Research Article

Design and Synthesis of Some Novel Oxadiazole Derivatives and Evaluation of *In Vivo* Anti Inflammatory Activity Followed by Molecular Docking against Cox-II Enzyme

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ABSTRACT

Oxadiazole is a versatile heterocyclic nucleus which attracted a wide attention of the medicinal chemists in search for new therapeutic molecules. Out of its possible isomers 1, 3, 4-oxadiazole was widely exploited for various applications as medicinal agents. The literature survey revealed that 1, 3, 4-oxadiazoles were reported to possess a wide range of pharmacological activities. The main aim and objective of the present research work was designed and synthesis of some novel 2, 5-disubstituted 1, 3, 4-oxadiazole derivatives and evaluation of in vivo anti inflammatory activity followed by molecular docking against COX II enzyme. Based on this, a new series of compounds had been planned to synthesize by reacting paraacetamidophenol, ethylchloroacetate, hydrazine monohydrate and various aromatic acids. In continuation of these research work on 2, 5-disubstituted 1, 3, 4-oxadiazole and above observation promoted to synthesize the title compounds AB1-AB8 with their potent biological activity. Molecular docking was performed to find out the binding affinity or molecular interaction energy (kcal/mol) of docked compounds. Lowest (negative value) energy of docked molecule indicated high binding affinity with the target protein. In silico molecular docking studies, the binding energies of the synthesized compounds were found to be AB1: -4.21; AB2: -5.21; AB3: -5.06; AB4: -3.96; AB5: -4.38; AB6: -3.45; AB7: -4.25; AB8: -3.83 (k.cal/ml); standard drug diclofenac sodium: -3.15 (k.cal/ml) which indicated that the compound had high binding affinity towards the target protein cyclooxygenase with PDB id 6COX (COX II). Anti inflammatory activity of each synthesized compound was evaluated by carrageenan induced paw oedema method. The activity was studied at 100 mg/kg body weight and their responses were measured at 30, 60, 120 and 180 minute. The in vivo experimental data displayed that the compound AB2, AB3, AB5 and AB7 possessed very good anti inflammatory activity among the eight synthesized compounds and all the compounds exhibited highest activity at 120 min. The percent protection (%) of the synthesized compounds were found to be AB1: 19.63±0.0294, AB2: 44.19±0.031**, AB3: 42.73±0.0351**, AB4: 19.04±0.0828ns, AB5: 39.53±0.0216*, AB6: 18.91±0.0310, AB7: 35.43±0.0623*, AB8: 18.84±0.0935ns, standard drug diclofenac sodium (DFS): 49.67±0.0095** etc.

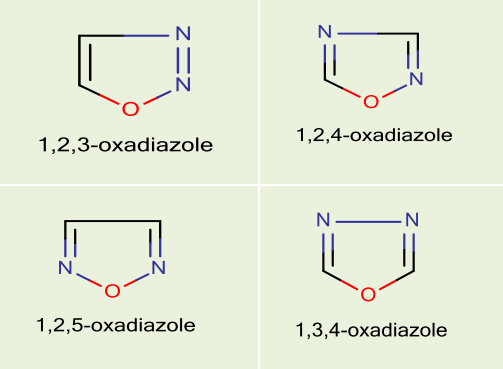
Keywords: Anti inflammatory; molecular docking; target protein; carrageenan; percent protection etc.

INTRODUCTION

Oxadiazoles are a class of heterocyclic aromatic chemical compound of the azole family; with the molecular formula $C_2H_2N_2O$. There are four isomers of oxadiazole depending on the position of nitrogen atom in the ring[1]. In chemistry, methine is a trivalent functional group =CH−, derived formally from methane. It consists of a carbon atom bound by two single bonds and one double bond, where one of the single bonds is to hydrogen. The group is also called methyne or methene; its IUPAC systematic name is methylylidene or methanylylidene.

Oxadiazole is derived from furan by replacement of two methine (-CH=) group by two pyridine type nitrogen (-N=)[2]. 1, 2, 4-Oxadiazole, 1, 2, 5-oxadiazole, and 1, 3, 4-oxadiazole are all known and appear in a variety of pharmaceutical drugs including raltegravir, butalamine, fasiplon, oxolamine, and pleconaril. The 1, 2, 3-isomer is unstable and ring-opens to form the diazoketone tautomer[3].

Table 1: Oxadiazole



Chemistry of oxadiazole

1,3,4-oxadiazole is a five membered heterocyclic aromatic compound containing two nitrogen atom at position three and four and one oxygen atom present at position one. 1,3,4 oxadiazole is thermally stable than other oxadiazoles, these oxadiazole are very important compound in medicinal chemistry due to their biological activities, during last few years. Oxadiazole, a very weak base due to inductive effect of the extra heteroatom. The replace of two -CH= groups in furan by two pyridine type

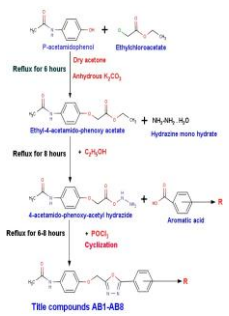
(-N=) lowers aromaticity of resulting oxadiazole ring to an extent that the oxadiazole ring exhibit character of conjugated diene. The electrophillic substitutions in oxadiazole ring are extremely difficult at the carbon atom because, the relatively low electron density on the carbon atom which can be attributed to electron withdrawal effect of the pyridine type nitrogen atom. If oxadiazole ring is substituted with electron-releasing groups, the attack of electrophiles occurs at nitrogen. The ring is generally resistant to nucleophilic attack. Furazan, or 1, 2, 5-oxadiazole, is an heterocyclic aromatic organic compound consisting of a five-atom ring containing 1 oxygen and 2 nitrogen atoms. The furazan ring system is also found in the steroid furazabol. Furazan and its derivatives are obtained from the oxime derivatives of 1, 2-diketones[4].

Biological activity

1, 3, 4-oxadiazoles a class of five member heterocyclic compounds were reported to possess a wide range of biological activities such as antimicrobial[5], anti-inflammatory[6], antifungal[7], anticonvulsant[8], anthelmintics[9], analgesic[10], insecticidal[11], local anesthetic[12], antidiarrheal[13], anticancer[14], hypoglycaemic[15], protease inhibitor-resistant HIV-1[16], vasorelaxant[17] activities.

MATERIALS AND METHOD

Synthetic scheme



Steps involved in the synthesis of target compounds[18]

Chemicals

The solvents and other chemicals which were used for the synthesis and purification of target compounds provided by institutional store and were of LR and AR grade.

Instrumentation

The melting points of the synthesized compounds were determined by open capillary tube method. The IR spectra of the synthesized compounds were recorded by ABB Bomen FT-IR spectrometer MB 104 IR spectra recorded with potassium bromide pellets. The ^{1}H-NMR spectra of synthesized compounds were recorded by using BRUKER NMR spectrometer in DMSO. The Mass spectra of synthesized compounds were recorded by JEOL GCmate. TLC method was used to determine the progress of the reaction. TLC plates are Pre-coated Silica gel (HF254-200 mesh) aluminium plates using ethyl acetate: n-hexane are used as solvent and visualized under UV-chamber. The IR, ^{1}H-NMR and MASS spectra were used to assign the structure of synthesized compounds.

Step 1: Ethyl-4-acetamido phenoxy acetate

A mixture of p-acetamido phenol (0.01 mol) and ethyl chloroacetate (0.01 mol) was refluxed by using dry acetone in presence of anhydrous potassium carbonate (K_2CO_3) for 6hrs. The reaction mixture was cooled and then poured in to crushed ice. The solid product obtained, these product was filtered, dried and recrystallized using ethanol.

Step 2: 4-Acetamido phenoxy acetyl hydrazide

A mixture of ethyl-4-acetamido phenoxy acetate (0.01 mol), hydrazine hydrate (0.01 mol) in ethanol (15 ml) was refluxed for 5-8 hrs. The reaction mixture was cooled and then poured in to crushed ice. The solid product was obtained; this product was filtered, dried and recrystallized from ethanol.

Step 3: 2-(4-Acetamidophenoxy methyl) -5-aryl substituted - 1, 3, 4-oxadiazole

A mixture of 4-Acetamido phenoxy acetyl hydrazide (0.01 mol) and various aromatic acids (0.01 mol) in phosphorus oxychloride (10 ml) was refluxed for 6-8 hours. The completion of the reaction process was monitored by TLC plates. The contents were cooled and poured into the crushed ice and then neutralized the reaction mixture with sodium bicarbonate solution and the solid product was obtained, the product was filtered, dried and recrystallized from ethanol[19].

Table 2: Physicochemical properties of synthesized compounds

Sl. No.	Compounds code	M. F	M. Wt	R_f value	m. p	Yield
1.	AB1	$C_{17}H_{16}N_4O_3$	324.33	0.77	116^{0}C	74.5 %.
2.	AB2	$C_{17}H_{13}Cl_2N_3O_3$	378.209	0.74	180^{0}C	69.9%
3.	AB3	$C_{17}H_{14}FN_3O_3$	327.309	0.75	189^{0}C	74%
4.	AB4	$C_{17}H_{14}BrN_3O_3$	388.215	0.65	183^{0}C	69%
5.	AB5	$C_{17}H_{13}BrN_4O_5$	433.213	0.64	166^{0}C	60%
6.	AB6	$C_{17}H_{14}N_4O_5$	354.31	0.72	171^{0}C	64%
7.	AB7	$C_{17}H_{13}N_5O_7$	399.31	0.68	204^{0}C	78%
8.	AB8	$C_{17}H_{13}N_5O_8$	415.31	0.72	215^{0}C	68%

Spectral data of synthesized compounds

Compound AB1

N-(4-{[5-(4-aminophenyl)-1,3,4-oxadiazol-2-yl]methoxy}phenyl) acetamide. IR (KBr) ν (cm^{-1}): 3393.16 cm^{-1} (Ar-NH), 1633.67 cm^{-1} (C=N), 1575.88 cm^{-1} (C=C), 1069.05 cm^{-1} (-C-O-C-), 3132.54 cm^{-1} (Ar-CH), 1249.43 cm^{-1}(Ar-NH_2), ^{1}H-NMR δ (ppm): 6.45-7.4 (s, 8H, Ar-H), 5.17 (s, 2H,-CH_2), 4.1(s, 2H, -NH_2), 2.05 (s,1H, -CH_3), 8.05 (s, 1H, -NH), Mass (m/e value) % relative abundance: 324.12 (M^+) (5.1), 310.87 (4) , 296.22 (8.25), 282.76 (2.2), 272.38(2.32), 262.6432 (7.3), 248.34 (11), 217.12 (15), 207.14 (7), 116.67 (18), 58.33(B).

Compound AB2

N-(4-{[5-(2,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl]methoxy}phenyl) acetamide.. IR (KBr) ν (cm^{-1}): 3381.92 cm^{-1} (Ar-NH), 1673.42 cm^{-1} (C=N), 1545.03 cm^{-1} (C=C), 1085.04 cm^{-1} (-C-O-C-), 687.47 cm^{-1} (C-Cl), 3115.62 cm^{-1} (Ar-CH), ^{1}H-NMR δ (ppm): 6.6-7.82(s, 8H, Ar-CH), 2.5 (s, 3H, -CH_3), 8.03(s, 1H, -NH), 5.22(s, 2H, -CH_2), Mass (m/e value) % relative abundance: 377.03 (M^+) (2.8), 333.16 (1.5), 325.42 (2.7), 286.43 (2.6), 183.26 (6), 160.62 (7), 140.65 (16), 115.64 (33), 95.53 (B).

Compound AB3

N-(4-{[5-(4-flurophenyl)-1,3,4-oxadiazol-2-yl]methoxy}phenyl) acetamide. IR (KBr) ν (cm^{-1}): 3392.09 cm^{-1} (Ar-NH), 1617.53 cm^{-1} (C=N), 1528.16 cm^{-1} (C=C), 1093.52 cm^{-1} (-C-O-C-), 1371.78 cm^{-1} (C-F), 3114.61 cm^{-1} (Ar-CH), ^{1}H-NMR δ (ppm): 2.21 (s, 1H, -CH_3), 8.09 (s, 1H, -NH), 5.21(s, 1H, -CH_2), 6.7-8.01(m, 8H, Ar-CH), Mass (m/e value) % relative abundance: 327.10 (M^+) (6.3), 310.37 (2.3), 299.57 (3), 282.87 (3.9), 266.22 (5), 249.61 (1.2), 232.72 (4), 104.86 (8.1), 75.50 (B).

Compound AB4

N-(4-{[5-(2-bromophenyl)-1,3,4-oxadiazol-2-yl]methoxy}phenyl) acetamide. IR (KBr) ν (cm^{-1}): 3286.82 cm^{-1} (Ar-NH), 1617.53 cm^{-1} (C=N), 1528.16 cm^{-1} (C=C), 1093.52 cm^{-1} (-C-O-C-), 687.47 cm^{-1} (C-Br), 3114.61 cm^{-1} (Ar-CH), ^{1}H-NMR δ (ppm): 2.21 (s, 1H, -CH_3), 8.09(s, 1H, -NH), 5.21(s, 1H, -CH_2), 6.7-8.01(m, 8H, Ar-CH), Mass (m/e value) % relative abundance: 387.02(M^+) (6.3), 310.37 (2.3), 299.57 (3), 282.87 (3.9), 266.22 (5), 249.61 (1.2), 232.72 (4), 104.86 (8.1), 75.60 (B).

Compound AB5

N-(4-{[5-(2-bromo,4-nitrophenyl)-1,3,4-oxadiazol-2-yl]methoxy}phenyl) acetamide. IR (KBr) ν (cm^{-1}): 3381.95 cm^{-1} (Ar-NH), 1684.44 cm^{-1} (C=N), 1586.2 cm^{-1} (C=C), 1064.25 cm^{-1} (-C-O-C-), 1365.57 cm^{-1}(N=O), 619.89 cm^{-1} (C-Br), 3130.43 cm^{-1} (Ar-CH), ^{1}H-NMR δ (ppm): 6.74-8.36(m, 7H, Ar-CH) ,5.31(s, 2H, -CH_2),2.31 (s, 1H, -CH_3), 8.16(s, 1H, -NH), Mass (m/e value) % relative abundance: 432.00 (M^+) (4), 388.71 (8.1), 362.27 (4.2), 233.28 (5), 217.31 (8.9), 182.52 (5), 96.79 (7), 78.82(B).

Compound AB6

N-(4-{[5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl]methoxy}phenyl)acetamide. IR (KBr) ν (cm^{-1}): 3382.43 cm^{-1} (Ar-NH), 1703.01 cm^{-1} (C=N), 1592.32 cm^{-1} (C=C), 1088.54 cm^{-1} (-C-O-C-), 1378.11 cm^{-1} (N=O), 3112.69 cm^{-1} (Ar-CH), ^{1}H-NMR δ (ppm): 6.41-7.8(m, 8H, Ar-CH), 2.42 (s, 3H, -CH_3), 8.13(s, 1H, -NH), 5.21(s, 2H, CH_2), Mass (m/e value) % relative abundance: 354.09 (M^+) (3.8), 335.16 (4.8), 302.39 (3.1), 287.43 (3.7), 249.58 (7.1), 226.00 (5.8), 204.96 (6.7), 127.56 (13.1), 103.69 (9), 89.93 (B).

Compound AB7

N-(4-{[5-(3,5-dinitrophenyl)-1,3,4-oxadiazol-2-yl]methoxy}phenyl) acetamide. IR (KBr) ν (cm^{-1}): 3382.02 cm^{-1} (Ar-NH), 1677.79 cm^{-1} (C=N), 1530.6 cm^{-1} (C=C), 1089.68 cm^{-1} (-C-O-C-), 1372.45 cm^{-1} (N=O), 1523.12 asym cm^{-1} (N=O), 3117.5 cm^{-1} (Ar-CH), ^{1}H-NMR δ (ppm): 6.83-8.42(m, 8H, Ar-CH), 5.35(s, 2H,-CH_2), 2.07 (s, 1H,-CH_3), 8.24 (s, 1H, -NH), Mass (m/e value) % relative abundance: 399.08 (M^+) (5), 388.76 (13), 380.25 (8), 261.63 (8), 182.52 (5), 167.62 (17), 156.56 (19), 81.97(B).

Compound AB8

N-(4-{[5-(2-hydroxy-3,5-dinitrophenyl)-1,3,4-oxadiazol-2-yl]methoxy}phenyl) acetamide. IR (KBr) ν (cm^{-1}): 3118.84 cm^{-1} (Ar-NH), 1654.42 cm^{-1} (C=N), 1541.89. cm^{-1} (C=C), 1368.45 cm-1 (N=O), 1528.45 asym. cm-1 (N=O),1090.01 cm^{-1} (-C-O-C-), 3118.84 cm^{-1} (Ar-CH), 3382.83 cm^{-1}(Ar-OH), ^{1}H-NMR δ (ppm): 6.7-7.6(s, 6H, Ar-CH), 2.11 (s, H, -CH_3), 8.00(s, 1H, -NH), 5.12(s, 1H, -CH_2), Mass (m/e value) %

relative abundance: 415.07(M) (11.1), 318.68 (16), 292.76 (7), 276.89 (20), 249.99 (8.2), 236.0277 (28.1), 203.2266 (76), 182.2587 (8), 134.4966 (32), 116.55 (B).

Computational Chemistry

Molecular docking

Molecular docking is defined as an optimization problem, which would describe the "best-fit" orientation of a ligand that binds to a particular protein of interest. During the course of the process, the ligand and the protein adjust their conformation to achieve an overall "best-fit" and this kind of conformational adjustment resulting in the overall binding is referred to as "induced fit. The aim of the molecular docking to achieve an optimized conformation for both the protein and the ligand and to achieve relative orientation between protein and ligand such that free energy of overall system is minimized. The application of docking are the hit identification – docking combined with a scoring function can be used to quickly screen large databases of potential drugs in silico to identify molecules that are likely to bind to protein target of interest and the lead optimization docking can be used to predict in where and in which relative orientation a ligand binds to a protein. This information may in turn be used to design more potent and selective analogues[20].

Scoring functions

In the fields of computational chemistry and molecular modelling, scoring functions are fast approximate mathematical methods used to predict the strength of the non-covalent interaction (also referred to as binding affinity) between two molecules after they have been docked. Structure-based drug design attempts to use the structure of proteins as a basis for designing new ligands by applying the principles of molecular recognition. Selective high affinity binding to the target is generally desirable since it leads to more efficacious drugs with fewer side effects. Thus, one of the most important principles for designing or obtaining potential new ligands is to predict the binding affinity of a certain ligand to its target (and known antitargets) and use the predicted affinity as a criterion for selection[21].

One early general-purposed empirical scoring function to describe the binding energy of ligands to receptors was developed by Böhm[22, 23]. This empirical scoring function took the form:

$$\Delta G_{bind} = \Delta G_0 + \Delta G_{hb} \sum n - bonds + \Delta G_{ionic} \sum_{ionic-int} + \Delta G\ lipophilic\ |A| + \Delta G_{rot} NROT$$

Where

ΔG_0: Empirically derived offset that in part corresponds to the overall loss of translational and rotational entropy of the ligand upon binding.

ΔG_{hb}: Contribution from hydrogen bonding.

ΔG_{ionic}: Contribution from ionic interactions.

ΔG_{lip}: Contribution from lipophilic interactions where $|A_{lipo}|$ is surface area of lipophilic contact between the ligand and receptor.

ΔG_{rot}: Entropy penalty due to freezing a rotatable in the ligand bond upon binding.

A more general thermodynamic "master" equation is as follows:

$$G_{bind} = -RTlnK_d$$

$$K_d = \frac{[Ligand][Receptor]}{[Complex]}$$

$$\Delta G_{bind} = \Delta G_{desolvation} + \Delta G_{motion} + \Delta G_{configuration} + \Delta G_{interaction}$$

Where

Desolvation: Enthalpic penalty for removing the ligand from solvent

Motion: Entropic penalty for reducing the degrees of freedom when a ligand binds to its receptor

Configuration: Conformational strain energy required to put the ligand in its "active" conformation

Interaction: Enthalpic gain for "resolvating" the ligand with its receptor

Computational analysis of synthesized compounds

Job Id: NRS/0015/11/2016; **PDB Code:** 6COX

Structure of target protein COX II

Crystalline structure of the target protein cyclooxygenase with PDB id 6COX (COX II) was retrieved from protein data bank and protein clean-up process was done and essential missing hydrogen atom were been added. Different orientation of the lead molecules AB1 to AB8 along with standard drug ciprofloxacin with respect to the target protein was evaluated by Auto dock program and the best dock pose was selected based on the interaction study analysis.

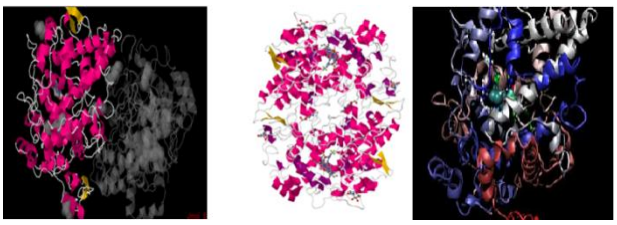


Figure 1: Structure of cyclooxygenase II (6COX)

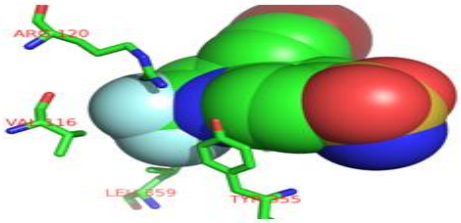


Figure 2: Ball and stick model of cyclooxygenase II (6COX)

Experimental Pharmacology

Experimental animals and standard drug

White female albino Wister rats weighing about 150-200 gm was used. They were obtained from the animal house of Anurag Pharmacy College, Kodad-508206 and Telangana State. They were kept under observation for about 7 days before onset of experiment to exclude any intercurrent infection, had free access to normal diet and water. The experimental protocol was approved by IAEC (Institutional Animal Ethics Committee) of CPCSEA: 1712/P0/a/13/CPCSEA. The standard drug diclofenac sodium was purchased from retail local shop.

Protocol for the study of acute oral toxicity of synthesized compounds[24]

In the present study acute oral toxicity of the synthesized compounds were performed by acute toxic class method according to OECD guideline-423. In this method the toxicity of synthesized compounds were tested using a step wise procedure, each step using three rat of single sex (female or male). The rats were fasted prior to dosing (food but water should be with held) for three to four hours. Following the period of fasting the animal should be weighted and synthesized compound were administered orally at a dose 2000 mg / kg body weight. Animals were observed individually after dosing at least once during the first 30 min; periodically during the first 24 h with special attention giving during the first 4 h and daily thereafter, for total of 14 days. As know mortality observed with the above dose. Test compound dose reduced by specific intervals. The mortality was not observed at the dose 2000 mg / Kg. So 100 mg /Kg body weight was selected for their pharmacological evaluation.

Experimental protocol for the evaluation of anti inflammatory activity[25, 26]

Method

Carrageenan induced paw oedema method in rats.

Requirements

Animal Rats (150-200 g). The animals were divided in to ten groups and each group contains six animals.

Drugs

Carrageenan (Prepare 1% w/v solution and inject 0.1 ml below the planter region). Diclofenac sodium 20 mg/kg, i. p. prepared a stock solution containing 5 mg/ml of the drug and injects 0.5 ml/100 g of the body weight of the animal.

Equipment (Plethysmograph)

It is a simple apparatus containing mercury. The mercury displacement due to dipping of the paw can be directly read from scale attached to the mercury column or adjusting the mercury level in arm B to the original A level by moving arm B up/down and noting the volume required to bring the level in both the arms equal.

Procedure

1. Weigh the animal and number them.
2. Make a mark on both the hind paws (right and left) just beyond tibio-tarsal junction, so that every time the paw is dipped in mercury column up to the fixed mark to ensure constant paw volume.
3. Note the initial paw volume (both right and left) of each rat by mercury displacement method.
4. Divide the animals in to 10 groups each comprising of at least six rats. To one group inject saline and to the second group inject diclofenac sodium and group AB1-AB8 administered synthesized compounds through oral route.
5. After 30 min inject 0.1 ml of 1% (w/v) carrageenan in the planter region of the left paw of control as well as diclofenac sodium and drug treated group. The right paw will serve as reference non-inflamed paw for comparison.
6. Note the paw volume of legs of control, diclofenac sodium and extract (sample) treated rats 30, 60, 120 and 180 min after carrageenan challenge.
7. Calculate the % difference in the right and left paw volumes of each animal of control, diclofenac sodium and drug- treated group. Compare the mean % change in paw volume in control, diclofenac sodium and test compounds treated animals and express as percent oedema inhibition by the compounds. The percentage inhibition of paw oedema was calculated by using the following formula:

Percentage protection = [(control-test)/control] ×100.

RESULTS AND DISCUSSION

Chemistry

The synthesis of target compounds (AB1-AB8) N-(4-{[substituted phenyl)-1, 3, 4-Oxadiazole-2-yl]methoxy}phenyl) acetamide were carried out by reacting Para acetamidophenol, ethylchloro acetate, hydrazine monohydrate and various aromatic acids. The synthesized compounds were characterized by IR, NMR, and Mass spectroscopy. The progress of the reaction was monitored by TLC using solvent systems of different polarities. TLC plates are pre-coated silica gel (HF254-200 mesh) aluminium and spots were visualized under U.V chamber and the proposed structures of the synthesized compounds were ascertained by spectral data. All the synthesized compounds having the following solubility profile: Insoluble in water, slightly soluble in chloroform, ethanol, and freely soluble in DMF, DMSO.

Docking results analysis

Table 3: Docking results analysis

C.C	EFEB (k.cal/ml)	EIC (Ki)	vdw+H-bond+de (k.cal/ml)	EE (k.cal/ml)	TIME (k.cal ml)	Fr (%)	IS
AB1	-4.21	818.24 µM	-4.00	+0.01	-6.36	50	502.257
AB2	-5.21	152.49 µM	-7.05	-0.16	-7.21	50	686.32
AB3	-5.06	195.09 µM	-5.77	-1.47	-7.23	50	477.441
AB4	-3.96	1.26 mM	-3.87	-2.11	-5.98	50	463.157
AB5	-4.38	616.75 µM	-4.87	-1.45	-6.32	50	597.255
AB6	-3.45	2.94 mM	-4.78	-0.09	-4.86	50	467.084
AB7	-4.25	767.19 µM	-6.11	-0.04	-6.15	50	562.375
AB8	-3.83	1.55 mM	-5.57	-0.28	-5.85	50	629.71
DFS	-3.15	4.94 mM	-4.00	+0.01	3.99	50	416.955

C.C = Compounds code. EFEB = Est. Free Energy of Binding. EIC = Est. Inhibition Constant. Vdw + H-bond + dE = Vender walls + Hydrogen bond + Dessolve energy. EE = Electrostatic Energy. TIME = Total Intermolecular Energy. Fr = Frequency. IS = Interaction Surface.

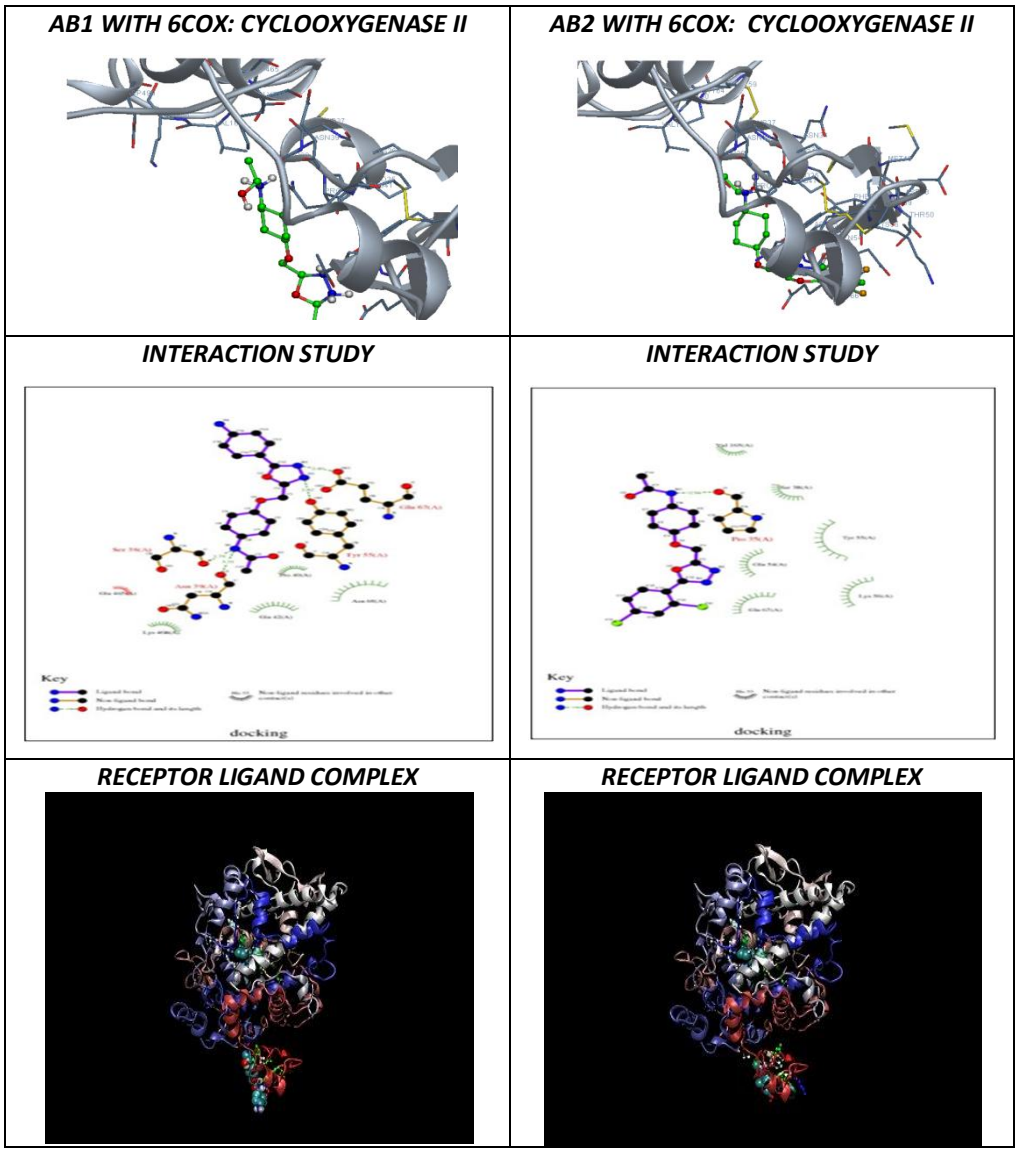


Figure 3: Compound AB1 and AB2 with 6COX interaction.

Evaluation of *in vivo* anti inflammatory activity

Table 4A: Anti Inflammatory Activity of the Synthesized Compounds (100 mg/kg)

Treatment groups	Paw volume (cm) as measured by mercury displacement at			
	30 min		60 min	
	MEAN±SD	% P	MEAN±SD	% P
CT(Control)	0.605±0.0057	-	0.70	-
AB1	0.5525±0.0095	14.25±0.0206	0705±0.0057	19.63±0.0294
AB2	0.5325±0.005*	26.42±0.0264*	0.585±0.0057**	44.19±0.031**
AB3	0.55±0.0816*	18.17±0.0129*	0.505±0.0057**	42.73±0.0351**
AB4	0.565±0.0057^{ns}	13.48±0.0316^{ns}	0.61±0.0081^{ns}	19.04±0.0828^{ns}
AB5	0.545±.0057*	18.05±0.06702*	0.5325±0.005*	39.53±0.0216*
AB6	0.5825±0.0095	14.27±0.033	0.68±0.0081	18.91±0.0310
AB7	0.555±0.0057	17.54±0.105*	0.5575±0.0095*	35.43±0.0623*
AB8	0.5675±0.005^{ns}	14.17±0.04163^{ns}	0.615±0.2082^{ns}	18.84±0.0935^{ns}
DFS	0.4905±0.0005**	40.1211±0.234**	0.485±0.0057**	49.67±0.0095**

Table -4B: Anti Inflammatory Activity of the Synthesized Compounds (100 mg/kg)

Treatment groups	Paw volume (cm) as measured by mercury displacement at			
	120 min		180 min	
	MEAN±SD	% P	MEAN±SD	% P
CT(Control)	0.905±0.0057	-	0.9725±0.00957	-
AB1	0.5525±0.005*	50.25±0.4406*	0.595±0.0173*	46.23±0.0263*
AB2	0.4925±0.005***	58.38±0.0244***	0.5625±0.005**	51.72±0.0294**
AB3	0.5075±0.0095***	57.55±0.0191***	0.5825±0.005*	49.64±0.0264**
AB4	0.5425±0.005*	48.77±0.4701*	0.6325±0.015	45.03±0.0655
AB5	0.51±0.0081***	57.15±0.0189***	0.5825±0.0095*	47.58±0.0206**
AB6	0.5775±0.0189	47.72±0.0435	0.66±0.00816	44.4±0.0377
AB7	0.525±0.0057***	56.79±0.0191***	0.5975±0.0095*	45.6±0.0129**
AB8	0.605±0.0191	49.3±0.3327	0.645±0.00577	44.59±0.0925
DFS	0.4525±0.005***	60.56±0.0275***	0.495±0.00577**	51.42±1.275**

P<0.001= ***, highly significant. P<0.01= **, moderate significant. P<0.05= *, significant. P>0.05= ns. Values are expressed as MEAN ±SD of animals. The data were statistically analysed by ONE WAY ANOVA followed by Tukey Kramer multiple comparison test.

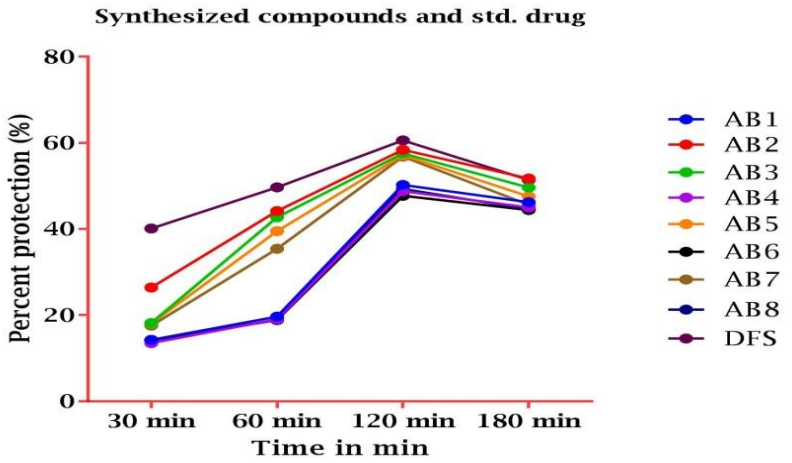


Figure 4: Comparison of percent protection of synthesized compounds in different treatment groups.

Molecular docking studies

Most of the scoring functions in molecular docking are physics-based molecular mechanics force fields that estimate the energy of the binding pose; a low (negative) energy indicates a stable system and thus a likely binding interaction. Molecular docking is performed to find out the binding affinity or molecular interaction energy (kcal/mol) of docked compounds. Lowest (negative value) energy of docked molecule indicates high binding affinity with the target protein/compound. In silico molecular docking studies the binding energy of synthesized compounds (AB1-AB8) were found to be -4.21; -5.21; -5.06; -3.96; -4.38; -3.45; -4.25; -3.83 which indicated that the compound had high binding affinity towards the cyclooxygenase II (6COX) protein and inhibit the function 6COX protein in comparison with standard drug diclofenac sodium (-3.15).

Acute oral toxicity studies

(i) Acute oral toxicity studies were performed according to the OECD guideline 423 method.

(ii) This method has been designed to evaluate the substance at the fixed doses and provide information both for hazard assessment and substance to be ranked for hazard classification purposes.

(iii) The each compound was administered initially at a dose of 2000 mg/kg b. w and 1% CMC (p. o) and observed 14 days mortality due to acute toxicity.

(iv) Careful observation were made at least thrice a day for the effect on CNS, ANS, motor activity, salivation and other general signs of toxicity were also observed and recorded.

(v) Since no sign of toxicity observed at 2000 mg/kg b. w. to the group of animals, the LD50 value of the test compound expected to exceed 2000 mg/kg b. w. and represented as class 5 (2000 mg/kg < LD50 < 2500 mg/kg).

(vi) From the toxicity studies the data revealed that all the synthesized compounds proved to be non toxic at tested dose levels and well tolerated by the experimental animals as there LD50 cut of values > 2000 mg/kg b. w. So for the evaluation of *in vivo* pharmacological activity 100 mg/kg body weight was selected.

Evaluation of *in vivo* anti inflammatory activity

Anti-inflammatory activity of the each compound was evaluated by carrageenan induced paw oedema method. The activity was studied at 100 mg/kg b. w. p. o. And then their responses were measured at 30, 60, 120 and 180 min. From the above experimental data displayed that the compound AB2, AB3, AB5 and AB7 possessed very good anti-inflammatory activity among the eight synthesized compounds which was also proved by in silico molecular docking studies with reference to binding energies and interaction studies with the target porotein 6COX: Cyclooxygenase II. All the compounds exhibited highest activity at 120 min**.** The percent protection of the synthesized compounds were found to be AB1: 19.63±0.0294, AB2: 44.19±0.031**, AB3: 42.73±0.0351**, AB4: 19.04±0.0828^{ns}, AB5: 39.53±0.0216*, AB6: 18.91±0.0310, AB7: 35.43±0.0623*, AB8: 18.84±0.0935^{ns}, standard drug diclofenac sodium (DFS): 49.67±0.0095** etc.

CONCLUSION

We here reported that the compound AB2, AB3, AB5 and AB7 possessed very good anti-inflammatory activity among the eight synthesized compounds and all the compounds exhibited highest activity at 120 min and docking studies also proved that these four compounds have the higher binding affinity towards target protein cyclooxygenase with PDB id 6COX (COX II) and known to be inhibitor of COX II.

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