Journal of Molecular Structure 1200 (2020) 127092

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

Microwave assisted amberlite-IRA-402 (OH) ion exchange resin catalyzed synthesis of new benzoxazole scaffolds derived from antiinflammatory drugs aceclofenac and mefenamic acid as potential therapeutic agents for inflammation

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ARTICLE INFO

Article history:
Received 27 May 2019
Received in revised form
15 September 2019
Accepted 17 September 2019
Available online 20 September 2019

Keywords:
Benzoxazole
Amberlite-IRA-402 (OH)
Anti-inflammatory
COX-2
Antioxidant

ABSTRACT

An efficient microwave assisted synthesis of 2-substituted benzoxazole derivatives from antiinflammatory drugs aceclofenac and mefenamic acid using amberlite-IRA-402 (OH) ion exchange resin as a base catalyst were reported. The synthesized compounds were purified and characterized by ^{1}H NMR, ^{13}C NMR and Mass spectroscopy. *In silico* molecular docking studies were carried out to predict the binding affinity of the synthesized benzoxazole derivatives with COX-2 protein. Molecular Docking analysis showed that compounds **4** and **7** possess excellent binding affinity towards COX-2 with a docking score of −11.6 and −10.4 kcaL/mol respectively. The results obtained from *in vitro* antiinflammatory studies towards membrane stabilization and proteinase inhibitory activities showed that the synthesized benzoxazole compounds **4** and **7** possess better efficacy when compared to that of standards aceclofenac and etodolac with a percentage inhibition of 74.22 ± 0.15, 70.64 ± 0.24 for membrane stabilization and 75.19 ± 0.12, 71.80 ± 0.49 for proteinase enzyme assay at 100 μmoL/L. The synthesized compounds **4** and **7** were also evaluated for antioxidant activity which showed good inhibition (70.16 ± 0.31 and 68.25 ± 0.49) at 100 μmoL/L which was on Par to that of standard ascorbic acid.

1. Introduction

Inflammation due to exposure of tissues and cells to harmful stimuli initiates the release of chemicals from tissues, migrating cells and so on results in inflammatory diseases and may leads in neurodegenerative disease or may progress into cancer [1,2]. In recent years chronic inflammatory diseases represents one of the most significant causes of death throughout the world. The prevalence of diseases like heart disorders, cancer, stroke, chronic respiratory diseases, obesity and diabetes associated with chronic inflammation is estimated to rise in the next 25 years [Fig. 1]. Sustained vigorous inflammation can sometimes lead to cellular damage or hyperplasia which subsequently leads to the overproduction of reactive oxygen species (ROS) from inflammatory cells [3–5]. The rate of ROS induced DNA damage is more during chronic inflammation and this process substantially causes depletion of cellular antioxidants. Currently used non-steroidal anti-inflammatory drugs (NSAIDS) have the risk of acute myocardial infarction and other serious coronary heart diseases [6]. Though numerous effective anti-inflammatory agents are available in market it is still the challenge of the chemist to develop more effective and less toxic agents to treat the signs and symptoms of acute inflammation, long-term consequences of chronic inflammatory diseases and effective in the prevention of free radical-mediated damage [7,8].

Over the past decades benzoxazole derivatives have gained a lot of significance because of their versatile applications as intermediates for the design and synthesis of new pharmacologically active compounds [9–11]. Benzoxazoles are the structural isosteres of nucleic bases adenine, guanine which allow them to interact easily with the biological receptors. A slight change in the substitution pattern of benzoxazole nucleus causes distinguishable

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https://doi.org/10.1016/j.molstruc.2019.127092
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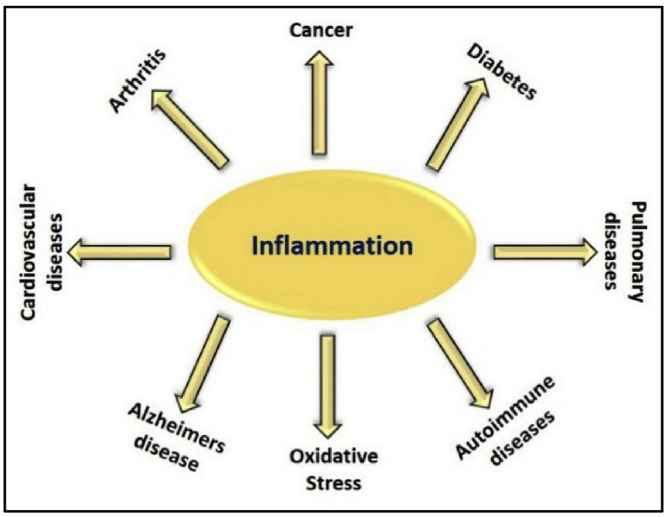


Fig. 1. Inflammation and its associated diseases.

difference in their pharmacological activities [12]. Recent studies clearly reveal the biological importance of benzoxazoles and its related analogues like antiinflammatory [13–15], anti-cancer [16], diuretic [17], herbicidal [18], hypoglycemic [19], anti-histaminic [20], antiviral [21], antimicrobial [22], antiallergic [23], anti-convulsants [24], elastase inhibitors [25], antitubercular agents [26], antifungal [27], 5-HT3 receptor agonists [28], melatonin receptor agonists [29], amyloid fibril inhibitors [30] and Rho kinase inhibitors [31]. These examples highlights the level of interest in the synthetic approaches of new benzoxazole derivatives and have prompted many researchers to explore the potential applicability of this important pharmacophoric scaffold.

Among the widely used therapeutic drugs, NSAIDS are considered as significant agents primarily used for the treatment of inflammation and arthritis. Antiinflammatory drugs presently available for the treatment of various inflammatory disorders have more adverse or undesirable side effects [32]. In recent years therapeutically active substitutions have been incorporated in benzoxazole scaffold to yield antiinflammatory drug candidates and these hybrid molecules displays significant pharmacological and biological activity [Fig. 2] [33–36]. The high therapeutic efficacy of benzoxazole related drugs have encouraged medicinal chemists to synthesize large varieties of novel anti-inflammatory agents [37]. The incorporation of benzoxazole nucleus is an important strategy in drug discovery research [38]. This class of molecules has broadened the scope in remedying various dispositions in clinical medicine. In the present work new potential therapeutic benzoxazole scaffolds were synthesized by incorporating two antiinflammatory drugs aceclofenac and mefenamic acid under microwave irradiation in the presence of amberlite-IRA-402 (OH) ion exchange resin as a base cum catalyst [39].

2. Experimental

2.1. Materials and method

Reagents and solvents were sourced commercially and used without additional purification. Thin layered chromatography (TLC) was carried out on preparative plates of silica gel. Visualization was made using iodine chamber. Purification of the synthesized compounds were performed by column chromatography using silica gel (100–200 mesh). Melting points were measured on Elchem Microprocessor based DT apparatus using an open capillary tube and are corrected with standard benzoic acid. The NMR spectra were recorded on a Bruker Avance III–400 MHz spectrometer using TMS as internal standard (chemical shifts δ in ppm). Mass was recorded on Finnigan Mat 8230 Mass Spectrometer.

The synthetic strategy leading to the target compounds **4** and **7** from aceclofenac and mefenamic acid bulk drugs is illustrated in Scheme: 1-2. The compounds 2-((2-(benzo[*d*]oxazol-2-yl) phenyl) amino)-2-oxoethyl 2-(2-((2,6-dichlorophenyl)amino)phenyl)acetate **4** and N-(2-(benzo [*d*]oxazol-2-yl)phenyl)-2-((2,3-dimethylphenyl)amino)benzamide **7** were prepared by dissolving aceclofenac **1** and mefenamic acid **5** in THF and the corresponding acid chloride formation was carried out in a microwave by using thionyl chloride followed by the reaction of 2-(benzo [*d*] oxazol-2-yl)aniline **3** in the presence of amberlite-IRA-402 (OH) ion exchange resin as a base catalyst.

2.2. General procedure for the synthesis of benzoxazole scaffolds 4 and 7

Synthesis of 2-((2-(benzo[*d*]oxazol-2-yl) phenyl)amino)-2-oxoethyl 2-(2-((2,6-dichlorophenyl) amino)phenyl)acetate, **4** and N-(2-(benzo [d]oxazol-2-yl)phenyl)-2-((2,3-dimethylphenyl) amino) benzamide, **7**.

A mixture of aceclofenac (6 mmol, 2.12 g) and thionyl chloride (7 mmol, 3.5 mL) was taken in a reaction vessel mixed thoroughly in THF and then irradiated in a microwave oven at 420 W for 90 min afforded 2-chloro-2-oxoethyl 2-(2-((2,6-dichlorophenyl)amino) phenyl)acetate, **2**. Similarly a mixture of mefenamic acid (6 mmol, 1.45 g) and thionyl chloride (7 mmol, 3.14 mL) was taken in a reaction vessel mixed thoroughly in THF and then irradiated in a microwave oven at 340 W for 45 min afforded 2-((2,3-dimethylphenyl)amino)benzoyl chloride, **6**. Completion of the reaction was determined by the stoppage of SO_2 gas. Removal of excess of thionyl chloride was carried out by distillation under reduced pressure. Further a base trap was connected in order to neutralize the excess vapour evolved. To the compounds **2** and **6** (in-situ) 2-(benzo [*d*] oxazol-2-yl)aniline **3** (6 mmol, 1.26 g) and amberlite-IRA-402 (OH) ion exchange resin (20 mg) was added and subjected to microwave oven at a power of 340 W for 2 h. The completion of the reaction was monitored by thin layer chromatography (TLC) using Petroleum ether: EtOAc (3:1). After completion, the catalyst was filtered off and the mixture was diluted with EtOAc, washed with water followed by brine and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the resulting solid was filtered, dried and purified using column chromatography to afford compounds **4** and **7**.

2.3. Spectral data of the synthesized compounds 4 and 7

2-((2-(benzo[d]oxazol-2-yl)*phenyl)amino)-2-oxoethyl 2-(2-((2,6-dichlorophenyl) amino) phenyl) acetate*, **4** light brown solid, 84.2% yield, mp 216–218 °C. IR (KBr pellets, cm^{-1}) υ: 751.22, 790.76, 1123.46, 1191.93, 1277.75, 1356.83, 1608.52, 1672.17, 1756.07, 3315.41. ^{1}H NMR ($CDCl_3$, ppm, 400 MHz) δ: 3.76 (2H, s, CH_2), 4.54 (2H, s, CH_2), 4.99 (1H, s, NH), 6.37–6.39 (1H, d, $J = 8.0$ Hz, CH), 6.45 (1H, s, NH), 6.63–6.67 (1H, t, $J = 8.4$ Hz, CH), 6.78–6.80 (2H, d, $J = 8.4$ Hz, CH), 6.94–6.98 (2H, m, CH), 7.07–7.09 (3H, m, CH), 7.14–7.17 (3H, m, CH), 7.48–7.51 (2H, d, $J = 8.4$ Hz, CH), 7.71–7.75 (1H, m, CH). ^{13}C NMR ($CDCl_3$, ppm, 100 MHz) δ: 34.06, 43.72, 114.79, 124.71, 125.72, 126.02, 127.81, 129.61, 130.06, 130.77, 131.05, 131.95, 132.17, 133.07, 133.42, 134.07, 143.43, 146.60, 156.25, 167.63, 172.46. HRMS [EI^+] Calcd for $C_{29}H_{21}Cl_2N_3O_4$ *m/z* 545.0909 [M^+], found 545.0994.

N-(2-(benzo [d]oxazol-2-yl)*phenyl)-2-((2,3-dimethylphenyl) amino) benzamide*, **7** pale brown solid, 88.4% yield, mp 262–265 °C.

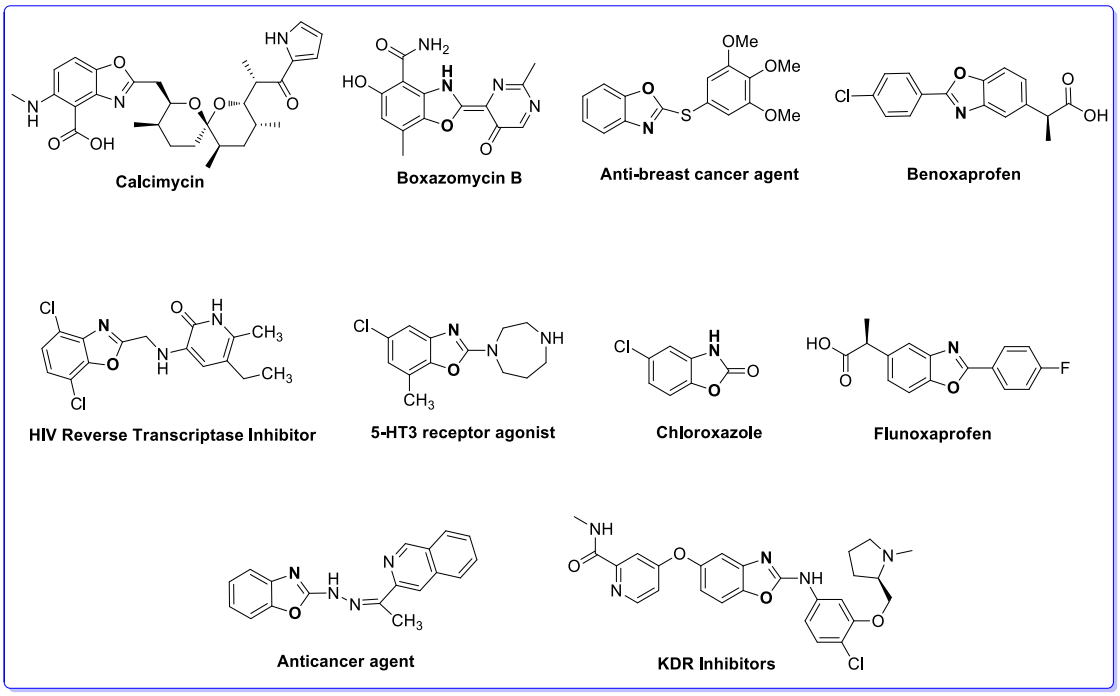
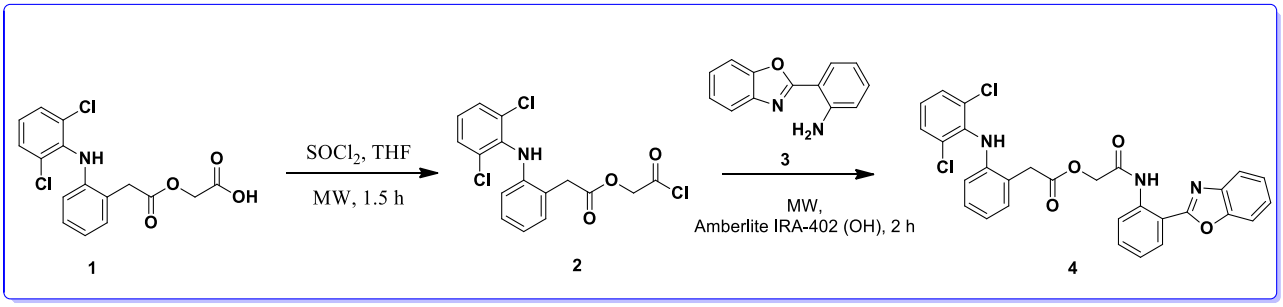


Fig. 2. Various drugs possessing benzoxazole nucleus.



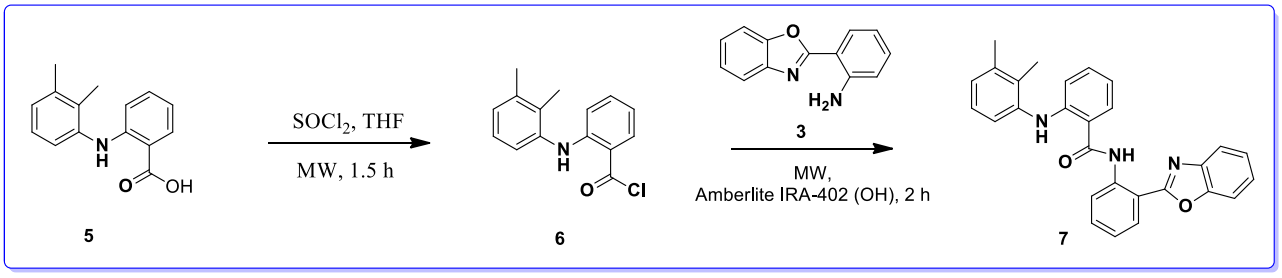
Scheme: 1. Synthesis of 2-((2-(benzo[*d*]oxazol-2-yl)phenyl)amino)-2-oxoethyl 2-(2-((2,6-dichloro phenyl)amino)phenyl)acetate, **4**.

IR (KBr pellets, cm^{-1}) υ: 751.22, 790.76, 1123.46, 1191.93, 1277.75, 1356.83, 1608.52, 1672.17, 1756.07, 3315.41. ^{1}H NMR ($CDCl_3$, ppm, 400 MHz) δ: 2.12 (3H, s, CH_3), 2.34 (3H, s, CH_3), 5.03 (1H, s, NH), 6.45 (1H, s, NH), 6.69–6.70 (2H, d, $J = 5.6$ Hz, CH), 6.94–6.98 (2H, t, $J = 7.2$ Hz, CH), 7.38–7.41 (3H, m, CH), 7.48–7.51 (3H, m, CH), 8.38–8.40 (1H, d, $J = 8.0$ Hz, CH). ^{13}C NMR ($CDCl_3$, ppm, 100 MHz) δ: 17.92, 22.48, 116.35, 122.56, 124.83, 125.31, 126.12, 131.14, 133.48, 134.15, 136.51, 137.67, 141.81, 145.39, 147.20, 151.74, 162.31, 165.15. HRMS [EI$^+$] Calcd for $C_{28}H_{23}N_3O_2$ *m/z* 433.1790 [M^+], found 433.9245.

2.4. Pharmacology

2.4.1. In-vitro anti-inflammatory studies

In the present work *in-vitro* anti-inflammatory studies were carried out by two methods such as membrane stabilization test



Scheme: 2. Synthesis of N-(2-(benzo[*d*]oxazol-2-yl)phenyl)-2-((2,3-dimethylphenyl)amino) benzamide, **7**.

and proteinase inhibitory test as per the reported method [40,41].

2.4.1.1. Membrane stabilization test. Preparation of red blood cells (RBCs) suspension:

After the consent from a healthy volunteer fresh human blood (10 mL) was collected and transferred to heparinized centrifuged tubes. Collected blood was subjected to centrifugation at 3500 rpm for 10 min and washed with equal volume of saline solution for three times. The volume of blood was measured and reconstituted to 10% by using normal saline.

Heat induced hemolysis:

Equal volume of test samples of various concentrations (25, 50 and 100 μmoL/L) and 10% v/v RBC suspension (2 mL) were transferred to centrifuged tubes. In control test tube instead of drug only saline was added. Etodolac was taken as standard drug. All centrifuge tubes were incubated in water bath at 56 °C for 30 min. Reaction mixture was then centrifuged at 2500 rpm for 5 min and absorbance of the supernatant was taken at 560 nm. The experiment was performed in triplicates and the Percent membrane stabilization activity was calculated by:

$$I\%(\text{percentage inhibition}) = [AB - AA/AB] \times 100$$

Where AB = absorption of blank sample

AA = absorption of sample

2.4.1.2. Proteinase inhibitory activity. The reaction mixture (2 mL) containing 0.06 mg trypsin, 1 mL of 20 mM Tris HCl buffer (pH 7.4) and 1 mL test sample of different concentrations was added. Etodolac was taken as standard drug. The mixture was incubated at 37 °C for 5 min and then 1 mL of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 2 mL of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank.

The experiment was performed in triplicate and the percentage inhibition of proteinase inhibitory activity was calculated by:

$$I\%(\text{percentage inhibition}) = [AB - AA/AB] \times 100$$

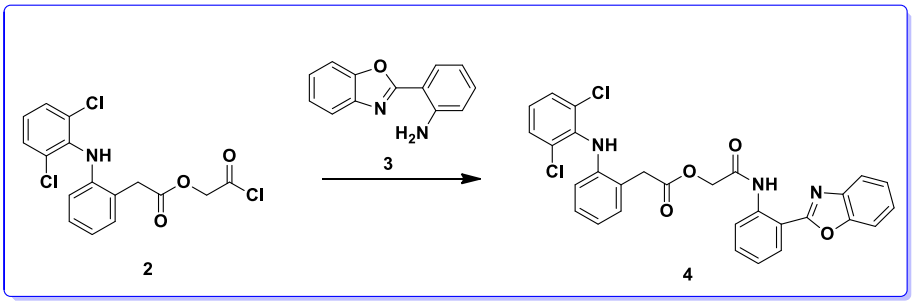
Where AB = absorption of blank sample

AA = absorption of sample

2.4.2. Antioxidant studies

The generation of different reactive oxidant species during inflammation generally stimulates the progress of a state of oxidative stress, with major biological implications. Oxidative stress plays an essential role in the development and perpetuation of inflammation, and thus contributes to the pathophysiology of a number of debilitating illnesses, such as diabetes, cancer, cardiovascular diseases and neurodegenerative processes. Oxidants affect all stages of the inflammatory response, including the release by damaged tissues of molecules acting as endogenous danger signals. So there is a need for antioxidants which effectively terminate these signals by removing free radical intermediates, and inhibit cell death and other oxidation reactions during inflammatory processes. In present study the antioxidant efficacy of benzoxazole derivatives were screened for their free radical scavenging activity

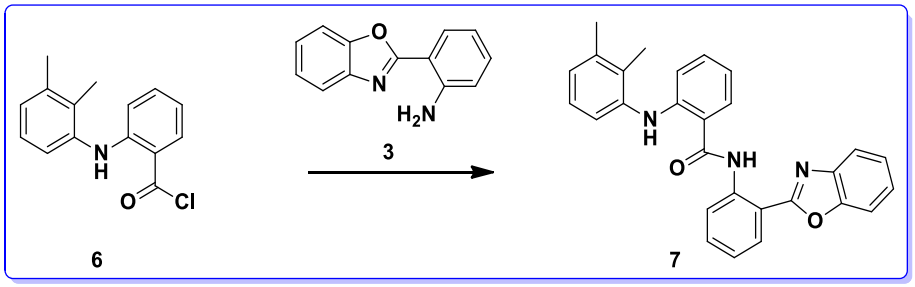
Table: 1
Screening of effective base and solvent combination in the synthesis of 2-((2-(benzo[*d*]oxazol-2-yl)phenyl)amino)-2-oxoethyl2-(2-((2,6-dichlorophenyl)amino)phenyl) acetate, **4** under the presence and absence of microwave irradiation[a].



Entry	Base	Solvent	Conventional		Microwave	
			Time (h)	Yield (%)[b]	Time	Yield (%)[b]
1	Pyridine	DMF	12	41	8 h	55
2	Et_3N	DMF	8	55	4 h	70
3	DIPEA	DMF	14	47	8 h	60
4	Amberlite IRA-402 (OH)	DMF	5	60	3 h	78
5	KOH	DMF	10	45	6 h	62
6	K_2CO_3	DMF	12	52	7 h	68
7	Amberlite IRA-402 (OH)	Acetonitrile	10	59	5 h	72
8	Amberlite IRA-402 (OH)	Toluene	8	45	3 h	65
9	Amberlite IRA-402 (OH)	THF	5	67	2 h	84
10	Amberlite IRA-402 (OH)	DCM	10	58	5 h	65
11	Amberlite IRA-402 (OH)	Ethanol	5	60	2 h	75
12	Amberlite IRA-402 (OH)	Acetone	8	55	3 h	70

[a] Reaction conditions: Compound **2** - 1 mmol, Compound **3** - 1 mmol; Base: 1.5 mmol; Amberlite IRA-402 (OH): 50 mg.
[b] isolated yield.

Table 2
Screening of effective base and solvent combination in the synthesis of N-(2-(benzo [d]oxazol-2-yl)phenyl)-2-((2,3-dimethylphenyl) amino) benzamide, 7 under the presence and absence of microwave irradiation[a].



Entry	Base	Solvent	Conventional		Microwave	
			Time (h)	Yield (%)[b]	Time	Yield (%)[b]
1	Pyridine	DMF	12	45	8 h	66
2	Et_3N	DMF	8	68	4 h	78
3	DIPEA	DMF	14	52	8 h	67
4	Amberlite IRA-402 (OH)	DMF	5	64	3 h	75
4	KOH	DMF	10	55	6 h	69
5	K_2CO_3	DMF	12	62	7 h	70
6	Amberlite IRA-402 (OH)	Acetonitrile	10	65	5 h	78
7	Amberlite IRA-402 (OH)	Toluene	8	48	3 h	75
8	Amberlite IRA-402 (OH)	THF	5	70	2 h	88
9	Amberlite IRA-402 (OH)	DCM	10	64	5 h	68
10	Amberlite IRA-402 (OH)	Ethanol	5	67	3 h	78
11	Amberlite IRA-402 (OH)	Acetone	8	61	3 h	74

[a] Reaction conditions: Compound 6 - 1 mmol, Compound 3 - 1 mmol; Base: 1.5 mmol; Amberlite IRA-402 (OH): 50 mg.
[b] Isolated yield.

by using the DPPH radical assay as per the reported method [42].

2.4.2.1. DPPH radical scavenging assay. Free radical scavenging is one of the familiar mechanisms through which antioxidants inhibits oxidation. In the present study, the radical scavenging ability of all the synthesized compounds was determined spectrophotometrically using the stable DPPH radical scavenging method. DPPH assay is a quite simple and standard method for evaluating antioxidant activity. One of the best advantages of this assay is the commercial availability of DPPH radical, so there is no need of it to be generated in advance like in other assays. Compound solutions were prepared by dissolving an appropriate amount of each compound in ethanol. The solution of DPPH in ethanol was prepared just before UV measurements. 3 mL of sample and 1 mL of DPPH solutions were mixed and kept in the dark for 30 min at room temperature and then the decrease in absorption was measured at 517 nm. For the control absorption blank sample containing the same amount of ethanol and DPPH solution was measured. Ascorbic acid was used as standard. The experiment was carried out in triplicate. Radical scavenging activity was calculated by following formula:

$$I\%(\text{percentage inhibition}) = [AB - AA/AB] \times 100$$

Where AB = absorption of blank sample

AA = absorption of sample

2.4.3. In silico molecular docking studies

The docking analysis of the synthesized benzoxazole derivatives **4**, **7** along with standards etodolac, aceclofenac and mefenamic acid were investigated using Autodock tools (ADT) v1.5.4 and Autodock v4.2 program. The COX-2 protein (ID-5IKR) in complex with mefenamic acid was taken from protein data bank. To run the docking process, searching grid map extended over the selected COX-2 protein was used and polar hydrogens were added to the ligand moieties. Kollman charges were allocated and atomic solvation parameters were added. Polar hydrogen charges of the Gasteiger-type were assigned and the non-polar hydrogens were merged with the carbons and the internal degrees of freedom and torsions were set. In the present version COX-2 protein was kept rigid, and ligand allowed to move freely. The rototranstional area in to which the ligand is allowed to move freely around the active

Table 3
Optimization of the amount of catalyst in the synthesis of in the synthesis of 2-((2-(benzo[*d*]oxazol-2-yl)phenyl)amino)-2-oxoethyl2-(2-((2,6-dichlorophenyl)amino) phenyl) acetate, 4 and N-(2-(benzo [d]oxazol-2-yl)phenyl)-2-((2,3-dimethylphenyl) amino) benzamide, 7 under the presence of microwave irradiation.

Entry	Catalyst (mg)	Compound 4		Compound 7	
		Time (h)	Yield (%)[b]	Time	Yield (%)[b]
1	5.0	4.0	65	4.0	68
2	10.0	3.5	73	3.5	76
3	15.0	3.0	79	3.0	82
4	20.0	2.0	84	2.0	88
5	25.0	2.0	84	2.0	88
6	30.0	2.0	84	2.0	88
7	50.0	2.0	84	2.0	88
8	No catalyst	18.0	15	17.0	18

[a]Reaction conditions: Compound 2, 6 - 1 mmol, Compound 3 - 1 mmol; THF; Microwave-340 W.
[b] Isolated yield.

Table 4
In silico molecular docking results for antiinflammatory efficacy of synthesized benzoxazole derivatives and standards.

Entry	Ligand	Autodock score	Lipophilicity	Hydrophobicity
1	4	−11.6	−3.6	−1.4
2	7	−10.4	−3.0	−0.5
3	Std[a]	−8.9	−3.3	−0.8
4	Std[b]	−10.2	−3.5	−0.6
5	Reference ligand (Std[c])	−9.2	−2.9	−0.5

[a] Etodolac.
[b] Aceclofenac.
[c] Mefenamic acid.

sphere is restricted to 10 Å. Affinity maps for the entire atom types present, as well as an electrostatic map was computed with a grid spacing of 0.375 A°. For each ligand, a docking experiment consisting of 100 simulations was performed and the analysis was based on binding free energies and root mean square deviation (RMSD) values and the ligand molecules were then ranked in the order of increasing docking energies. The analysis of hydrophobic and lipophilic interaction of ligands towards COX-2 protein was carried by using ALOGPS 2.1 and Biovia discovery studio visualizer 4.5.

3. Results and discussion

3.1. Synthesis

The synthesis of 2-((2-(benzo[*d*]oxazol-2-yl)phenyl)amino)-2-oxoethyl-2-(2-((2,6-dichloro phenyl)amino)phenyl)acetate, **4** and N-(2-(benzo [*d*]oxazol-2-yl)phenyl)-2-((2,3-dimethylphenyl) amino) benzamide, **7** as depicted in scheme **1-2** is hitherto unreported in literature. Synthesis of compounds **4** and **7** was initially carried out by conventional method through heating on a water bath for 7 h but the conversion of the desired product was less. On

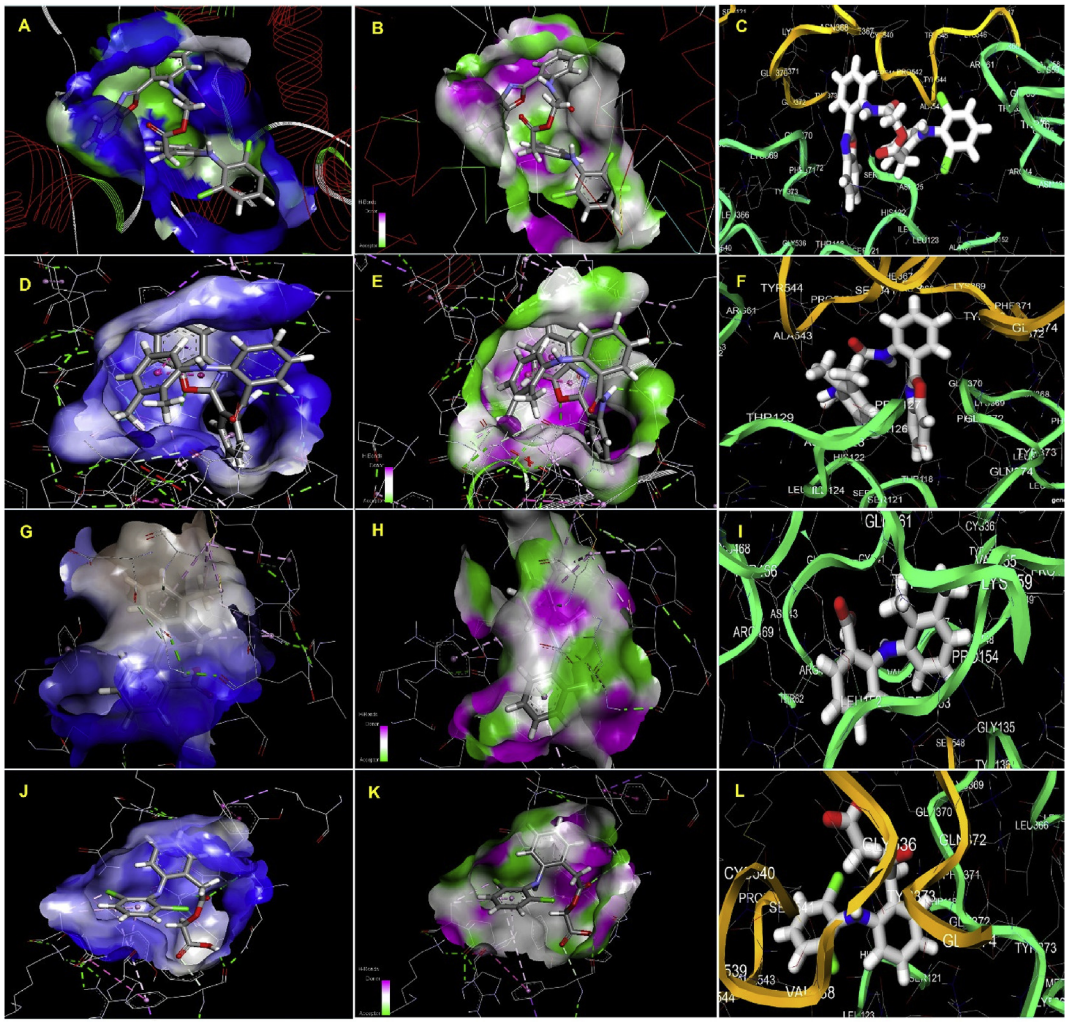


Fig. 3. In silico molecular docking studies of benzoxazole derivatives as COX-2 inhibitors. [A-C] The binding interactions of 2-((2-(benzo[*d*]oxazol-2-yl)phenyl)amino)-2-oxoethyl2-(2-((2,6-dichlorophenyl)amino)phenyl)acetate, 4 [D-F] The binding interactions of N-(2-(benzo[*d*]oxazol-2-yl)phenyl)-2-((2,3-di methyl phenyl) amino) benzamide, 7 [G-I] The binding interaction of standard mefanamic acid [J-L] The binding interaction of aceclofenac.

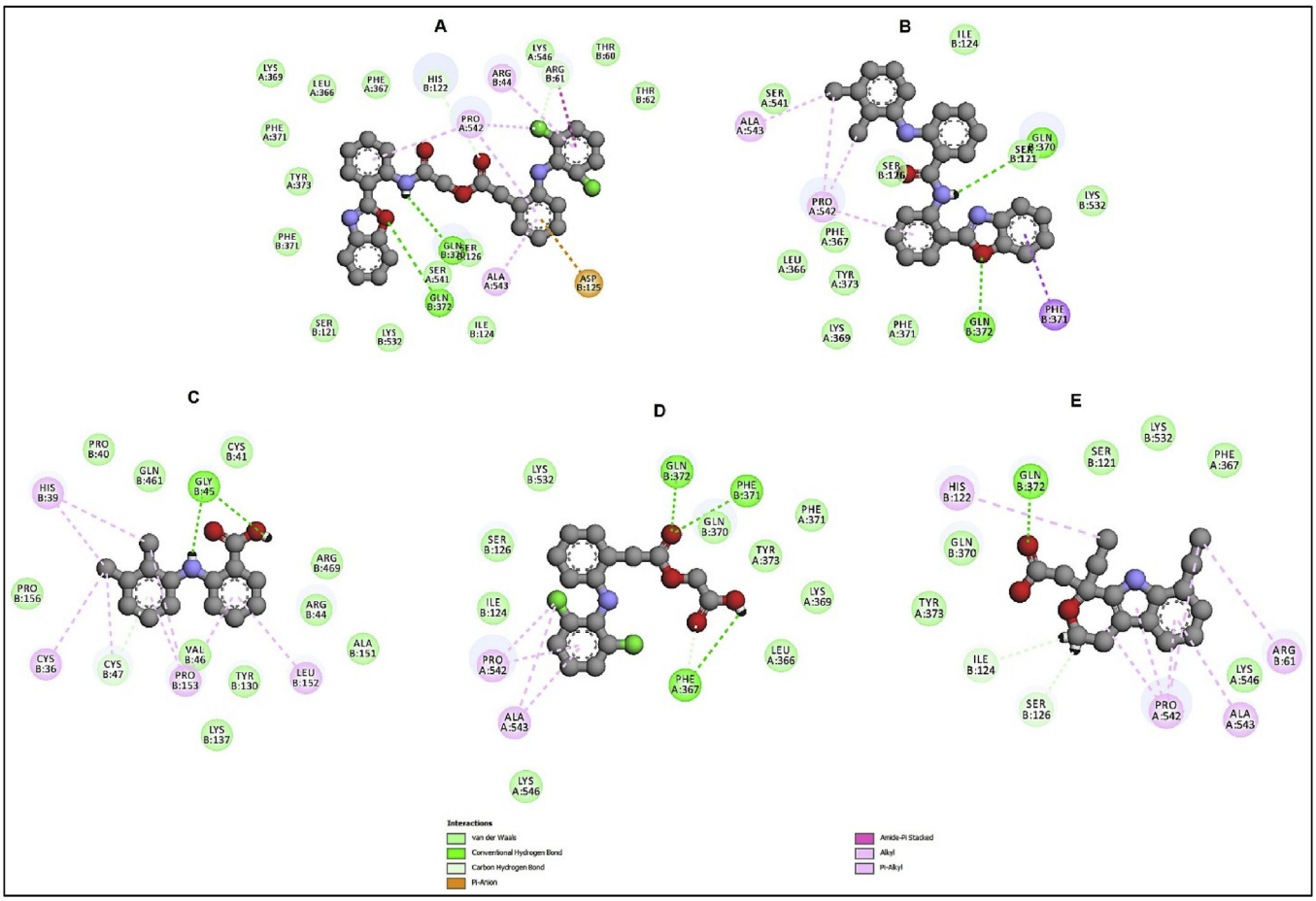


Fig. 4. Various interactions of synthesized benzoxazole derivatives 4, 7 and standards with COX-2 amino acids. [A] 2-((2-(benzo[*d*]oxazol-2-yl)phenyl)amino)-2-oxoethyl2-(2-((2,6-dichloro phenyl)amino)phenyl)acetate, 4 [B] N-(2-(benzo[*d*]oxazol-2-yl)phenyl)-2-((2,3-di methyl phenyl) amino) benzamide, 7 [C] Standard mefanamic acid [D] Standard aceclofenac [E] Standard etodolac.

the other hand when the same reaction was carried out in a microwave at an appropriate time leads to the formation of the products **4** and **7** with high yield and purity. The formation of the product **4** as confirmed from ^{1}H NMR showed 16 protons in the aromatic region and the peak at 6.45 δ corresponds to amide −NH group whereas the singlet appears at 4.99 δ corresponds to −NH group of the aceclofenac. The appearance of two singlets at 3.76 and 4.54 δ respectively related to two methylene groups of the aceclofenac. The change in the position of chemical shift towards deshielding region 4.54 δ for the CH_2 group was mainly attributed to the presence of neighboring electronegative oxygen atom. The ^{13}C NMR spectra of compound **4** shows 30 carbons in which the peaks appears at 172.46 and 167.63 ppm corresponds to C═O groups. The appearance of peak at 156.25 ppm related to the C−Cl group of the aceclofenac and the peaks at 34.06, 43.72 ppm corresponds to the methylene groups of the corresponding product **4**. Furthermore, the product **4** was confirmed by HRMS, which gave the molecular ion peak at *m*/*z* 545.0909 which matches with the molecular formula $C_{29}H_{21}Cl_2N_3O_4$ (see, Figs. S1−S3 in supporting information).

Similarly the formation of the product **7** as confirmed from the ^{1}H NMR showed 16 protons in the aromatic region and the peak at 6.45 δ corresponds to amide −NH group whereas the singlet appears at 5.03 δ corresponds to −NH group of the mefenamic acid. The appearance of two singlets at 2.12 and 2.34 δ respectively related to two methyl groups of the mefenamic acid. The ^{13}C NMR spectra of compound **7** shows 28 carbons in which the peaks appears at 165.15 corresponds to C═O group. The appearance of peaks at 22.48, 17.92 ppm corresponds to the methyl groups of the corresponding product **7** and the signals at 116.15−151.74 ppm corresponds to aromatic carbons. Furthermore, the product **7** was confirmed by HRMS, which gave the molecular ion peak at *m*/*z* 433.1790 which matches with the molecular formula $C_{28}H_{23}N_3O_2$ (see, Figs. S4−S6 in supporting information).

3.2. Optimization of the reaction conditions

To afford increased selectivity and improvise yields, microwave

Table 5
Membrane stabilizing inhibitory activity of benzoxazole derivatives and standards.

S.No	Compound	Concentration (μmol/L)		
		25	50	100
1.	4	25.78 ± 0.69	39.26 ± 0.72	74.22 ± 0.15
2.	7	22.36 ± 0.14	35.17 ± 0.41	70.64 ± 0.24
3.	Std[a]	21.28 ± 0.20	32.64 ± 0.22	65.42 ± 0.36
4.	Std[b]	24.92 ± 0.75	37.63 ± 0.15	72.18 ± 0.39
5.	Std[c]	20.86 ± 0.11	31.48 ± 0.52	58.79 ± 0.45

[a] Etodolac.
[b] Aceclofenac.
[c] Mefenamic acid.

Table 6
Proteinase inhibitory efficacy of benzoxazole derivatives and standards.

S.No	Compound	Concentration (μmol/L)		
		25	50	100
1.	4	27.64 ± 0.35	40.10 ± 0.55	75.19 ± 0.12
2.	7	23.95 ± 0.84	36.04 ± 0.19	71.80 ± 0.49
3.	Std[a]	22.87 ± 0.25	34.55 ± 0.76	69.04 ± 0.51
4.	Std[b]	26.14 ± 0.98	39.76 ± 0.10	72.48 ± 0.55
5.	Std[c]	21.90 ± 0.66	32.50 ± 0.17	67.02 ± 0.48

[a] Etodolac.
[b] Aceclofenac.
[c] Mefenamic acid.

irradiation has been established as an important technique. To find appropriate reaction medium, various bases like Pyridine, Et_3N, KOH, DIPEA and K_2CO_3 were employed along with amberlite IRA-402 (OH) in the synthesis of compounds **4** and **7** by using DMF as a solvent. The reactions proceeded in the presence of all the bases with yields ranging from 55 to 78% and the maximum yield of 78% was observed with amberlite IRA-402 (OH)-DMF combination. With amberlite IRA-402 (OH) as a base, the reactions were also performed in different solvents like toluene, acetonitrile, dichloromethane, tetrahydrofuran, ethanol and acetone (Tables 1 and 2). The dielectric constant of solvent molecules plays a major role in the distribution of charges during the course of the reaction. It generally measures the solvent ability to minimize strength of the electric field associated with corresponding charged particle. Polar solvents with high dielectric constant tend to donate hydrogens more effectively and forms hydrogen bonding with the reactants [43]. After screening different solvents and bases highest yield of 2-((2-(benzo[*d*]oxazol-2-yl) phenyl) amino)-2-oxoethyl 2-(2-((2,6-dichlorophenyl) amino)phenyl)acetate, 4 (84%) and N-(2-(benzo[d]oxazol-2-yl)phenyl)-2-((2,3-dimethylphenyl) amino) benzamide, 7 (88%) was obtained with amberlite IRA-402 (OH) as a base and tetrahydrofuran as a solvent.

In a pursuit to increase the yields, efforts were done to optimize catalyst loading in the reaction under microwave irradiation at 340 W. Results obtained clearly indicated that increase in the concentration of the catalyst loaded the yield in the formation of the product increased gradually and maximum yield was obtained when 20 mg of the catalyst used (Table 3). Moreover, further increase in the amount of catalyst failed to increase the yield; therefore, 20 mg of amberlite IRA-402 (OH) was found to be the optimal quantity for this reaction under the present experimental conditions.

3.3. Reusability of the catalyst

The recovery and reusability of amberlite-IRA-402 (OH) catalyst are one of the important advantages which make it more suitable for commercial applications. In this perspective, reusability and recovery efficiency were examined in the synthesis of compound **4** and **7** under optimized conditions. After completion of the reaction the catalyst was recovered by filtration, washed with hot ethanol thoroughly, dried at 90 °C for 5 h and reused as such for subsequent model reaction under similar reaction conditions. Recovered amberlite-IRA-402 (OH) exhibited good catalytic activity even up to four cycles without any change in reaction time and only a minimal decrease in the yield was observed which could be attributed to the loss of catalyst during recycling.

3.4. Molecular docking simulations

The results obtained from molecular docking analysis of compounds 4 and 7 along with standards showed that compound 4 and 7 possess good binding interaction towards COX-2 protein with binding energy of −11.6 and −10.4 k cal/mol which was greater than standard etodolac (−8.9 k cal/mol), aceclofenac (−10.2 k cal/mol) and mefanamic acid (−9.2 k cal/mol) (Table 4 and Fig. 3). The binding interactions of ligands 4, 7 and standards with various amino acids of COX-2 protein was illustrated using Biovia discovery studio visualizer 4.5. (Fig. 4). The effective binding affinities of compound 4 and 7 was mainly attributed due to the synergistic efficacy of both benzoxazole and antiinflammatory drugs (Aceclofenac and Mefenemic acid) which further increases the hydrophobic and lipophilic interactions of the ligands. The hydrophobic interaction is very important interaction in the binding site because most interaction in the binding site are hydrophobic even less stronger than hydrogen bonding and ionic interaction. Increase in lipophilic nature makes the molecule more polarized and accordingly the London dispersion forces increases which are quite necessary for the lipophilic substances to interact within

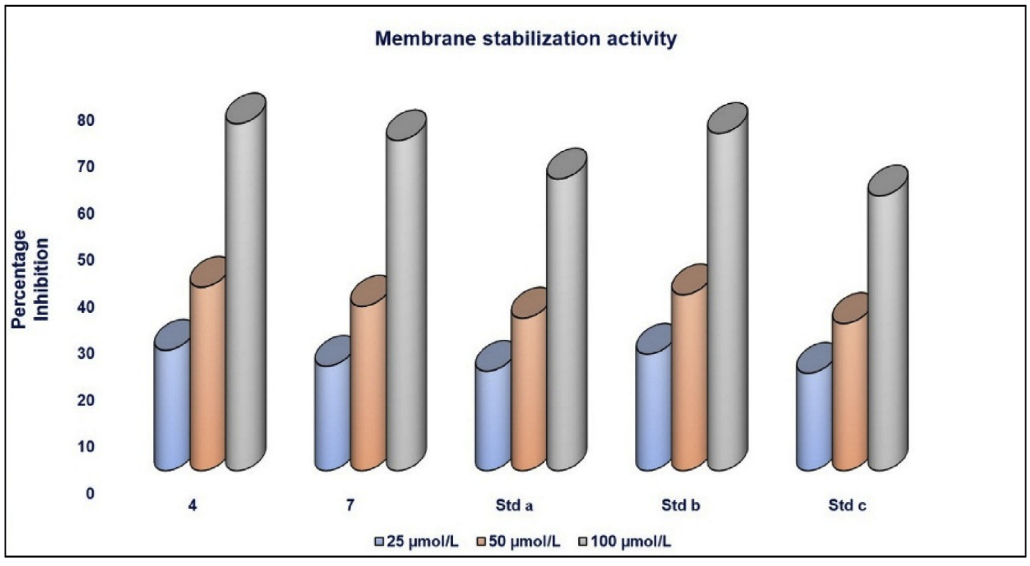


Fig. 5. Membrane stabilization efficacy of synthesized benzoxazole derivatives 4, 7 and standards.

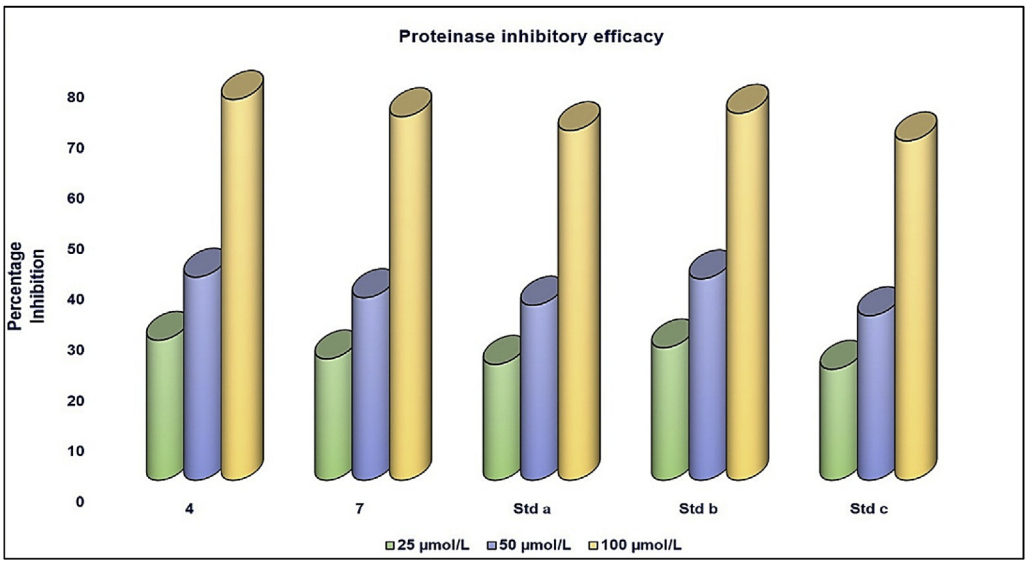


Fig. 6. Proteinase inhibitory efficacy of synthesized benzoxazole derivatives 4, 7 and standards.

themselves and other amino acid residues in the receptor.

3.5. Pharmacology

The results obtained from *in vitro* antiinflammatory activity showed that the synthesized benzoxazole derivatives 4 and 7 showed good efficacy with percentage inhibition of 74.22 ± 0.15, 70.64 ± 0.24 for membrane stabilization and 75.19 ± 0.12, 71.80 ± 0.49 for proteinase enzyme assay at a concentration of 100 µmoL/L which was on par to that of standard aceclofenac and

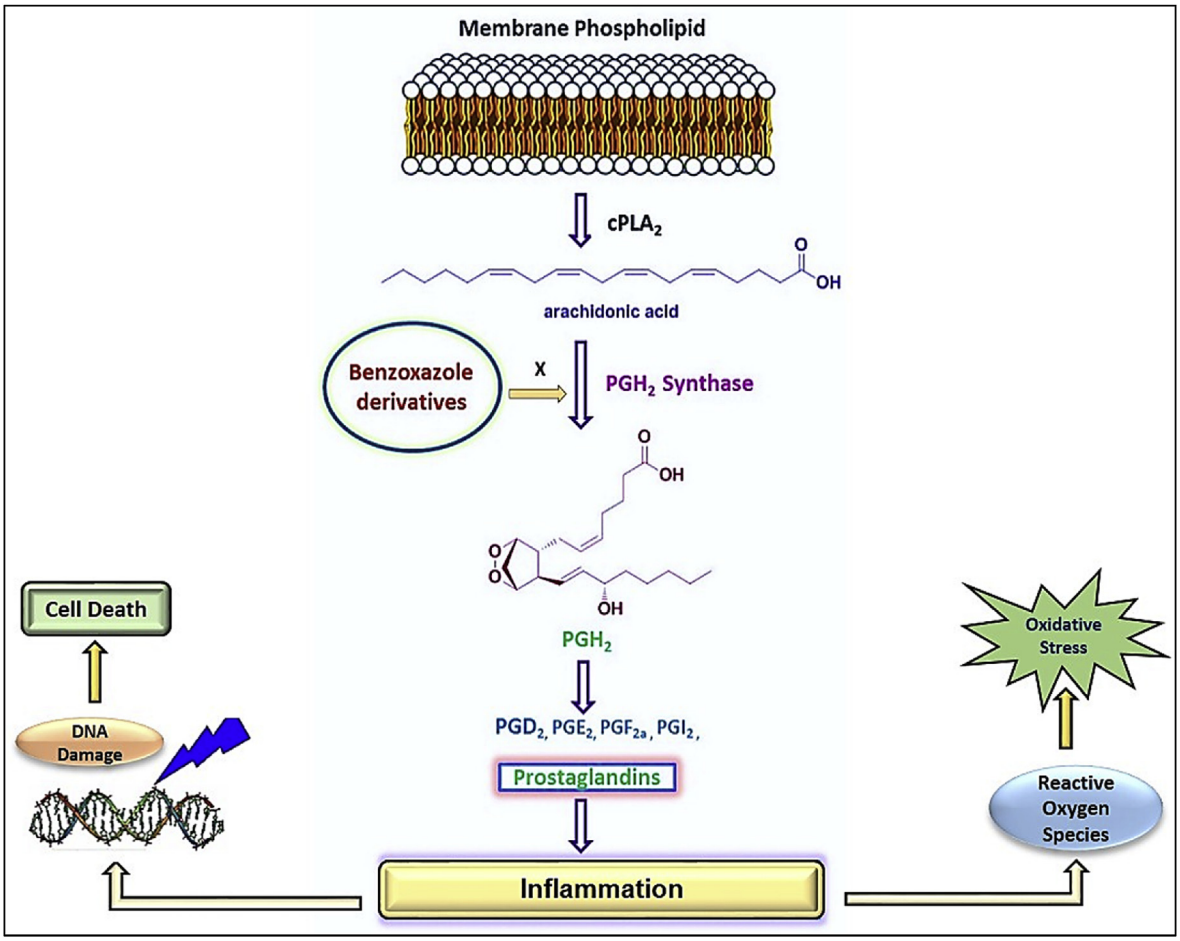


Fig. 7. Plausible antiinflammatory mechanism of synthesized benzoxazole derivatives.

Table 7
DPPH radical scavenging efficacy of benzoxazole derivatives and standard.

Compound	Percentage Inhibition			
	Concentration (μmol/L)			
	10	50	100	IC_{50}
4	12.27 ± 0.29	34.29 ± 0.10	70.16 ± 0.31	1.88
7	10.64 ± 0.15	32.55 ± 0.72	68.25 ± 0.49	1.91
Std[a]	16.65 ± 1.29	40.20 ± 0.33	79.05 ± 0.14	1.72

[a] Ascorbic acid.

greater than that of standard etodolac and mefenamic acid (Tables 5 and 6 and Figs. 5 and 6). Benzoxazole derivatives shows antiinflammatory efficacy mainly by protecting the human erythrocyte membrane against lysis induced by heat. Injury caused to human erythrocyte membrane will render the cell more susceptible to secondary damage through free radical induced lipid peroxidation. From the results obtained from molecular docking simulations it is clear that the synthesized benzoxazole compounds 4 and 7 acts as antagonists against COX-2 protein thereby prevents the synthesis of Prostaglandin H2 synthase which is essential for the release of prostaglandins during the process of inflammation. In addition to this it also reduces oxidative damage by quenching toxic products of fatty acid lipid peroxidation thereby preventing further tissue damage (Fig. 7).

Reduction of DPPH radicals can be observed by decrease in absorbance at 517 nm. The synthesized benzoxazoles derivatives 4 and 7 reduced DPPH radicals significantly. Activity of benzoxazole derivatives were compared with commercial antioxidant Ascorbic acid. The IC_{50} value was calculated for each compound along with standard ascorbic acid as represented in Table 4. The scavenging activity increased with increasing concentrations of the test samples. The IC_{50} values for compounds 4 and 7 were found to be 1.88, 1.91 respectively which were comparable to the IC_{50} value (1.72) of standard Ascorbic acid (Table 7). As per structural and chemical features it is quite obvious that structural variations brings about bioactivity in addition to this it also alters the biological properties of the molecules in regular trend.

4. Conclusion

In conclusion novel benzoxazole derivatives were synthesized and screened pharmacologically for its antioxidant, antiinflammatory studies. The synergistic effect of both benzoxazole and antiinflammatory drugs (aceclofenac and mefenamic acid) significantly enhanced the pharmacological properties of compound 4 and 7. Molecular docking studies clearly revealed the antiinflammatory efficacy of synthesized benzoxazole derivatives and their interaction with COX-2 protein. In this modern era in order to treat the signs and symptoms of oxidative stress during chronic and acute inflammatory diseases there is a need to develop an effective therapeutic drug candidate with less side effects. Hence in the present work novel benzoxazole derivatives were designed and their antiinflammatory and antioxidant efficacy was explored successfully.

Acknowledgements

One of the author Dr.Gangadhara Angajala would like to gratefully acknowledge Kalasalingam Academy of Research and Education (KARE) for their constant encouragement and support and providing all the necessary facilities for carrying out this study. The authors also thank DST-VIT-FIST, SIF-VIT for carrying out NMR analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molstruc.2019.127092.

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