



ORIGINAL ARTICLE

Molecular modeling, synthesis, characterization and pharmacological evaluation of benzo[d]oxazole derivatives as non-steroidal anti-inflammatory agents



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Abstract A series of N-(2-(4-chlorobenzyl)benzo[d]oxazol-5-yl)-3-substituted-propanamide (**3a–3n**) were synthesized and evaluated for their acute and chronic anti-inflammatory potential. The structure of the compounds was elucidated by elemental and spectral (IR, ¹H NMR and MS) analysis. The synthesized compounds (at a dose of 20 mg/kg b.wt. p.o.) have shown their ability to provide 45.1–81.7% protection against carrageenan-induced paw edema, in comparison with diclofenac sodium (69.5%) and ibuprofen (64.7%). The most active compounds **3a**, **3l** and **3n** were screened for chronic anti-inflammatory activity (cotton-pellet-induced granuloma) and to study their ulcerogenic activity. Compounds **3a**, **3l** and **3n** showed 48.4%, 39.3% and 44.0% protection against cotton pellets-induced granuloma compared to diclofenac sodium (60.2%). The tested compounds were less ulcerogenic than the ibuprofen. Molecular modeling studies suggest that these compounds have strong interaction with the COX-2 enzyme, which is responsible for the activity. © 2015 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Non-steroidal anti-inflammatory drugs are among the most widely used of all therapeutic agents. These drugs are often taken without prescription for minor aches and pains. Several anti-inflammatory agents are available in the market and none of these is ideal in controlling or modifying the signs and symptoms of inflammation. Gastrointestinal bleeding, ulceration and complications are the main complications of these drugs; therefore the emphasis on minimizing these risks has been one of the greatest challenges to develop much safer

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drugs (Borne et al., 2013). In our recent research projects related to amide and heterocyclic compounds, the substituted benzo[d]oxazoles have attracted much attention due to their utilization as analgesic and anti-inflammatory (Pilli et al., 1994; Gokhan et al., 1996; Paramashivappa et al., 2003; Sondhi et al., 2006; Seth et al., 2014), antifungal (Vinsona et al., 2006; Kim et al., 2010), anti-bacterial (Yildiz-Oren et al., 2004; Gadegoni et al., 2013), anti-tubercular agent (Arisoy et al., 2013) and in Alzheimer's disease (Chun et al., 2008). These activities probably result from its planar and compact structure. All anti-inflammatory agents act by the inhibition of the cyclooxygenase (COX) enzyme. There are two isoforms of cyclooxygenase enzyme; COX-1, which is found primarily in blood vessels, kidney and stomach, and COX-2, which is responsible for induction of inflammation. All, over-the-counter anti-inflammatory agents inhibit both COX-1 and COX-2 at varying degrees. COX-1 has short binding site, while COX-2 enzyme has long binding active site. The anti-inflammatory and analgesic properties of NSAIDs are derived from COX-2 inhibition and the common side effect such as gastrointestinal bleeding is due to inhibition of COX-1.

The molecular docking approach is one of the most rational and authentic approaches in the drug design and discovery for studying the molecular interaction of small molecules. The docking involves two main components such as search algorithm and the scoring functions. The search algorithm involves the positioning of the molecular conformations in the active site while the scoring function determines the most energetically favorable orientation of the molecule. Based on the reported studies and the substantial need for superior anti-inflammatory activity we have designed the hybrid compounds of benzo[d]oxazole containing $-\text{CONHCH}_2\text{CH}_2-\text{N}$, which can interact with the possible binding site (Tyr-355, Arg-120) of the COX-2 enzyme. Molecular modeling studies were performed in order to prove whether COX-2 was a possible target for the synthesized compounds or not. With this theory, we are reporting the synthesis, characterization and biological evaluation of title compounds for their acute and chronic anti-inflammatory activity as well as their ulcerogenic potential. In this study we have used Autodock 4.2 (Morris et al., 2009) for docking of the synthesized compounds on COX-2 crystal structure (PDB: 3NT1) with (Duggan et al., 2010) binding site which was also previously reported by many authors (Raghavendra et al., 2011, 2013; Basile et al., 2012). Additionally we also have calculated and compared the binding affinities of these compounds with standard reference molecules.

2. Material and methods

2.1. Chemistry

Nuclear magnetic resonance spectra (NMR) were recorded on Bruker, Avance DPX-300 spectrometer (300 MHz) using CDCl_3 . Chemical shifts are reported in ppm related to tetramethylsilane (TMS) as the internal standard. Infrared (IR) spectra were recorded in potassium bromide (Merck, Darmstadt, Germany) disks, using Perkin Elmer IR spectrophotometer. Melting points (mp) were recorded using Stuart Scientific electro-thermal melting point apparatus (UK), and are

uncorrected. Mass spectra were recorded on a LC-MS-LCQ (Agilent, Advantage-Max) instrument equipped with electrospray ion (ESI) source and elemental analyses were carried out on a Heraeus Carlo Erba-1108 elemental analyzer. Purity of the compounds and progress of the reaction were monitored using TLC and compounds were purified using column chromatography. Starting material, reagents and solvents were purchased from Sigma Aldrich Chemical Co., USA, and Himedia (Mumbai, India) and were used without further purification.

2.2. Synthesis

2.2.1. The title compounds were prepared using the synthetic strategy described in Scheme 1. Compound 5-amino-2-(4-chloro benzyl)-benzo[d]oxazole (1) was acetylated with chloropropionyl chloride in dry benzene. The second reaction was carried out by refluxing 3-chloro-N-(2-(4-chloro-benzyl)-benzo[d]oxazol-5-yl)-propionamide (2) with different secondary amine or heterocyclic compound in dry condition for 6 hours to give final compounds in variable yield between 30% and 70%.

2.3. Pharmacological evaluation

2.3.1. Animals

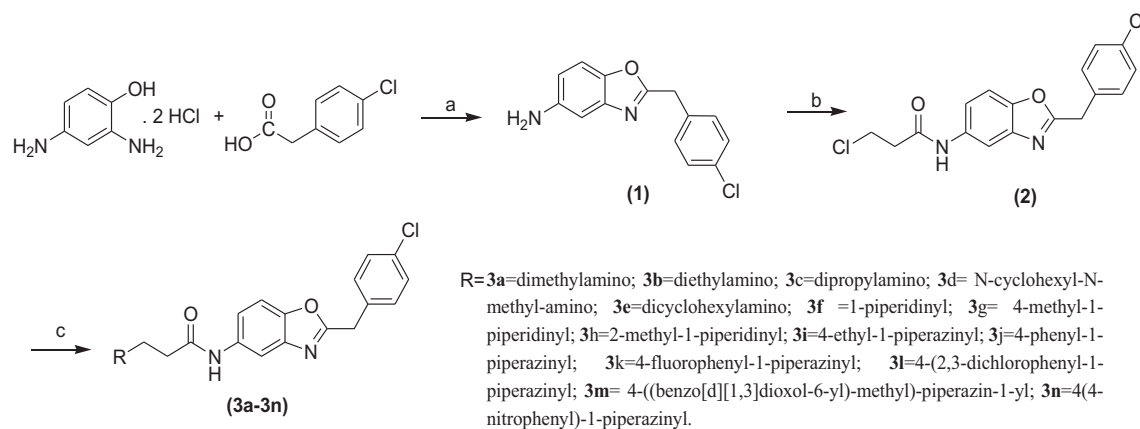
The animals were procured from the Animal House Center, Faculty of Pharmacy, and were divided and housed in different cages at $25 \pm 2^\circ\text{C}$, relative humidity of 50–65%, under 12 h light and dark cycles; they were fed standard animal feed. All animals were acclimatized for a week before use. All the experiments were conducted after receiving the approval from University Animal Ethics Committee. The acute anti-inflammatory activity of the compounds was evaluated on Wistar rats as described by Winter et al. (1962) while chronic anti-inflammatory activity of a few compounds was studied according to the cotton pellet granuloma method of D'arcy et al. (1960). The ulcerogenic activity of compounds (3a, 3l and 3n; 60 mg/kg b.wt.) and ibuprofen (60 mg/kg b.wt.) was performed according to Cioli et al. (1979).

2.3.2. Acute anti-inflammatory activity

The animals (albino rats) were divided into groups of four. Synthesized compounds were given orally (20 mg/kg b.wt.) and the paw volume was determined plethysmographically (Ugo-Basel, Italy). The control group received equivalent volume of normal saline. The reference group received ibuprofen (20 mg/kg b.wt. p.o.). After half an hour the carrageenan (0.1 mL of 1.0% w/v solution) in sterile saline was injected into the subplantar tissue of the rat's right hind paw. The paw volume was determined at hourly intervals for 3 h (0, 1, 2, 3 h). The percent of inhibition of inflammation was determined using the following formula: % inhibition = $100 \times (1 - V_s/V_c)$, where ' V_c ' represents edema volume in control and ' V_s ' edema volume in the group treated with sample.

2.3.3. Cotton pellets induced granuloma

Sterile cotton-pellets (10 mg each) were implanted subcutaneously in the back axilla regions of each rat under ketamine hydrochloride (100 mg/kg i.m.) anesthesia. The animals



Scheme 1 Synthesis of benzo[d]oxazole derivatives **3a–3n**. Reagent and conditions: (a) PPA, 2 h, 80 °C; (b) chloropropionyl chloride, dry benzene; (c) different secondary amines, benzene, reflux.

in the test groups were treated orally with synthesized compounds (**3a**, **3l** or **3n**; 20 mg/kg) for 7 consecutive days, while the control group received normal saline (vehicle control). The diclofenac sodium was used as reference drug (10 mg/kg body weight). Scarification was made after 8th day and the cotton-pellets were removed, freed from extraneous tissues. The wet pellets were weighed and then dried in a vacuum oven at 60 °C for 4 h to a constant weight; after that the dried pellets were weighed again. Increment in the dry weight of the pellets was taken as a measure of granuloma formation.

2.3.4. Acute ulcerogenic activity

Wistar rats (150–200 g) were used in the present experiment. Three times the anti-inflammatory dose of standard/compounds was used. Rats ($n = 5$) were divided into different groups. Compounds (60 mg/kg b.wt.) or ibuprofen (60 mg/kg b.wt.) was given orally. Control group received only normal saline. The animals were fasted 18 h prior to administration of test compounds. After 6 h of the drug treatment the rats were sacrificed. Stomach was removed and opened along the greater curvature. Inner lining was washed with normal saline and examined by magnifying glasses (5 \times). The number of ulcers and severity index was calculated using the scores: 0 = no lesions; 1 = superficial ulcers; 2 = deep ulcers; 3 = perforation; 4 = severe perforation.

2.4. Molecular modeling and docking studies

To understand the mechanism of anti-inflammatory activity of the compounds, docking simulation (Morris et al., 1998) was performed on X-ray crystal structure of COX-2 protein (PDB code: 3NT1; resolution 1.73 Å; Duggan et al., 2010) using Autodock 4.2 (Morris et al., 2009). The protein was prepared by removing B chain, nonreactive water molecules, and ligand molecules other than crystal ligand (naproxen in COX-2). The synthesized molecules were built as pdb files using PRODRG2 server (Schuttelkopf and van Aalten, 2004). Gasteiger charges and Kollman united atom charges were added to the ligand and receptor, respectively. The atomic solvation parameters were assigned using the ADDSOL utility of Autodock 4.2. The grid calculation was performed using Autogrid4 program, in which a box dimension of 22.5 Å and

grid spacing of 0.375 Å parameters were set. The generated box size allows each member of the tested compound to rotate freely in order to find the conformation with the best binding free energy. LGA is used as a global optimizer and energy minimization for docking simulation. Prior to docking the synthesized molecules into target, crystal structure of naproxen was used to perform control docking to ensure whether the docking method was valid. The amino acid residues that interact with naproxen produced by the re-docking process were then compared with amino acid residues that bind the crystal structure; a root-mean-square deviation (RMSD) that was less than or equal to 2.0 Å was defined as reasonable criteria (Accelrys-Software-Inc, 2012). The resulted RMSD value obtained was 0.910 Å. This indicated that the parameter set for docking was suitable for reproducing the X-ray structure. Moreover, the re-docking results for naproxen revealed that ligand occupies COX-2 receptor-binding pockets and performs similar intermolecular interaction with surrounding amino acids.

3. Results and discussion

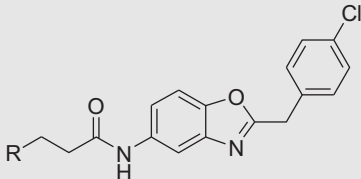
IR spectroscopic data reveal that synthesized compounds give characteristic signal at their assigned places. The presence of $-\text{NH}-\text{CO}-$ linkage between 1655 and 1685 cm^{-1} and NH stretching between 3350 and 3420 cm^{-1} indicates the synthesis of the compounds. ^1H NMR spectra of all compounds are in agreement with the suggested structure. Methylene (CH_2) protons of $-\text{NHCOCH}_2\text{CH}_2-$ were observed as triplet between 2.3 and 3.4 ppm, which confirms the synthesis of the target compounds (**3a–3n**). In case of compound **2**, these protons were deshielded and observed between 2.8 and 4.10 ppm due to the presence of $-\text{Cl}$. The signals for the benzylic proton were recorded as singlet between 3.50 and 4.25 ppm, while aromatic protons were observed between 6.90 and 7.20 ppm as doublet, with coupling constant (J value) $\sim 6-7$ Hz. The NH protons were observed as D_2O exchangeable protons.

The pharmacological data imply that 80% of the compounds were found to possess appreciable anti-inflammatory activity as compared to standard drugs, while 13% of compounds hold iota of similar properties (Table 1). Upon critical introspection of synthesized compounds, it has been found










that compounds bearing the dimethyl groups (**3a**, 72%), 4-methylpiperidine (**3g**, 72.7%) and substituted piperazine (**3l** and **3n**, dichlorophenyl, 4-nitrophenylpiperazine) exhibited more protection than standard drugs against carrageenan induced inflammation, while replacement of the dimethyl groups with diethyl (**3b**, 63.4%), dipropyl (**3c**, 66.6%), dipropyl (**3e**, 60.5%), or 2-methyl-piperidine (**3h**, 57.3%) reduces activity. The compounds bearing piperidine, phenyl piperazine

and 4-fluorophenylpiperazine groups exhibited activity similar to standard drug. Ethyl piperazine derivative (**3i**, 45.1%) was least active among all the compounds studied. Data reveal that compounds studied are capable to reduce the acute inflammation more than the chronic induced by cotton-pellet (**Table 1**). The ulcerogenic activity of ibuprofen (60 mg/kg b.wt.) and compounds (**3a**, **3l** and **3n**; 60 mg/kg b.wt.) was performed according to [Cioli et al. \(1979\)](#). The tested compounds showed

Table 1 Acute (carrageenan induced paw edema) and chronic (cotton pellet granuloma) anti-inflammatory activity of the synthesized compounds.



(3a-3n)

Compd.	R	Mean increase in paw volume (ml) (Mean ± SEM)	% Inhibition ^a	Cotton pellets-induced granuloma method ^a			
				Weight of moist cotton Pellet (mg) (Mean ± SEM)	% Activity ^a	Weight of dry cotton Pellet (mg) (Mean ± SEM)	% Activity ^a
3a	—N—(CH ₃) ₂	0.115 ± 0.020	72.0	165.25 ± 0.85	31.1	30.33 ± 0.52	48.4
3b	—N—(CH ₂ CH ₃) ₂	0.150 ± 0.030	63.4	—	—	—	—
3c	—N—(CH ₂ CH ₂ CH ₃) ₂	0.137 ± 0.037	66.6	—	—	—	—
3d	—N—C ₆ H ₁₁ CH ₃	0.150 ± 0.020	63.4	—	—	—	—
3e	—N—(C ₆ H ₁₁) ₂	0.162 ± 0.047	60.5	—	—	—	—
3f	—N— 	0.125 ± 0.025	69.5	—	—	—	—
3g	—N— 	0.112 ± 0.026	72.7	—	—	—	—
3h	—N— 	0.175 ± 0.025	57.3	—	—	—	—
3i	—N— 	0.225 ± 0.014	45.1	—	—	—	—
3j	—N— 	0.129 ± 0.025	68.5	—	—	—	—
3k	—N— 	0.130 ± 0.034	68.3	—	—	—	—
3l	—N— 	0.092 ± 0.012	77.6	152.38 ± 0.90	36.5	35.68 ± 0.85	39.3
3m	—N— 	0.212 ± 0.031	48.3	—	—	—	—
3n	—N— 	0.075 ± 0.014	81.7	144.25 ± 1.11	39.9	32.88 ± 0.66	44.0
Diclof. Na	—	0.125 ± 0.014	69.5	98.90 ± 0.86	59.0	23.38 ± 0.75	60.2
Ibuprofen	—	0.142 ± 0.025	64.7	—	—	—	—
Control	—	0.402 ± 0.020	—	239.38 ± 0.63	—	58.75 ± 0.48	—

p values were compared with control group (3 h after inducing edema.) (Tukey's Test). Anti-inflammatory activity was assessed as the percent inhibition of carrageenan-induced edema or granuloma between animals of control group and animals pretreated with reference drug – diclofenac sodium (10 mg/kg), Ibuprofen (20 mg/kg) or synthesized compounds (20 mg/kg, b.wt.).

^a *p* < 0.05 (significant difference). Number of animals (rats) in each group = 4.

Table 2 Ulcerogenic activity of the synthesized compounds in rats.

Compd.	Ulcerogenic activity ^a (severity index) ^b , (Mean ± SD)
3a (60 mg/kg)	2.20 ± 0.84
3l (60 mg/kg)	2.00 ± 0.71
3n (60 mg/kg)	1.60 ± 0.55
Ibuprofen (60 mg/kg)	2.80 ± 0.45
Control (normal saline)	–

^a Number of animals in each group = 5.

^b $p < 0.05$ (significant difference).

severity index ranging from 1.60 to 2.20 while the standard drug ibuprofen exhibited high severity index of 2.80 (Table 2). The ulcerogenic effects of tested compounds were appreciably lower than the ibuprofen.

In molecular modeling and docking studies, the intermolecular interaction between naproxen crystallized with COX-2 was studied and analyzed. Two critical hydrogen bonds were created between naproxen and Tyr-355 and Arg-120 in COX-2 active site (Fig. 1a). In order to simplify the analysis compounds were used to prepare intermolecular interaction with COX-2 (Table 3). The amino acid Arg-120 was shown to interact with all the synthetic compounds by hydrogen bond interaction as shown in Table 3 and Fig. 1b–e. Compound **3g** (Fig. 1b) was found to interact with Arg-120, Tyr-355 and Ser-530 by hydrogen bond interaction. Compounds **3i** (Fig. 1c) and **3m** (Fig. 1d) interact with Arg-120 & Ser-530 and Arg-120 & Ser-119, respectively. Some compounds, such as **3e**, **3i** and **3m** do not interact with Tyr-355, and interestingly they are among the lowest percent inhibition of COX-2 (Table 3), while compound **3n** (Fig. 1e) has the highest percent inhibition among all the compounds. In addition to Tyr-355 and Arg-120, compound **3n** can interact with Lys-83 and Ser-471. This will lead to more tight binding; hence, it showed the highest activity. This may indicate the importance of hydrogen bond interaction with Arg-120 and Tyr-355.

4. Conclusion

In conclusion, a series of *N*-(2-(4-chlorobenzyl)-benzo[d]oxazol-5-yl)-3-substituted-propanamide (**3a–3n**) were synthesized and evaluated for their anti-inflammatory potential. The experimental data of present study indicate that the synthesized compounds are more effective in acute inflammatory conditions than in chronic one. The tested compounds exhibited less ulcerogenic activity than the reference drug. Interestingly, the docking results were in agreement with that of the anti-inflammatory activity and against cyclooxygenase enzyme where the important structural features, spatial arrangement and binding interaction required for activity were determined. *In vivo* studies revealed that compounds **3a**, **3l** and **3n** have significant anti-inflammatory activities comparable to standard drugs. Compound **3n** (4-nitrophenyl-piperazine derivative), has interaction with Lys-83 and Ser-471 in addition to Tyr-355 and Arg-120 which might be contributing for significant activity. Compounds studied in this study have clearly indicated that the presence of benzo[d]oxazole and substituted

propanamide moiety has positive impact on anti-inflammatory activities. In conclusion, this series of compound can be explored more for further development of novel anti-inflammatory drugs.

5. Experimental

5.1. Chemistry

5.1.1. 3-chloro-*N*-(2-(4-chloro-benzyl)-benzo[d]oxazol-5-yl)-propanamide (**2**)

It was prepared by stirring 5-amino-2-(4-chloro benzyl)-benzo[d]oxazole (**1**, 10 mM, Yildiz-Oren et al., 2004) dissolved in dry benzene (20 ml) and chloropropionyl chloride (11 mM) for 2 h, then the reaction mixture was refluxed for 1 h. After completion of reaction, the contents were poured on crushed ice. The precipitated product was collected, washed with 1% (w/v) potassium carbonate solution and ice cold water, and dried in vacuum and recrystallized from dichloromethane.

5.1.2. General procedure for synthesis of *N*-(2-(4-chloro benzyl)-benzo[d]oxazol-5-yl)-3-substituted-propanamide (**3a–3n**)

A mixture of **2** (5 mM) in dry benzene (50 ml) and respective secondary amine or heterocyclic compounds (10 mM) was refluxed for 6–8 h. On cooling the amine hydrochloride, which was crystallized out was filtered and separated. The organic phase was washed with distilled water to remove the last traces of hydrochloride and dried on sodium sulfate (exsiccated). Benzene was removed under reduced pressure. Crude product was collected and recrystallized using ethanol (90%).

5.1.3. *N*-(2-(4-chlorobenzyl)-benzo[d]oxazol-5-yl)-3-(dimethylamino)-propanamide (**3a**)

Yield 30%, mp 124 °C; IR (cm⁻¹) (KBr): 3285.62 (NH), 2930.67 (CH), 1685.67 (–CONH–), 1622.25, 1460.30 (C=C), 1425.30 (–CH), 1152.45 (Ar–Cl), 736.91 (oop). ¹H NMR (300 MHz, CDCl₃): δ 1.25 (s, 6H, 2× CH₃), 2.23–2.26 (t, 2H, $J = 9$ Hz, –COCH₂), 3.31–3.34 (t, 2H, $J = 9$ Hz, –CH₂–N), 4.18 (s, 2H, benzylic), 7.40 (d, 2H, $J = 6$ Hz, Ar–H), 7.58–7.62 (br/m, 2H, Ar–H), 7.67–7.70 (d, 1H, $J = 9$ Hz, H-7), 8.00–8.05 (m, 2H, H-4, H-6), 8.13 (s, 1H, NH). LC-MS-LCQ (ESI, positive mode) for C₁₉H₂₀ClN₃O₂: m/z 357.8 [M]⁺, 358.9 [M + 1]⁺. Anal. calc for C₁₉H₂₀ClN₃O₂; C, 63.77; H, 5.63; N, 11.74; Found C, 63.74; H, 5.62; N, 11.75.

5.1.4. *N*-(2-(4-chloro benzyl)-benzo[d]oxazol-5-yl)-3-(diethylamino)propanamide (**3b**)

Yield: 50%, mp 118 °C; IR (cm⁻¹, KBr): 3287.9 (N–H), 1664.9 (–CONH–), 2924.5 (–CH), 1622.1, 1483.5 (Aromatic C=C), 1426.1 (–CH–), 1217.9 (C–N), 1092.3 (Ar–Cl), 1017.1 (C–O), 763.4 (oop). ¹H NMR (300 MHz, CDCl₃): δ 1.19 (t, 6H, $J = 6$ Hz, 2× CH₃), 2.10 (t, 2H, $J = 3$ Hz, –CO–CH₂–), 2.36–2.41 (q, 4H, $J = 3$ Hz, 2× CH₂), 2.89 (t, 2H, $J = 3$ Hz, –CH₂–N), 3.49 (s, 2H, benzylic), 7.58 (d, 2H, $J = 6$ Hz, Ar–H), 7.78 (d, 2H, $J = 6$ Hz, Ar–H), 7.92–7.93 (d, 1H, $J = 3$ Hz, H-7), 8.01–8.09 (m, 2H, H-4, H-6), 8.26 (s, 1H, NH). Anal. Calc for C₂₁H₂₄ClN₃O₂; C, 65.36; H, 6.27; N, 10.89; Found C, 65.35; H, 6.28; N, 10.90.

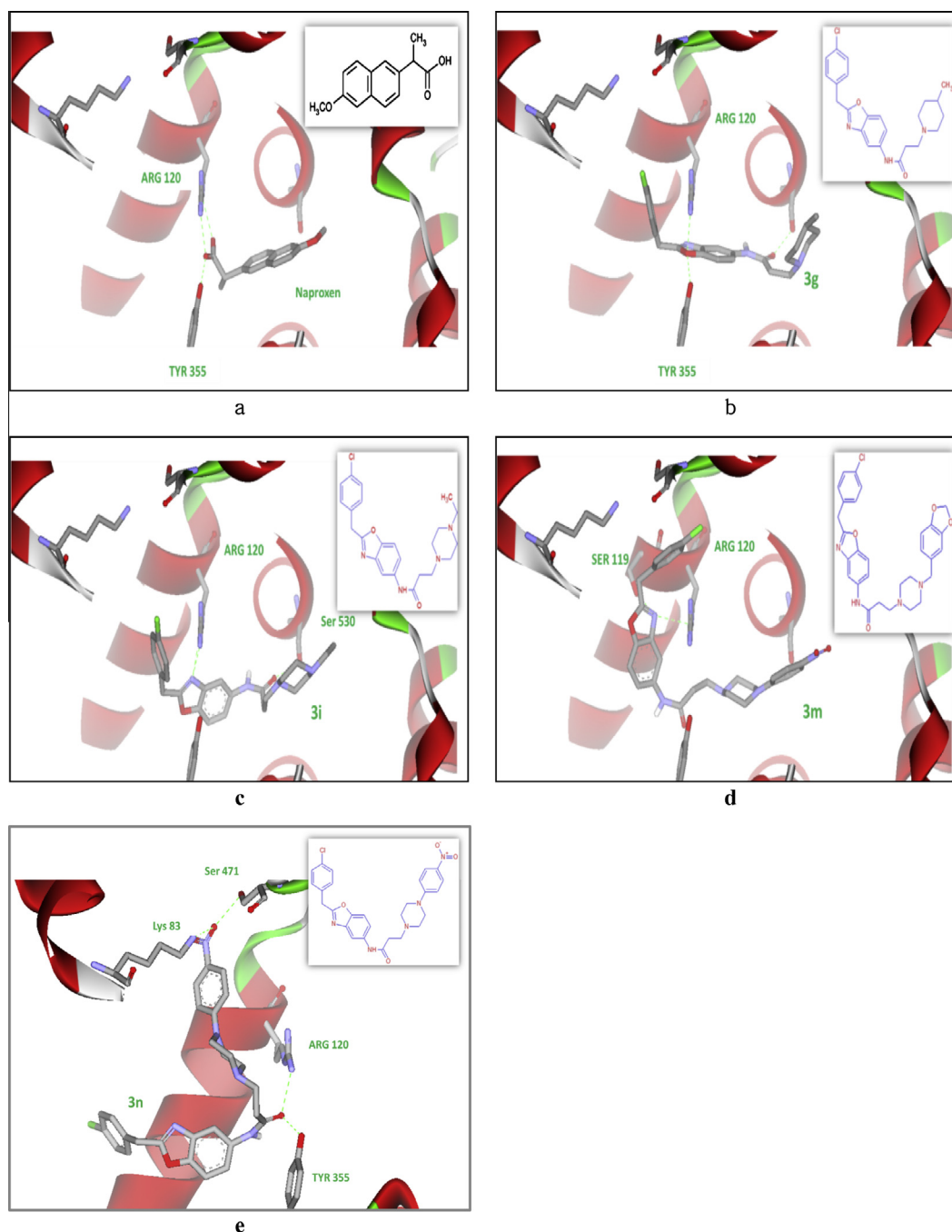


Figure 1 Solid ribbon representation of intermolecular interaction between (a) Naproxen, (b) **3g**, (c) **3i**, (d) **3m**, (e) **3n** and COX-2 (PDB = 3NT1) crystal structures. Created by Discovery Studio v3 visualizer (Accelrys-Software-Inc, 2012).

5.1.5. *N*-(2-(4-chlorobenzyl)-benzo[d]oxazol-5-yl)-3-(di propyl-amino)propanamide (3c)

Yield 54%, mp 122 °C; IR (cm⁻¹, KBr): 3356.82 (N—H), 2932.57 (—CH), 1670.67 (—CONH—), 1628.25, 1521.30 (C=C), 1486.30 (—CH), 1245.43 (C—N), 1050.23 (C—O), 800.91 (oop). ¹H NMR (300 MHz, CDCl₃): δ 0.98 (t, 6H, *J* = 3 Hz, 2× CH₃), 1.64–1.72 (m/br, 4H, 2× —CH₂), 2.48–2.50 (m, 6H, 3× —CH₂), 2.70 (t, 2H, *J* = 5 Hz, —CO—CH₂), 3.99 (s, 2H, benzylic), 7.32 (d, 2H, *J* = 8 Hz, Ar—H), 7.56 (m/br, 2H, Ar—H), 7.73–7.81 (d, 1H, *J* = 8 Hz, H-7), 7.93–8.03 (m, 2H, H-4, H-6), 8.32 (s, 1H, NH). Anal.

Calc for C₂₃H₂₈ClN₂O₂; C, 66.74; H, 6.82; N, 10.15; Found C, 66.70; H, 6.80; N, 10.12.

5.1.6. *N*-(2-(4-chlorobenzyl)-benzo[d]oxazol-5-yl)-3-(*N*-cyclo-hexyl-*N*-methylamino)-propanamide (3d)

Yield 58%; mp 140 °C; IR (cm⁻¹, KBr): 3209.62 (N—H), 2923.88 (—CH Stretching), 1664.45 (—CONH—), 1623.95, 1525.30 (Aromatic C=C), 1483.15 (—CH bend), 1157.21 (C—N), 1091.63 (C—O), 800.40 (oop). ¹H NMR (300 MHz, CDCl₃): δ 1.11–1.42 (m/br, 6H, 3× CH₂, cy.hex.), 1.72 (m, 4H, 2× CH₂, cy.hex.), 2.18 (s, 3H, CH₃), 2.84 (t, 2H, *J* = 6 Hz,

Table 3 Free energy contribution and Hydrogen bond interaction for synthetic compounds with COX-2 binding site.

Comp.	(ΔG) kcal/mol	No. of bonds	Amino acids contributing in hydrogen bonding
Naproxen	-5.97	2	Arg-120, Tyr-355
3a	-9.06	2	Arg-120, Tyr-355
3b	-9.65	3	Arg-120, Tyr-355, Ser-530
3c	-9.11	3	Arg-120, Tyr-355, Ser-530
3d	-10.5	2	Arg-120, Tyr-355
3e	-11.67	2	Arg-120, Ser-530
3f	-10.09	3	Arg-120, Tyr-355, Ser-530
3g	-9.96	3	Arg-120, Tyr-355, Ser-530
3h	-10.77	3	Arg-120, Tyr-355, Ser-530
3i	-9.05	2	Arg-120, Ser-530
3j	-10.90	2	Arg-120, Tyr-355
3k	-10.00	2	Arg-120, Tyr-355
3l	-9.24	2	Arg-120, Tyr-355
3m	-9.99	2	Arg-120, Ser-119
3n	-10.93	4	Arg-120, Tyr-355, Ser-471 and Lys-83

CO—CH₂), 3.32–3.38 (m, 1H, —CH, cy.hex.), 3.90 (t, 2H, $J = 6$ Hz, —CH₂—N), 4.23 (s, 2H, benzylic), 7.11 (d, 2H, $J = 7$ Hz, Ar—H), 7.49–7.54 (m, 2H, Ar—H), 7.72 (d, 1H, $J = 6$ Hz, H-7), 7.86–7.91 (m, 2H, H-4, H-6), 8.28 (s, 1H, NH). Anal Calc for C₂₄H₂₈ClN₃O₂; C, 67.67; H, 6.63; N, 9.87; Found C, 67.61; H, 6.59; N, 9.85.

5.1.7. *N*-(2-(4-chlorobenzyl)-benzo[d]oxazol-5-yl)-3-(dicyclohexylamino)propanamide (**3e**)

Yield 54%, mp 135 °C; IR (cm⁻¹, KBr): 3225.62 (N—H), 2965.67 (—CH), 1674.67(—CONH—), 1600.25, 1522.30 (C=C), 1475.30 (—CH), 1246.48 (C—N), 1092.45 (C—Cl), 1025.23 (C—O), 746.91 (oop). ¹H NMR (300 MHz, CDCl₃): δ 0.99–1.35 (m, 12H, 6 \times CH₂, dicy.hex.), 1.67–1.92 (m, br, 8H, 4 \times CH₂, dicy.hex.), 2.24–2.34 (m, 2H, $J = 6$ Hz, —CO—CH₂), 2.57 (t, 2H, $J = 6$ Hz, —CH₂), 3.62 (t, 2H, $J = 6$ Hz, —CH₂), 4.15 (s, 2H, benzylic), 7.11(d, 2H, $J = 6$ Hz, Ar—H), 7.59–7.62 (br/m, 2H, Ar—H), 7.73–7.75 (d, 1H, $J = 6$ Hz, H-7), 7.91–7.95 (m, 2H, H-4, H-6), 8.30 (s, 1H, NH). Anal Calc for C₂₉H₃₆ClN₃O₂; C, 70.50; H, 7.34; N, 8.50; Found C, 70.10; H, 7.32; N, 8.40.

5.1.8. *N*-(2-(4-chlorobenzyl)-benzo[d]oxazol-5-yl)-3-(piperidin-1-yl)propanamide (**3f**)

Yield 60%, mp 154 °C; IR (cm⁻¹, KBr): 3266.62 (N—H), 2966.67 (—CH), 1666.67 (—CONH), 1602.25, 1525.30 (C=C), 1466.30 (—CH bend), 1245.48 (C—N), 1125.23 (C—O), 748.91 (oop). ¹H NMR (300 MHz, CDCl₃): δ 1.66–1.71 (m, 6H, 3 \times CH₂), 2.34 (m, 4H, 2 \times CH₂), 2.64 (t, 2H, $J = 18$ Hz, —CO—CH₂), 3.69 (t, 2H, $J = 3$ Hz, —CH₂), 4.20 (s, 2H, —benzylic), 6.67 (d, 2H, $J = 6$ Hz, Ar—H), 6.97 (d, 2H, $J = 3$ Hz, Ar—H), 7.32–7.35 (d, 1H, $J = 9$ Hz, H-7), 7.65–7.67 (br/m, 2H, H-4, H-6), 8.01 (s, 1H, NH). Anal. Calc for C₂₂H₂₄ClN₃O₂; C, 66.14; H, 6.08; N, 10.56; Found C, 65.97; H, 6.03; N, 10.47.

5.1.9. *N*-(2-(4-chlorobenzyl)-benzo[d]oxazol-5-yl)-3-(4-methyl-piperidin-1-yl)propanamide (**3g**)

Yield 60%, mp 162 °C; IR (cm⁻¹, KBr): 3358.62 (N—H), 3015.25 (—CH), 1684.67 (—CONH—), 1610.25, 1522.30 (C=C), 1470.20 (—CH), 1278.58 (C—N), 1115.23 (C—O) 756.91 (oop). ¹H NMR (300 MHz, CDCl₃): δ 1.01–1.02 (d, 3H, $J = 6$ Hz, —CH₃), 1.63–1.73 (m, 4H, —CH₂, pipd.), 1.98–2.21 (m, 1H, —CH, pipd.), 2.55 (t, 4H, $J = 6$ Hz, —CH₂—, pipd.), 2.71 (t, 2H, $J = 6$ Hz, —CO—CH₂), 3.06 (t, 2H, $J = 3$ Hz, —CH₂—), 4.24 (s, 2H, benzylic), 7.31 (d, 2H, $J = 6$ Hz, Ar—H), 7.56 (d, 2H, $J = 6$ Hz, Ar—H), 7.68–7.70 (d, 1H, $J = 6$ Hz, H-7), 7.81–7.83 (m, 2H, H-4, H-6), 8.22 (s, 1H, NH). Anal Calc for C₂₃H₂₆ClN₃O₂; C, 67.06; H, 6.36; N, 10.20; Found C, 66.91; H, 6.03; N, 10.11.

5.1.10. *N*-(2-(4-chlorobenzyl)-benzo[d]oxazol-5-yl)-3-(2-methyl-piperidin-1-yl)propanamide (**3h**)

Yield 60%, mp 158 °C; IR (cm⁻¹, KBr): 3356.62 (N—H), 3005.67 (—CH), 1674.67 (—CONH—), 1606.25, 1422.30 (C=C), 1465.30 (—CH bend), 1248.48 (C—N), 1122.23 (C—O) 766.81 (oop). ¹H NMR (300 MHz, CDCl₃): δ : 1.01–1.03 (d, 3H, $J = 6$ Hz, —CH₃), 1.48 (br, 2H, pipd.), 2.06–2.16 (m, 4H, $J = 9$ Hz, pipd.), 2.56 (t, 2H, $J = 6$ Hz, —CO—CH₂), 2.72 (br/m, 2H, pipd.), 2.82–2.95 (m, br, 1H, pipd.), 3.08 (t, 2H, $J = 6$ Hz, —CH₂—), 3.90 (s, 2H, benzylic), 7.46 (d, 2H, $J = 6$ Hz, Ar—H), 7.53–7.59 (m, 3H, Ar—H, H-7), 7.63–7.66 (m, 2H, H-4, H-6), 8.01 (s, 1H, NH). Anal Calc for C₂₃H₂₆ClN₃O₂; C, 67.06; H, 6.36; N, 10.20; Found C, 66.91; H, 6.03; N, 10.11.

5.1.11. *N*-(2-(4-chlorobenzyl)-benzo[d]oxazol-5-yl)-3-(4-ethyl piperazin-1-yl)propanamide (**3i**)

Yield 54%, mp 132 °C; IR (cm⁻¹, KBr): 3286.2 (N—H), 1668.6 (—CONH—), 2930.5 (—CH), 1624.1, 1527.1 (C=C), 1483.9 (—CH), 1159.1 (C—N), 1093.1 (C—O) 757.5 (oop). ¹H NMR (300 MHz, CDCl₃): δ 1.13 (t, 3H, $J = 6$ Hz, —CH₃), 2.48 (t, 2H, $J = 12$ Hz, —CO—CH₂), 2.58 (q, 2H, $J = 6$ Hz, —N—CH₂), (m/br, 8H, pipz.), 2.79 (t, 2H, —CH₂—), 3.82 (s, 2H, benzylic), 7.57 (d, 2H, $J = 6$ Hz, Ar—H), 7.63 (d, 2H, $J = 6$ Hz, Ar—H), 7.86–7.88 (d, 1H, $J = 6$ Hz, H-7), 7.94–7.96 (m, 2H, H-4, H-6), 8.86 (s, 1H, NH). Anal Calc for C₂₃H₂₇ClN₄O₂; C, 63.99; H, 6.10; N, 13.57; Found C, 63.16; H, 5.89; N, 13.14.

5.1.12. *N*-(2-(4-chlorobenzyl)-benzo[d]oxazol-5-yl)-3-(4-phenyl piperazin-1-yl)propanamide (**3j**)

Yield 60%, mp 128 °C; IR (cm⁻¹, KBr): 3311.62 (N—H), 2955.67 (—CH), 1684.67 (—CONH—), 1615.25, 1520.30 (C=C), 1445.30 (—CH), 1235.48 (C—N), 1188.23 (C—O) 800.21 (oop). ¹H NMR (300 MHz, CDCl₃): δ 2.09 (t, 2H, $J = 3$ Hz, —CO—CH₂), 2.50 (br/m, 4H, pipz.), 2.78 (t, 2H, $J = 6$ Hz, —CH₂—), 3.45 (br, 4H, pipz.), 3.90 (s, 2H, benzylic), 6.67 (m, 3H, $J = 12$ Hz, H_b, H_d, H_f, phenyl pipz.), 6.92 (d, 2H, $J = 7$ Hz, Ar—H), 7.25 (m, 2H, H_c, H_e, phenyl pipz.), 7.56 (d, 2H, $J = 7$ Hz, Ar—H), 7.84–7.88 (d, 1H, $J = 12$ Hz, H-7), 8.08–8.15 (m, 2H, H-4, H-6), 8.46 (s, 1H, NH). Anal calc for C₂₇H₂₇ClN₄O₂; C, 68.27; H, 5.73; N, 11.80; Found C, 67.91; H, 5.17; N, 11.18.

5.1.13. *N*-(2-(4-chlorobenzyl)-benzo[d]oxazol-5-yl)-3-(4-(4-fluorophenyl)piperazin-1-yl)propanamide (**3k**)

Yield 65%, mp 140 °C; IR (cm⁻¹, KBr): 3355.62 (N—H), 2865.47 (—CH), 1664.67 (—CONH—), 1600.25, 1415.30 (C=C), 1455.30 (—CH), 1288.48 (C—N), 1178.23 (C—O) 789.11 (oop). ¹H NMR (300 MHz, CDCl₃): δ 2.32 (t, 2H, *J* = 6 Hz, —CH₂), 2.84 (t, 4H, *J* = 6 Hz, pipz.), 3.33 (t, 2H, *J* = 9 Hz, —CH₂—), 3.90 (t, 4H, *J* = 3 Hz, pipz.), 4.22 (s, 2H, benzylic), 6.61–6.62 (m, 2H, H_b, H_f, fluoroph.), 6.84–6.89 (br, 2H, H_c, H_e, fluoroph.), 7.11 (d, 2H, *J* = 6 Hz, Ar—H), 7.49 (d, 2H, *J* = 7 Hz, Ar—H), 7.62–7.65 (d, 1H, *J* = 9 Hz, H-7), 7.86–7.95 (m, 2H, H-4, H-6), 8.28 (s, 1H, NH). Anal cal for C₂₇H₂₆ClFN₄O₂; C, 65.78; H, 5.32; N, 11.37; Found C, 65.73; H, 5.31; N, 11.35.

5.1.14. *N*-(2-(4-chlorobenzyl)-benzo[d]oxazol-5-yl)-3-(4-(2,3-dichlorophenyl)piperazin-1-yl)propanamide (**3l**)

Yield 70%, mp 152 °C; IR (cm⁻¹, KBr): 3355.22 (N—H), 2955.67 (—CH), 1660.67 (—CONH—), 1636.25, 1422.30 (C=C), 1415.40 (—CH), 1295.48 (C—N), 1000.23 (C—O) 799.91 (oop). ¹H NMR (300 MHz, CDCl₃): δ 2.12 (t, 2H, *J* = 6 Hz, —CO—CH₂—), 2.43 (t, 4H, *J* = 6.0 Hz, pipz.), 2.99 (t, 2H, *J* = 6.0 Hz, —CO—CH₂—), 3.28 (t, 4H, *J* = 3.0 Hz, pipz.), 3.72 (s, 2H, benzylic), 6.88(d, 1H, *J* = 3.0 Hz, H_f), 7.10 (d, 1H, *J* = 6.0 Hz, H_d), 7.42 (m, 1H, H_c), 7.60 (d, 2H, *J* = 3 Hz, Ar—H), 7.69 (d, 2H, *J* = 6 Hz, Ar—H), 7.73(d, 1H, *J* = 3 Hz, H-7), 8.01–8.03 (br/m, 2H, H-4, H-6), 8.14 (s, 1H, NH). Anal calc for C₂₇H₂₅Cl₃N₄O₂; C, 59.63; H, 4.63; N, 10.30; Found C, 59.58; H, 4.59; N, 10.01.

5.1.15. *N*-(2-(4-chlorobenzyl)-benzo[d]oxazol-5-yl)-3-(4-((benzo[d][1,3]dioxol-6-yl)-methyl)-piperazin-1-yl)propanamide (**3m**)

Yield 60%, mp 150 °C; IR (cm⁻¹, KBr): 3226.62 (N—H), 3000.67 (—CH), 1670.67 (—CONH—), 1610.25, 1422.30 (C=C), 1458.30 (—CH), 1310.48 (C—N), 1100.23 (C—O) 745.91 (oop). ¹H NMR (300 MHz, CDCl₃): δ 2.18 (t, 2H, *J* = 3 Hz, —CO—CH₂), 2.60–2.75 (br, 8H, pipz.), 2.84 (t, 2H, *J* = 6 Hz, —CH₂—), 3.90 (s, 2H, benzylic), 4.18 (s, 2H, benzylic), 5.80 (s, 2H, H_b), 6.31 (s, 1H, H_d), 6.45 (dd, 2H, H_g, H_f), 7.27–7.39 (m, 3H, Ar—H, H-7), 7.69–7.77 (m, 4H, Ar—H, H-4, H-6), 7.90 (s, 1H, NH). Anal calc for C₂₉H₂₉ClN₄O₄; C, 65.35; H, 5.48; N, 10.51; found C, 65.19; H, 5.40; N, 10.49.

5.1.16. *N*-(2(4-chloro-benzyl)-benzo[d]oxazol-5-yl)-3-(4-(4-nitrophenyl)-piperazin-1-yl)propanamide (**3n**)

Yield 65%, mp 148 °C; IR (cm⁻¹, KBr): 3402.62 (N—H), 2850.67 (—CH), 1664.67 (—CONH—), 1600.25, 1424.30 (C=C), 1466.30 (—CH bend), 1262.48 (C—N), 1145.23 (C—O) 765.91 (oop). ¹H NMR (300 MHz, CDCl₃): δ 2.38 (t, 2H, *J* = 3 Hz, —CO—CH₂), 2.79 (t, 4H, *J* = 3 Hz, pipz.), 3.02 (t, 2H, *J* = 6 Hz, —CH₂), 3.90 (t, 4H, *J* = 6 Hz, pipz.), 4.20 (s, 2H, benzylic), 6.48–6.51 (m, 2H, H_b, H_f), 6.77 (d, 2H, *J* = 6 Hz, Ar—H), 7.38 (d, 2H, *J* = 6 Hz, Ar—H), 7.46–7.62 (m, 3H, H-4, H-6, H-7), 7.88–7.91 (t, 2H, *J* = 9 Hz, H_c, H_e, Ar—H), 8.28 (s, 1H, NH). LC-MS-LCQ (ESI, positive mode) 521.0 [M + 1]⁺. Anal. calc. for C₂₇H₂₆ClN₅O₄; C, 62.37; H, 5.04; N, 13.47; found C, 62.35; H, 5.04; N, 13.48.

5.2. Statistical analysis

Experimental data are expressed as mean. Statistical difference between the treated and the control groups was evaluated by one way analysis of variance (AVOVA) followed by the Tukey's test as a post-ANOVA (GraphPad Prism 5, San Diego, CA, USA) to determine the statistical significance. A *P* value < 0.05 was considered statistically significant.

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References

- Accelrys-Software-Inc., San Diego: Accelrys Software Inc., 2012.
- Arisoy, M., Temiz-Arpaci, O., Kaynak-Onurdag, F., Ozgen, S., 2013. Novel benzoxazoles: synthesis and antibacterial, antifungal, and antitubercular activity against antibiotic-resistant and -sensitive microbes. *Z. Naturforsch C*. 68, 453–460.
- Basile, L., Alvarez, S., Blanco, A., Santagati, A., Granata, G., Di Pietro, P., Guccione, S., Munoz-Fernandez, Á.M.F., 2012. Sulfonilamidothiopyrimidone and thiopyrimidone derivatives as selective COX-2 inhibitors: synthesis, biological evaluation, and docking studies. *Eur. J. Med. Chem.* 57, 149–161.
- Borne, R., Levi, M., Wilson, N., 2013. Nonsteroidal anti-inflammatory drugs. In: Foye's Principles of Medicinal Chemistry, seventh ed. Wolter Kluwer: Lippincott Wiliam & Wilkins, Philadelphia.
- Chun, Y.S., Lim, S.J., Oh, S.J., Moon, D.H., Kim, D.J., Cho, C.G., Yoo, K.H., 2008. Synthesis of functionalized benzoxazoles and their binding affinities to Aβ42 fibrils. *Bull. Korean Chem. Soc.* 29, 1765.
- Cioli, V., Putzolu, S., Rossi, V., Scorza Barcellona, P., Corradino, C., 1979. The role of direct tissue contact in the production of gastrointestinal ulcers by anti-inflammatory drugs in rat. *Toxicol. Appl. Pharmacol.* 50, 283–289.
- D'arcy, P.F., Howard, E.M., Muggleton, P.W., Townsend, S.B., 1960. The antiinflammatory action of griseofulvin in experimental animals. *J. Pharm. Pharmacol.* 12, 659–665.
- Duggan, K.C., Walters, M.J., Musee, J., Harp, J.M., Kiefer, J.R., Oates, J.A., Marnett, L.J., 2010. Molecular basis for cyclooxygenase inhibition by the non-steroidal anti-inflammatory drug naproxen. *J. Biol. Chem.* 285, 34950–34959.
- Gadegoni, H., Manda, S., Rangu, S., 2013. Synthesis and screening of some novel 2-[5-(substituted phenyl)-[1,3,4]oxadiazol-2-yl]-benzoxazoles as potential antimicrobial agents. *J. Korean Chem. Soc.* 57, 221–226.
- Gokhan, N., Erdogan, H., Tel, B., Demirdamar, R., 1996. Analgesic and antiinflammatory activity screening of 6-acyl-3-piperazinomethyl-2-benzoxazolinone derivatives. *Eur. J. Med. Chem.* 31, 625–628.
- Kim, B.J., Kim, J., Kim, Y.K., Choi, S.Y., Choo, H.Y.P., 2010. Synthesis of benzoxazole amides as novel antifungal agents against *Malassezia furfur*. *Bull. Korean Chem. Soc.* 31, 1270–1275.
- Morris, G.M., Goodsell, D.S., Halliday, R.S., Huey, R., Hart, W.E., Belew, R.K., Olson, A.J., 1998. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J. Comput. Chem.* 19, 1639–1662.
- Morris, G.M., Huey, R., Lindstrom, W., Sanner, M.F., Belew, R.K., Goodsell, D.S., Olson, A.J., 2009. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J. Comput. Chem.* 30, 2785–2791.

- Paramashivappa, R., Phani Kumar, P., Subba Rao, P.V., Srinivasa Rao, A., 2003. Design, synthesis and biological evaluation of benzimidazole/benzothiazole and benzoxazole derivatives as cyclooxygenase inhibitors. *Bioorg. Med. Chem. Lett.* 13, 657–660.
- Pilli, H.G., Özkanlı, F., Şafak, C., Erdoğan, H., Ünlü, S., Gümüsel, B., Demirdamar, R., 1994. 6-Acyl-2-benzoxazolinone-3-yl-acetamide and aceto-nitrile derivatives with analgesic activities. *Die Pharmazie* 49, 63–64.
- Raghavendra, N.M., Jyothsna, A., Venkateswara Rao, A., Subrahmanyam, C.V.S., 2011. Synthesis, pharmacological evaluation and docking studies of N-(benzo[d]thiazol-2-yl)-2-(piperazin-1-yl)-acetamide analogs as COX-2 inhibitors. *Bioorg. Med. Chem. Lett.* 22, 820–823.
- Raghavendra, N.M., Ramakrishna, K., Sirisha, V., Divya, P., Venkateswara Rao, A., 2013. Computer aided discovery of potential anti-inflammatory (s)-naproxen analogs as COX-2 inhibitors. *Med. Chem.* 9, 553–559.
- Schuttelkopf, A.W., van Aalten, D.M.F., 2004. PRODRG: a tool for high-throughput crystallography of protein–ligand complexes. *Acta Crystallogr. D Biol. Crystallogr.* 60, 1355.
- Seth, K., Garg, S.K., Kumar, R., Purohit, P., Meena, V.S., Goyal, R., Banerjee, U.C., Chakraborti, A.K., 2014. 2-(2-Arylphenyl)benzoxazole as a novel anti-inflammatory scaffold: synthesis and biological evaluation. *ACS Med. Chem. Lett.* 5, 512–516.
- Sondhi, S.M., Singh, N., Kumar, A., Lozach, O., Meijer, L., 2006. Synthesis, anti-inflammatory, analgesic and kinase (CDK-1, CDK-5 and GSK-3) inhibition activity evaluation of benzimidazole/benzoxazole derivatives and some Schiff's bases. *Bioorg. Med. Chem.* 14, 3758–3765.
- Vinsova, J., Cermakova, K., Tomeckova, A., Ceckova, M., Jampilek, J., Cermak, P., Kunes, J., Dolezal, M., Staud, F., 2006. Synthesis and antimicrobial evaluation of new 2-substituted 5,7-di-tert-butylbenzoxazoles. *Bioorg. Med. Chem.* 14, 5850–5865.
- Winter, C.A., Risely, E.A., Nuss, G.W., 1962. Carrageenin-induced edema in hind paw of the rat an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med.* 111, 544–547.
- Yildiz-Oren, I., Tekiner-Gulbas, B., Yalcin, I., Temiz-Arpaci, O., Ak-Sener, E., Altanlar, N., 2004. Synthesis and antimicrobial activity of new 2-[p-substituted-benzyl]-5-[substituted-carbonylamino]benzoxazoles. *Arch. Pharm.* 337, 402–410.