, CH $_{arom.}$), 7.81–7.86 (m, 4H, $CH_{arom.}$), 7.94–7.97 (m, 3H, $CH_{arom.}$), 8.33 (s, 1H, $CH_{arom.}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 18.75, 43.74, 55.66, 106.32, 119.20, 119.42, 124.42, 126.17, 126.98, 127.10, 127.42, 127.66, 127.95, 128.05, 128.27, 128.91, 129.39, 129.64, 132.77, 133.85, 134.26, 136.33, 157.25, 157.71, 172.07. Elemental Analysis Calcd. (%) for $C_{25}H_{22}N_2O_3$ (398.45): C, 75.36; H, 5.57; N, 7.03. Found: C, 75.52; H, 5.47; N, 6.93.

4.1.2. General procedure for the synthesis of 5-[1-(6-Methoxy-2-naphthyl) ethyl]-3-aryl-1,2,4-oxadiazole (4b-g)

To a solution of naproxen **1** (10 mmole, 1.2 g) in 30 ml acetonitrile, the *N,N'*-carbonyldiimidazole **CDI** (11 mmole, 1.2 g) was added and the mixture was stirred at room temperature for 30 mins. The respective amidoximes **2b-g** (10 mmole) was then added and stirred 3 hrs. After that, the reaction mixture was refluxed for 24 hrs (monitored with TLC) and cooled to room temperature. The formed precipitate **4b-g** was collected by filtration, dried and recrystallized from ethanol.

4.1.2.1. 5-[1-(6-Methoxy-2-naphthyl)ethyl]- 3-(4-chlorophenyl)-1,2,4-oxadiazole (4b). Yield 89%; beige solid; m.p.: 122–123 °C. IR (ATR) ν_{max} 3080 (CH aromatic), 2981, 2938, 2902 (CH aliphatic), 1602 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.81–1.82 (d, j = 7.1 Hz, 3H, CH-$\underline{CH_3}$), 3.87 (s, 3H, OCH_3), 4.76–4.81 (q, j = 7.2 Hz, 1H, $\underline{CH}$-CH_3), 7.16–7.19 (d, j = 8.9 Hz, 1H, $CH_{arom.}$), 7.30 (s, 1H, $CH_{arom.}$), 7.47–7.49 (d, j = 8.6 Hz, 1H, $CH_{arom.}$), 7.60–7.62 (d, j = 8.4 Hz, 2H, $CH_{arom.}$), 7.81–7.83 (d, j = 7.9 Hz, 3H, $CH_{arom.}$), 8.00–8.02 (d, j = 8.4 Hz, 2H, $CH_{arom.}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 19.87, 37.90, 55.65, 106.30, 119.39, 125.49, 126.22, 126.36, 127.90, 128.87, 129.27, 129.71, 129.80, 134.01, 135.60, 136.75, 157.92, 167.33, 182.88. Elemental Analysis Calcd. (%) for $C_{21}H_{17}ClN_2O_2$ (364.82): C, 69.14; H, 4.70; N, 7.68. Found: C, 68.94; H, 4.71; N, 7.69.

4.1.2.2. 5-[1-(6-Methoxy-2-naphthyl)ethyl]-3-(4-methoxyphenyl)-1,2,4-oxadiazole (4c). Yield 87%; beige solid; m.p.: 120–121 °C. IR (ATR) ν_{max} 3053 (CH aromatic), 2982, 2938, 2841 (CH aliphatic), 1604 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.80–1.82 (d, j = 7.1 Hz, 3H, CH-$\underline{CH_3}$), 3.84 (s, 3H, OCH_3 for phenyl), 3.87 (s, 3H, OCH_3 for naphthyl), 4.73–4.79 (q, j = 7.1 Hz, 1H, $\underline{CH}$-CH_3), 7.09–7.11 (d, j = 8.5 Hz, 2H, $CH_{arom.}$), 7.17–7.19 (d, j = 8.9 Hz, 1H, $CH_{arom.}$), 7.31 (s, 1H, $CH_{arom.}$), 7.47–7.49 (d, j = 8.6 Hz, 1H, $CH_{arom.}$), 7.82–7.83 (m, 3H, $CH_{arom.}$), 7.94–7.96 (d, j = 8.5 Hz, 2H, $CH_{arom.}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 19.92, 37.87, 55.67, 55.84, 106.32, 115.10, 118.94, 119.39, 126.18, 126.39, 127.89, 128.87, 129.21, 129.72, 133.99, 135.79, 157.90, 162.18, 167.81, 182.30. Elemental Analysis Calcd. (%) for $C_{22}H_{20}N_2O_3$ (360.40): C, 73.32; H, 5.59; N, 7.77. Found: C, 73.21; H, 5.50; N, 7.67.

4.1.2.3. 5-[1-(6-Methoxy-2-naphthyl)ethyl]- 3-(3,4-dimethoxyphenyl)-1,2,4-oxadiazole (4d). Yield 84%; white solid; m.p.: 80–82 °C. IR (ATR) ν_{max} 3062, 3012 (CH aromatic), 2981, 2938, 2840 (CH aliphatic), 1603 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.78–1.80 (d, j = 7.2 Hz, 3H, CH-$\underline{CH_3}$), 3.82 (s, 6H, $2OCH_3$ for phenyl), 3.86 (s, 3H, OCH_3 for naphthyl), 4.72–4.77 (q, j = 7.2 Hz, 1H, $\underline{CH}$-CH_3), 7.09–7.12 (d, j = 8.4 Hz, 1H, $CH_{arom.}$), 7.15–7.17 (d, j = 8.8 Hz, 1H, $CH_{arom.}$), 7.29 (s, 1H, $CH_{arom.}$), 7.44–7.47 (d, j = 10.9 Hz, 2H, $CH_{arom.}$), 7.59–7.61 (d, j = 7.8 Hz, 1H, $CH_{arom.}$), 7.80–7.83 (d, j = 8.6 Hz, 3H, $CH_{arom.}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 19.92, 37.89, 55.67, 56.10 ($2OCH_3$), 106.31, 110.20, 112.43, 118.95, 119.40, 121.07, 126.16, 126.36, 127.91, 128.87, 129.72, 133.98, 135.79, 149.48, 151.98, 157.90, 167.94, 182.28. Elemental Analysis Calcd. (%) for $C_{23}H_{22}N_2O_4$ (390.43): C, 70.75; H, 5.68; N, 7.17. Found: C, 70.71; H, 5.53; N, 7.10.

4.1.2.4. 5-[1-(6-Methoxy-2-naphthyl)ethyl]- 3-(1,3-benzodioxol-5-yl)-1,2,4-oxadiazole (4e). Yield 81%; beige solid; m.p.: 128–130 °C. IR (ATR) ν_{max} 3049 (CH aromatic), 2974, 2936, 2904 (CH aliphatic), 1604 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.79–1.81 (d, j = 7.1 Hz, 3H, CH-$\underline{CH_3}$), 3.87 (s, 3H, OCH_3), 4.73–4.78 (q, j = 7.2 Hz, 1H, $\underline{CH}$-CH_3),), 6.13 (s, 2H, O-$\underline{CH_2}$-O), 7.06–7.08 (d, j = 8.1 Hz, 1H, $CH_{arom.}$), 7.17–7.19 (d, j = 8.9 Hz, 1H, $CH_{arom.}$), 7.31 (s, 1H, $CH_{arom.}$), 7.44–7.48 (m, 2H, $CH_{arom.}$), 7.57–7.59 (d, j = 8.1 Hz, 1H, $CH_{arom.}$), 7.82–7.83 (d, j = 6.1 Hz, 3H, $CH_{arom.}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 19.91, 37.86, 55.67, 102.29 (O-CH_2-O, disappeared by Dept-135), 106.31, 106.99, 109.37, 119.39, 120.34, 122.62, 126.19, 126.38, 127.89, 128.87, 129.71, 133.99, 135.72, 148.42, 150.44, 157.90, 167.75, 182.36. Elemental Analysis Calcd. (%) for $C_{22}H_{18}N_2O_4$ (374.39): C, 70.58; H, 4.85; N, 7.48. Found: C, 70.63; H, 4.78; N, 7.44.

4.1.2.5. 5-[1-(6-Methoxy-2-naphthyl)ethyl]-3-(4-nitrophenyl)-1,2,4-oxadiazole (4f). Yield 84%; yellow solid; m.p.: 114–116 °C. IR (ATR) ν_{max} 3093, 3062 (CH aromatic), 2991, 2905 (CH aliphatic), 1602 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.83–1.84 (d, j = 7.1 Hz, 3H, CH-$\underline{CH_3}$), 3.87 (s, 3H, OCH_3), 4.79–4.85 (q, j = 7.1 Hz, 1H, $\underline{CH}$-CH_3), 7.16–7.18 (d, j = 8.9 Hz, 1H, $CH_{arom.}$), 7.30 (s, 1H, $CH_{arom.}$), 7.48–7.50 (d, j = 8.6 Hz, 1H, $CH_{arom.}$), 7.81–784 (d, j = 9.3 Hz, 3H, $CH_{arom.}$), 8.24–8.26 (d, j = 8.6 Hz, 2H, $CH_{arom.}$), 8.36–8.38 (d, j = 8.6 Hz, 2H, $CH_{arom.}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 19.81, 37.93, 55.64, 106.29, 118.96, 119.39, 124.78, 126.26, 126.33, 127.93, 128.87, 129.71, 132.42, 134.01, 135.46, 149.59, 157.92, 166.84, 183.40. Elemental Analysis Calcd. (%) for $C_{21}H_{17}N_3O_4$ (375.38): C, 67.19; H, 4.56; N, 11.19. Found: C, 67.01; H, 4.51; N, 11.08.

4.1.2.6. 5-[1-(6-Methoxy-2-naphthyl)ethyl]- 3-naphthalene-2-yl-1,2,4-oxadiazole (4g). Yield 85%; white solid; m.p.: 118–120 °C. IR (ATR) ν_{max} 3050 (CH aromatic), 2987, 2939, 2904 (CH aliphatic), 1603 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.81–1.83 (d, j = 6.4 Hz, 3H, CH-$\underline{CH_3}$), 3.87 (s, 3H, OCH_3), 4.76–4.80 (q, j = 6.6 Hz, 1H, $\underline{CH}$-CH_3), 7.17–7.18 (d, j = 6.4 Hz, 1H, $CH_{arom.}$), 7.31 (s, 1H, $CH_{arom.}$), 7.47–7.57 (m, 4H, $CH_{arom.}$), 7.82–7.83 (m, 3H, $CH_{arom.}$), 8.02.-8.03 (m, 2H, $CH_{arom.}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 19.90, 37.90, 55.66, 106.30, 119.39, 126.20, 126.36, 126.61, 127.49, 127.91, 128.87, 129.67, 129.72, 132.00, 134.00, 135.71, 157.91, 168.12, 182.66. Elemental Analysis Calcd. (%) for $C_{21}H_{18}N_2O_2$ (330.38): C, 76.34; H, 5.49; N, 8.48. Found: C, 76.19; H, 5.42; N, 8.37.

4.2. Biological activity

4.2.1. In vitro cyclooxygenase (COX) inhibition assay

The ability of the test compounds listed in Table 1 to inhibit ovine COX-1 and COX-2 (IC_{50} value, μM) was determined using an enzyme immune assay (EIA) kit (Cayman Chemical, Ann Arbor, MI, USA) according to a previously reported method [30].

4.2.2. In vitro 15-lipoxygenase (LOX) inhibition assay

The inhibitory activity of the test compounds listed in Table 1 against soya bean 15-LOX (IC_{50} value, μM) was assessed using an enzyme immune assay (EIA) kit (catalogue no 760709, Cayman Chemical, Ann Arbor, MI, USA). The IC_{50} values of test compounds were determined according to the manufacturer's instructions as reported methods [38].

4.2.3. In vitro NO released amounts detection

To investigate whether the concentration of NO produced by these compounds was associated with their COX-2 inhibition activities, the levels of NO production in the cell lysates were determined according to the manufacturer's instructions as reported methods [39].

4.3. Molecular docking study

The molecular docking simulation was performed using CDOCKER imbedded into Discovery Studio Software [42]. The active sites were generated from the co-crystallized ligands (IMM and SC-558) within COX-1 and COX-2 protein structures (PDBcodes:1CX2). Automatic protein preparation module was used applying CHARMm force field. The binding site sphere has been defined automatically by the software and encompassed all key amino acid residues for binding interaction with the ligand: Arg513, Tyr385, Val349, Leu352, Gly526, Ala527 and Leu531 residues. The docked compounds were built using Chem.3D ultra 12.0 software [Chemical Structure Drawing Standard; Cambridge Soft corporation, USA (2010)], and copied to Discovery Studio. Ligands were prepared using “Prepare Ligands” protocol in Discovery Studio where hydrogen atoms were added at their standard geometry, optical isomers and 3D conformations were automatically generated. Docking was performed using CDOCKER protocol in Discovery Studio keeping the parameters at default. Each compound would retain 10 poses and the best scoring pose of the docked compounds was recognized. Receptor–ligand interactions of the complexes were examined in 2D and 3D styles.

5. Conclusion

In summary, **12** new final naproxen analogues containing 3-aryl-1,2,4-oxadiazoles moiety **(4b-g)** and their reaction intermediates aryl carboximidamides moiety **(3b-g)** were synthesized, characterized and evaluated *in vitro* as dual COXs/LOX inhibitors. Among the tested compounds, the aryl carboximidamide derivatives with 4-Cl, 4-OMe, 3,4-di-OMe and phenyl groups were the most potent as dual COX-2/15-LOX inhibitors than reference celecoxib and that compounds **3b**, **3c**, **3d** and **3g** could be considered as promising candidates for the future development as anti-inflammatory agents. Compound **3g** (aryl carboximidamide derivative) is promising lead compounds that had strong potential to be further developed as novel selective COX-2/15-LOX inhibitors. Further structural optimization of these promising anti-inflammatory agents especially is ongoing for more precise SAR information and agents that are more potent.

Acknowledgment

This research was partially supported by the Korea Institute of Science and Technology (2018 KIST School Partnership Research Grant).

References

[1] B. Cryer, NSAID-associated deaths: the rise and fall of NSAID-associated GI mortality, Am. J. Gastroenterol. 100 (8) (2005) 1694.
[2] W.L. Smith, I. Song, The enzymology of prostaglandin endoperoxide H synthases-1 and-2, Prostaglandins Other Lipid Mediat. 68 (2002) 115–128.
[3] G.A. FitzGerald, C. Patrono, The coxibs, selective inhibitors of cyclooxygenase-2, N. Engl. J. Med. 345 (6) (2001) 433–442.
[4] J.-Y. Fu, J. Masferrer, K. Seibert, A. Raz, P. Needleman, The induction and suppression of prostaglandin H2 synthase (cyclooxygenase) in human monocytes, J. Biol. Chem. 265 (28) (1990) 16737–16740.
[5] R.G. Kurumbail, A.M. Stevens, J.K. Gierse, J.J. McDonald, R.A. Stegeman, J.Y. Pak, D. Gildehaus, T.D. Penning, K. Seibert, P.C. Isakson, Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents, Nature 384 (6610) (1996) 644.
[6] L.J. Marnett, A.S. Kalgutkar, Cyclooxygenase 2 inhibitors: discovery, selectivity and the future, Trends Pharmacol. Sci. 20 (11) (1999) 465–469.
[7] J.K. Gierse, J.J. McDonald, S.D. Hauser, S.H. Rangwala, C.M. Koboldt, K. Seibert, A single amino acid difference between cyclooxygenase-1 (COX-1) and − 2 (COX-2) reverses the selectivity of COX-2 specific inhibitors, J. Biol. Chem. 271 (26) (1996) 15810–15814.
[8] G. Dannhardt, W. Kiefer, Cyclooxygenase inhibitors–current status and future prospects, Eur. J. Med. Chem. 36 (2) (2001) 109–126.
[9] P. Lipsky, Progress toward a new class of therapeutics: selective COX-2 inhibition: introduction and course description, J. Rheumatol.-Suppl. 24 (49) (1997) 1–5.
[10] N.E. Lane, Pain management in osteoarthritis: the role of COX-2 inhibitors, J. Rheumatol. Suppl. 49 (1997) 20–24.
[11] J. Goldstein, P. Correa, W. Zhao, A. Burr, R. Hubbard, K. Verburg, G. Geis, Reduced incidence of gastroduodenal ulcers with celecoxib, a novel cyclooxygenase-2 inhibitor, compared to naproxen in patients with arthritis, Am. J. Gastroenterol. 96 (4) (2001) 1019–1027.
[12] L. Laine, S. Harper, T. Simon, R. Bath, J. Johanson, H. Schwartz, S. Stern, H. Quan, J. Bolognese, R.O.E.S. Group, A randomized trial comparing the effect of rofecoxib, a cyclooxygenase 2–specific inhibitor, with that of ibuprofen on the gastroduodenal mucosa of patients with osteoarthritis, Gastroenterology 117 (4) (1999) 776–783.
[13] C. Hawkey, L. Laine, T. Simon, A. Beaulieu, J. Maldonado-Cocco, E. Acevedo, A. Shahane, H. Quan, J. Bolognese, E. Mortensen, Comparison of the effect of rofecoxib (a cyclooxygenase 2 inhibitor), ibuprofen, and placebo on the gastroduodenal mucosa of patients with osteoarthritis: A randomized, double-blind, placebo-controlled trial, Arthrit. Rheumat.: Off. J. Am. College Rheumatol. 43 (2) (2000) 370–377.
[14] U. Mahadevan, E.V. Loftus Jr, W.J. Tremaine, W.J. Sandborn, Safety of selective cyclooxygenase-2 inhibitors in inflammatory bowel disease, Am. J. Gastroenterol. 97 (4) (2002) 910–914.
[15] M.E. Turini, R.N. DuBois, Cyclooxygenase-2: a therapeutic target, Annu. Rev. Med. 53 (1) (2002) 35–57.
[16] V.K. Redasani, S.B. Bari, Synthesis and evaluation of mutual prodrugs of ibuprofen with menthol, thymol and eugenol, Eur. J. Med. Chem. 56 (2012) 134–138.
[17] J.L. Masferrer, B.S. Zweifel, P.T. Manning, S.D. Hauser, K.M. Leahy, W.G. Smith, P.C. Isakson, K. Seibert, Selective inhibition of inducible cyclooxygenase 2 in vivo is antiinflammatory and nonulcerogenic, Proc. Natl. Acad. Sci. 91 (8) (1994) 3228–3232.
[18] R.A. Lewis, K.F. Austen, R.J. Soberman, Leukotrienes and other products of the 5-lipoxygenase pathway: biochemistry and relation to pathobiology in human diseases, N. Engl. J. Med. 323 (10) (1990) 645–655.
[19] S. Crooks, D. Bayley, S. Hill, R. Stockley, Bronchial inflammation in acute bacterial exacerbations of chronic bronchitis: the role of leukotriene B4, Eur. Respir. J. 15 (2) (2000) 274–280.
[20] K. Rainsford, Leukotrienes in the pathogenesis of NSAID-induced gastric and intestinal mucosal damage, Agents Actions 39 (1) (1993) C24–C26.
[21] S. Laufer, Discovery and development of ML3000, Inflammopharmacology 9 (1–2) (2001) 101–112.
[22] N. Hudson, M. Balsitis, S. Everitt, C. Hawkey, Enhanced gastric mucosal leukotriene B4 synthesis in patients taking non-steroidal anti-inflammatory drugs, Gut 34 (6) (1993) 742–747.
[23] R. Mashima, T. Okuyama, The role of lipoxygenases in pathophysiology; new insights and future perspectives, Redox Biol. 6 (2015) 297–310.
[24] J. Martel-Pelletier, D. Lajeunesse, P. Reboul, J.-P. Pelletier, Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective non-steroidal anti-inflammatory drugs, Ann. Rheum. Dis. 62 (6) (2003) 501–509.
[25] C. Charlier, C. Michaux, Dual inhibition of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) as a new strategy to provide safer non-steroidal anti-inflammatory drugs, Eur. J. Med. Chem. 38 (7–8) (2003) 645–659.
[26] G. Moussa, R. Alaaeddine, L.M. Alaeddine, R. Nassra, A.S. Belal, A. Ismail, A.F. El-Yazbi, Y.S. Abdel-Ghany, A. Hazzaa, Novel click modifiable thioquinazolinones as anti-inflammatory agents: Design, synthesis, biological evaluation and docking study, Eur. J. Med. Chem. 144 (2018) 635–650.
[27] B.S. Orlek, F.E. Blaney, F. Brown, M.S.G. Clark, M.S. Hadley, J. Hatcher, G.J. Riley, H.E. Rosenberg, H.J. Wadsworth, P. Wyman, Comparison of azabicyclic esters and oxadiazoles as ligands for the muscarinic receptor, J. Med. Chem. 34 (9) (1991) 2726–2735.
[28] S. Borg, K. Luthman, F. Nyberg, L. Terenius, U. Hacksell, 1,2,4-Oxadiazole derivatives of phenylalanine: potential inhibitors of substance P endopeptidase, Eur. J. Med. Chem. 28 (10) (1993) 801–810.
[29] M.S. Malamas, J. Sredy, M. McCaleb, I. Gunawan, B. Mihan, D. Sullivan, Antihyperglycemic activity of new 1,2,4-oxadiazolidine-3,5-diones, Eur. J. Med. Chem. 36 (1) (2001) 31–42.
[30] S. Ningaiah, U.K. Bhadraiah, S. Keshavamurthy, C. Javarasetty, Novel pyrazoline amidoxime and their 1, 2, 4-oxadiazole analogues: Synthesis and pharmacological screening, Bioorg. Med. Chem. Lett. 23 (16) (2013) 4532–4539.
[31] J.L. Burgaud, E. Ongini, P. Del Soldato, Nitric oxide-releasing drugs: a novel class of effective and safe therapeutic agents, Ann. N.Y. Acad. Sci. 962 (1) (2002) 360–371.
[32] J.M. Cash, J.H. Klippel, Second-line drug therapy for rheumatoid arthritis, N. Engl. J. Med. 330 (19) (1994) 1368–1375.
[33] N.M. Davies, J.L. Wallace, Nonsteroidal anti-inflammatory drug-induced gastrointestinal toxicity: new insights into an old problem, J. Gastroenterol. 32 (1) (1997) 127–133.
[34] M.D. Mullican, M.W. Wilson, D.T. Conner, C.R. Kostlan, D.J. Schrier, R.D. Dyer, Design of 5-(3, 5-di-tert-butyl-4-hydroxyphenyl)-1, 3, 4-thiadiazoles,-1, 3, 4-oxadiazoles, and-1, 2, 4-triazoles as orally active, nonulcerogenic antiinflammatory agents, J. Med. Chem. 36 (8) (1993) 1090–1099.
[35] B. Tozkoparan, N. Gökhan, G. Aktay, E. Yeşilada, M. Ertan, 6-benzylidenethiazolo [3, 2-b]-1, 2, 4-triazole-5 (6h)-onessubstituted with ibuprofen: Synthesis, characterizationand evaluation of anti-inflammatory activity, Eur. J. Med. Chem. 35 (7–8) (2000) 743–750.
[36] B. Tozkoparan, E. Küpeli, E. Yeşilada, M. Ertan, Preparation of 5-aryl-3-alkylthio-l, 2, 4-triazoles and corresponding sulfones with antiinflammatory–analgesic activity, Bioorg. Med. Chem. 15 (4) (2007) 1808–1814.
[37] H.A. Mostafa, A.K.M.H. Alaa, K.T. Laurent, Synthesis and molecular docking of novel indole-2-carboxamide derivatives with anti-inflammatory and antinociceptive activities, J. Adv. Chem. 10 (2014) 3143–3159.
[38] M.H. Abdelrahman, B.G.M. Youssif, M.A. Abdelgawad, A.H. Abdelazeem, H.M. Ibrahim, A. Moustafa, L. Treamblu, S.N.A. Bukhari, Synthesis, biological evaluation, docking study and ulcerogenicity profiling of some novel quinoline-2-carboxamides as dual COXs/LOX inhibitors endowed with anti-inflammatory activity, Eur. J. Med. Chem. 127 (2017) 972–985.
[39] S.Z. Ren, Z.C. Wang, D. Zhu, X.H. Zhu, F.Q. Shen, S.Y. Wu, J.J. Chen, C.X. Zhu, Design, synthesis and biological evaluation of novel ferrocenepyrazole derivatives containing nitric oxide donors as COX-2 inhibitors for cancer therapy, Eur. J. Med. Chem. 157 (2018) 909–924.
[40] M.S. Abdelbaset, G.E.A. Abuo-Rahma, M.H. Abdelrahman, M. Ramadan, B.G.M. Youssif, S.N.A. Bukhari, M.F.A. Mohamed, M. Abdel-Aziz, Novel pyrrol-2(3H)-ones and pyridazin-3(2H)-ones carrying quinoline scaffold as anti-proliferative tubulin polymerization inhibitors, Bioorg. Chem. 80 (2018) 151–163.
[41] Accelrys Software Inc, Discovery Studio Modeling Environment, Release 2.5, Accelrys Software Inc., San Diego (2007).
[42] R.G. Kurumbail, A.M. Stevens, J.K. Gierse, J.J. McDonald, R.A. Stegeman, J.Y. Pak, D. Gildehaus, J.M. Miyashiro, T.D. Penning, K. Seibert, P.C. Isakson, W.C. Stallings, Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents, Nature 384 (6610) (1996) 644–648.