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Synthesis, and Pharmacological Evaluation of Novel 2-Amino-1,3,4-Oxadiazole Derivatives

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ABSTRACT

Background: Oxadiazole is one of the exciting heterocyclic nucleus and an important pharmacophore in the field of drug designing and discovery because of its diverse biological activities. The research conducted is focused on synthesis, biological activities and virtual screening of 2-(substituted phenyl)-1, 3, 4-oxadiazol-5-yl] benzamide derivatives. **Material and Method:** First 5-(substituted phenyl)-1, 3, 4- oxadiazol-2-amine was synthesized by reacting semicarbazide hydrochloride with 4-chlorobenzaldehyde and pyridine-3-carbaldehyde. These 2-amino-1, 3, 4-oxadiazole nuclei were further converted to corresponding amide derivatives by reaction with different benzoic acids in presence of dicyclohexyl carbodiimide (DCC) using acetonitrile as solvent. **Results:** Noval synthesized derivatives were assessed for potential anti-inflammatory activity. Compound 1c was found to be most potent cyclooxygenase inhibitor with maximum mean paw volume displacement 0.233 (±) 0.012. Anti-inflammatory activity of all derivatives was found to be average as compared to standard drug. Eight noval synthesized compounds were docked against cyclooxygenase-2 (5F1A) to establish their binding potential. *In-silico* studies showed the interaction between synthesized compounds and cyclooxygenase-2 enzyme depending on both hydrophobic forces and conventional hydrogen bonds. Highest binding affinity was found to be -9.6 Kcal/mol by compound 1c which is greater than the binding affinity of standard molecule diclofenac sodium. **Conclusion:** These results suggested that 2-[substituted phenyl)-1, 3, 4-oxadiazol-5-yl] benzamide might be used as lead compound for further designing and manipulating new potential cyclooxygenase-2 inhibitors.

Keyword: 2-amino-1, 3, 4-oxadiazole, Cycloxygenase, Anti-inflammatory

INTRODUCTION

Medicinal chemistry, being an inter-disciplinary are, involves the layout, designing, fusion and development of the diverse novel drug as an agent to be used for research purpose [1-3]. A fundamental immune response, inflammation, activated by the host that empowers the dismissal of destructive boosts in addition to restoration of broken tissue. Acute inflammation, being a part of innate immunity, is a principle host barrier categorized by redness, edema, aches, and hyperthermia [4]. Nowadays numerous types of inflammations have been recognized based on type of stimuli and diverse regulatory mechanisms that has an impact on typical human pathology and

physiology [5]. The enzyme cyclooxygenase-2 (COX-2) responsible for the production of thromboxane-A2, prostaglandins, and prostacyclin along with other physiological and pathological processes [6-8]. COX-2 is also responsible for the production of reactive oxygen species (ROS), and oxidative stress along with mediators (bradykinins, and histamines) [6, 9-11]. Researches have been shown that blockage of COX-2 receptor can actively responsible in the down streaming of the inflammatory responses [6, 12]. As per the reports, Acetylsalicylic acid was the first drug to be introduced as NSAID, and an anti-inflammatory drug as it acts on the unique receptors that involved in the process of inflammation [11]. NSAIDs being an extraordinary group of drugs including rofecoxib, and celecoxib that actively downregulate the synthesis of prostaglandins, thromboxane, and prostacyclin [13-15]. The long-term use of NSAIDs leads to the development of gastrointestinal effects which can be caused due to the inhibition of COX-2 and downregulation of protective prostaglandins [16]. The increased cardiovascular risks observed with the chronic use of NSAIDs as they also downregulate PgI_2 [17]. Now, celecoxib is the only selective COX-2 inhibitor accessible in U.S. to treat the inflammation [18].

The prime structure of COX-1 includes 602 amino acids while COX-2 isoform has 604 amino acids. The COX-1 and COX-2 isoforms have 60-65% sequence identity inside species and around 85-90% sequence identity amongst one-of-a-kind species [19].

The use of heterocyclic compounds such as oxadiazoles is considered as an important isomeric compound used as COX-2 inhibitor. The oxadiazoles exist in various isomeric forms such as 1,2,4-, (1) 1,2,5-, (2) 1,2,3- (3) and 1,3,4- (4) oxadiazoles respectively [20]. 1, 3, 4-oxadiazoles, a significant pharmacophore, with two nitrogen and a carbon atom has a significant potential of biodynamic moiety [21]. It has the potential of being an incorporates antimicrobial, anticonvulsant, anti-tubercular, hypoglycemic, vasodilator, analgesic and anti-inflammatory, anthelmintic, anti-oxidant, anticancer, antiviral, hemolytic, and anti-proliferative agent [22, 23]. 1, 3, 4-oxadiazole derivatives shows significant interaction with COX-2 [24]. Keeping view of Cox-2 enzymes importance we decided to synthesized noval 2-(substituted phenyl)-1, 3, 4-oxadiazol-5-yl] benzamide derivatives. The in silico molecular docking were performed to analyzed the binding mode of these compounds with the target protein. Further evaluate in vitro and in vivo studies.

MATERIALS AND METHODS

All reagents and solvents used in this work were obtained from commercial suppliers, including Aldrich Chemical Co. and Merck (Pty) Ltd, and were used without further purification. The progress of the reactions was routinely monitored by thin-layer chromatography (TLC) employing Merck silica gel 60 F_{254} plates coated on aluminum sheets with a thickness of 0.20 mm. Visualization of the TLC plates was carried out under ultraviolet light at wavelengths ranging from 254 to 366 nm.

Fourier-transform infrared (FTIR) spectra of the synthesized compounds were recorded using an Alpha Bruker FTIR spectrophotometer, and absorption frequencies are reported in cm^{-1}. Melting points were determined with a GallenKamp (SANYO) MPD.BM3.5 melting point apparatus and are reported without correction. Proton (^1H) and carbon (^13C) nuclear magnetic resonance spectra were obtained on a Bruker BioSpin 600 MHz spectrometer. Chemical shifts (δ) are expressed in parts per million (ppm) relative to the residual solvent signals of DMSO-d_6 (δ 2.50 ppm for ^1H and δ 39.5 ppm for ^13C). All NMR spectra were processed using MestReNova software. Mass spectrometric analysis was performed using electron ionization (EI) mode on a JEOL 600H-1 mass spectrometer.

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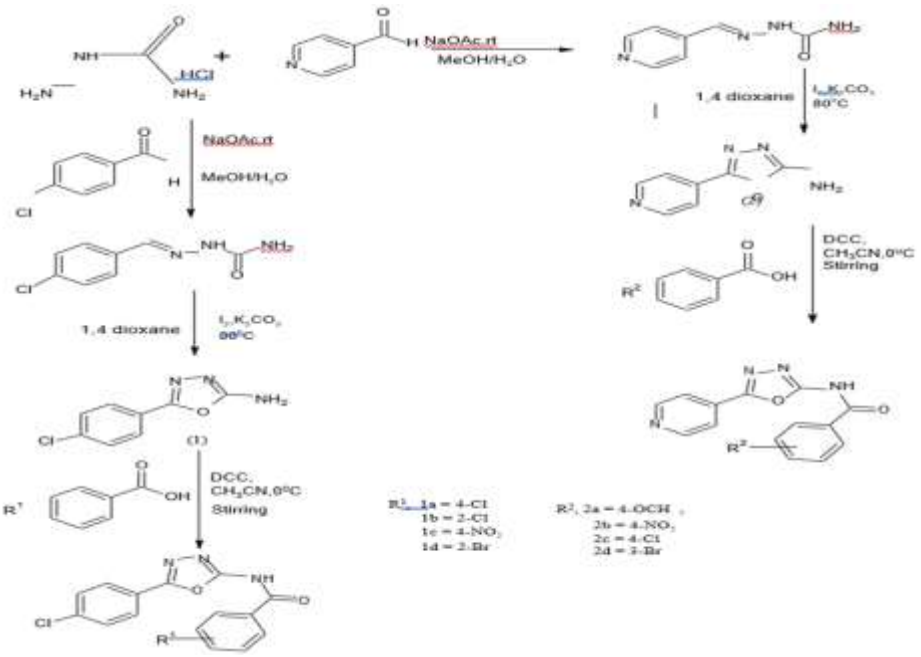

Figure 2: Synthesis of 2-amino 1, 3, 4-oxadiazole derivatives

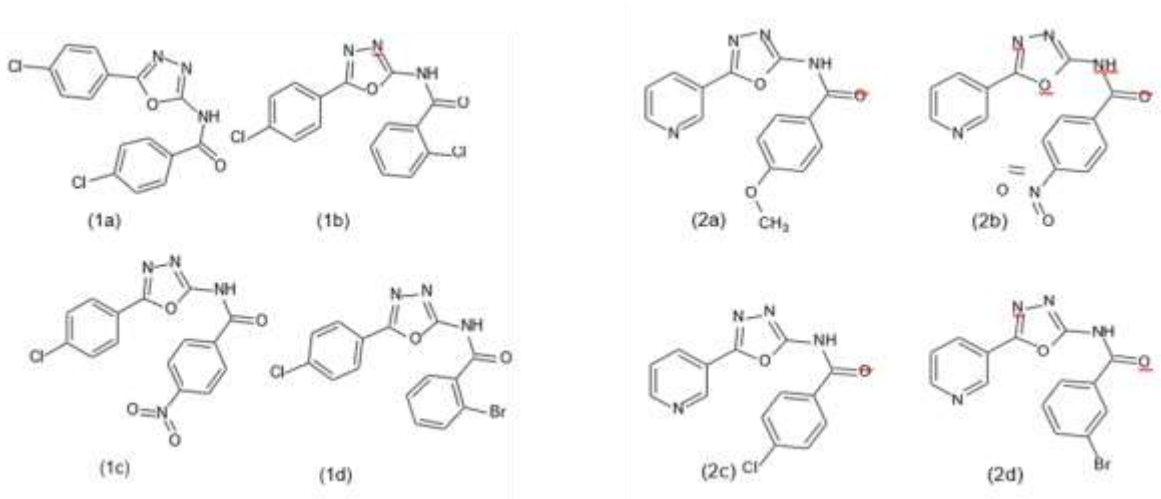

Figure 3: Structures of synthesized compounds 1a-1d, and 2a-2d

CHEMICALS

All the solvents and chemicals used were purchased from Sigma-Aldrich, Merck and Honeywell. No purification was carried out prior to their use. Semicarbazide.HCl, sodium acetate, potassium carbonate, iodine, sodium sulfate, salicaldehyde, pyridin-4-carboxaldehyde, 2-methoxy benzaldehyde, 4-chlorobenzaldehyde, 3-bromobenzoic acid, 4-chlorobenzoic acid, 4- nitrobenzoic acid, 4-methoxybenzoic acid, 2-chlorobenzoic acid, 3-hydroxybenzoic acid, N,N'- Dicyclohexylcarbodiimide (DCC), methanol, 1,4-dioxane, dicloromethane.

PURIFICATION AND CHARCTERIZATION

Compounds synthesized were washed with methanol and Thin- layer chromatography (TLC) (Merck TLC Silica gel 60 F254) was done to verify the purity of synthesized compounds. Characterization of all the synthesized compounds was done by spectrophotometric analysis with the help of Bruker ALPHA FTIR spectrometer with Eco ATR and 1HNMR was carried on Bruker AM300 spectrophotometer using DMSO as solvent. Gallen Kamp melting point apparatus was used for recording melting points.

GENERAL PROCEDURE FOR SYNTHESIS

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To a solution of aldehyde (0.5 mmol) in methanol (10 mL), an equimolar solution of semicarbazide·HCl (0.5 mmol) and sodium acetate (0.5 mmol) in water (10 mL) was added. The mixture was stirred, and the solvent was subsequently removed using a rotary evaporator. Potassium carbonate (1.5 mmol) followed by iodine (0.6 mmol) was added to the reaction flask, and the mixture was refluxed with stirring at 80 °C for 48 hours. Progress of the reaction was monitored by TLC. Upon completion, the mixture was cooled to room temperature, treated with 5% sodium thiosulfate, and the resulting precipitate was filtered and dried in a desiccator [25].

PREPARATION OF 2- [SUBSTITUTED PHENYL)-1, 3, 4-OXADIAZOL-5-YL] BENZAMIDE

Benzoic acid (6.7 mmol) and N, N' -Di-cyclohexyl carbodiimide (DCC, 6.8 mmol) were added in 30 mL of acetonitrile in a beaker. After stirring for 30 minutes, a creamy emulsion was formed. This mixture was gradually added to a solution of 2-amino 1, 3, 4-oxadiazole (13 mmol) in 20 mL of acetonitrile in a reaction flask. The resultant reaction mixture further stirred for 5 h at room temperature. After reaction completion as monitored through TLC the solution was filtered and acidified with 3–20 ml of 2M HCl. The HCl treated solutions were basified with 2 M NaOH and extracted with CHCl3 and dried under vacuum.

Carrageenan Induced Inflammatory Response

The anti-inflammatory activity of the newly synthesized compounds was evaluated *in vivo* using a carrageenan-induced paw edema model in rats. Following a 12-hour fast, animals were randomized into three treatment groups: a negative control (normal saline, 10 mL/kg), a test group (compounds 1a–1d and 2a–2d at 10 mg/kg), and a positive control (diclofenac sodium, 20 mg/kg). Thirty minutes post-treatment, acute inflammation was triggered via a sub-plantar injection of 0.1 mL of 1% carrageenan into the right hind paw. Edema was quantified plethysmographically at 0, 1, 2, 3, and 4 hours post-induction. Data, expressed as mean ± SEM, were analyzed by one-way ANOVA with Tukey's post-hoc test, comparing results to the saline control group [26,27].

Molecular docking studies were performed to evaluate the binding orientation and relative binding affinity of the synthesized 1,3,4-oxadiazole derivatives (1a–1d and 2a–2d) against the cyclooxygenase enzyme (COX-2; PDB ID: 5F1A), which was obtained from the RCSB Protein Data Bank [27]. The protein structure was prepared using Discovery Studio Visualizer (DSV) by removing co-crystallized ligands, water molecules, and other unwanted interactions. The chemical structures of all ligands were individually sketched using ChemSketch software and converted into compatible PDB and PDBQT formats for docking analysis [28]. AutoDock Vina was employed to perform the molecular docking simulations. The grid box for the human COX-2 active site was defined with dimensions of x = 98, y = 70, and z = 98 Å, using a grid spacing of 1.0 Å. Docking results were analyzed based on the lowest binding energy values (kcal/mol) and the most favorable binding conformations. The resulting ligand–protein complexes were visualized using Discovery Studio, and interacting amino acid residues were examined through both three-dimensional (3D) and two-dimensional (2D) interaction analyses.

ChemSketch and Molinspiration Cheminformatics, a web freeware tool, were utilized for the chemo- informatic evaluation of the noval synthesized compounds. Various parameters such as molecular formula, molecular weight, LogP value, number of hydrogen bond donors and acceptors, molar refractivity, PSA, density, polarizability, molar volume, surface tension and Lipinski rule validation were determined and reported. A structure of single compound is first uploaded to this web tool in mol format which creates an output as these parameters [29].

RESULTS AND DISCUSSION

Spectral Characterization of Synthesized Compounds

Chloro-N-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]benzamide was obtained as a white crystalline product with a yield of 35%, this compound exhibited a melting point of 126 °C. TLC analysis showed an Rf value of 0.25. IR (cm^{-1}) bands were observed at 3220 (N–H stretch), 1690 (C=N), 1600 (C=O), 1498 (N–H bending), 1380 (C=C), and 656 (C–X). The ^{1}H NMR spectrum (DMSO, δ ppm) displayed signals at 7.766–7.839 (multiplet, 4H, aromatic H of 4-chlorophenyl), 7.201–7.991 (multiplet, 4H, aromatic H), and 9.23 (singlet, 1H, amide N–H).

2-Chloro-N-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]benzamide was isolated as a white solid in 70% yield with a melting point of 147 °C. Its TLC Rf value was 1.4. IR (cm^{-1}) absorptions included 3300 (N–H stretch), 1710 (C=N), 1660 (C=O), 1489 (N–H bending), 1386 (C=C), and 750 (C–X). The ^{1}H NMR (DMSO, δ ppm) exhibited 7.766–7.839 (multiplet, 4H, 4-chlorophenyl aromatic H), 7.4–7.94 (multiplet, 8H, aromatic H), and 9.58 (singlet, 1H, amide N–H).

N-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]-4-nitrobenzamide (1C) a brown solid was obtained with a yield of 30% and melting point of 154 °C. TLC analysis gave Rf = 0.34. The IR spectrum (cm^{-1}) showed bands at 3000 (N–H stretch), 1700 (C=N), 1670 (C=O), 1508 (N–H bending), 1450 (C=C), 1315 (C–O), and 770 (C–X). The ^{1}H NMR (DMSO, δ ppm) displayed multiplets at 6.96–7.28 (4H, aromatic H of 4-chlorophenyl), 7.20–7.99 (8H, aromatic H), and a singlet at 10.83 (1H, amide N–H).

PHYSICAL DATA AND CHEMO-INFORMATICS OF SYNTHESIZED COMPOUNDS

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All the synthesized compounds were purified through recrystallization. All compounds were obtained in powder form. Melting points ranged from 90 ºC to 176 ºC. Percentage yield of compounds ranged from 30%- 70%. Compounds additionally fulfilled the Lipinski rule standards. Detailed physical properties and chemo-informatics properties are given in Table 1 and Table 2 respectively.

Table 1: Physical properties of synthesized compounds

Compound	Molecular formula	Molecular weight (g/mol	Melting point (ºC)	Physical state	Color	% yield
1a	$C_{15}H_9Cl_2N_3O_2$	334.15686	126	Solid	White	35
1b	$C_{15}H_9Cl_2N_3O_2$	334.15686	147	Solid	White	70
1c	$C_{15}H_9ClN_4O_4$	344.70936	154	Solid	Brown	30
1d	$C_{15}H_9BrClN_3O_2$	378.60786	168	Solid	White	35
2a	$C_{15}H_{12}N_4O_3$	296.28078	126	Solid	Brown	30
2b	$C_{14}H_9N_5O_4$	311.25236	144	Solid	Brown	40
2c	$C_{14}H_9BrN_4O_2$	345.15086	130	Solid	White	50
2d	$C_{14}H_9BrN_4O_2$	345.15086	165	Solid	White	70

Table 2 Chemo informatics analysis of synthesized compounds

Compound	No. of HBA	No. of HBD	Mol. Log*P*	Polar surface area (PSA) (Å^2)	Molar refractivity (cm^3)	Density (g/cm^3)	Surface tension (dyne/c)	Polarizability (cm^3)	Molar volume (cm^3)	Lipinski rule validation
1a	1	5	4.18	68.02	83.69± 0.3	1.473± 0.06	61.2± 0.3	33.17± 0.5 10^{-24}	226.7± 0.3	yes
1b	1	5	4.13	68.02	83.69± 0.3	1.473± 0.06	61.2± 0.3	33.17± 0.5 10^{-24}	226.7± 0.3	yes
1c	1	8	3.46	113.85	85.34± 0.3	1.521± 0.06	69.3± 0.3	33.83± 0.5 10^{-24}	226.6± 0.3	yes
1d	1	5	4.29	68.02	86.48± 0.3	1.639± 0.06	62.3± 0.3	34.28± 0.5 10^{-24}	230.9± 0.3	yes
2a	1	7	1.59	90.15	78.67± 0.3	1.346± 0.06	60.3± 0.3	31.18± 0.5 10^{-24}	220.0± 0.3	yes
2b	1	9	1.49	126.74	78.53± 0.3	1.497± 0.06	75.1± 0.3	31.13± 0.5 10^{-24}	207.8± 0.3	yes
2c	1	6	2.21	80.91	76.88± 0.3	1.445± 0.06	65.8± 0.3	30.48± 0.5 10^{-24}	208.0± 0.3	yes
2d	1	6	2.32	80.91	79.68± 0.3	1.626± 0.06	66.9± 0.3	31.58± 0.5 10^{-24}	212.2± 0.3	yes

FT-IR ῡ CM^{-1} SPECTRAL DATA OF SYNTHESIZED COMPOUNDS

Functional groups of synthesized compounds were validated using FTIR spectral data. The stretching vibrations of OH, stretching of N-H, C=O, C=C, bending of N-H, C=N and C-X were observed at expected wave number (Table. 3)

Table 3: FT-IR (ῡ cm^{-1}) spectral data of synthesized compounds

compound	N-H(Stretch)	C=O	N-H(bend)	C=C	C=N	C-X
1a	3220	1600	1498	1380	1690	650
1b	3300	1660	1489	1386	1680	750

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1c	3000	1670	1508	1450	1690	770
1d	3050	1640	1512	1400	1710	765
2a	3100	1600	1560	1450	1700	-
2b	3100	1600	1550	1440	1690	-
2c	3200	1670	1550	1420	1680	760
2d	3120	1645	1511	1420	1680	640

1H NMR (Δ PPM) SPECTRAL DATA OF SYNTHESIZED COMPOUNDS

Table 4 shows Chemical shift values found from proton NMR of the synthesized compounds. Protons of each compound are individually mentioned in the tables below. Multiplet of aromatic protons of 1a- 1d, protons observed at 6.76-7.83 ppm. Multiplet of aromatic protons of other benzoyl ring were observed in the range of 7.20-7.99 ppm. Singlet of N-H was observed at 9.23-10.21 ppm. Whereas Table 4 also shows Chemical shift values found from proton NMR of the synthesized compounds. Multiplet of pyr-aromatic protons of 2a-2d, observed at 6.42-9.09 ppm. Multiplet of aromatic protons of other benzoyl ring were observed in the range of 7.20-7.99 ppm. Singlet of N-H was observed at 9.20-11.52 ppm.

Table 4. ^{1}H NMR chemical shifts in ppm of compounds 1a-1d

Compound	Protons	Chemical Shift (ppm)	Multiplicity
1a	Ar-H	6.76-7.83	(m, 4H, J = 7.52 Hz)
	Ar-H	7.20-7.99	(m, 4H, J = 7.39 Hz)
	NH (Amide)	9.23	(s, 1H)
1b	Ar-H	7.76-7.83	(m, 4H, J = 8.62 Hz)
	Ar-H	7.40-7.94	(m, 4H, J = 8.08 Hz)
	NH (Amide)	9.58	(s, 1H)
1c	Ar-H	6.96-7.28	(m, 4H, J = 7.46 Hz)
	Ar-H	7.20-7.99	(m, 4H, J = 7.39 Hz)
	NH (Amide)	10.83	(s, 1H)
1d	Ar-H	7.76-7.83	(m, 4H, J = 7.32 Hz)
	Ar-H	7.39-7.67	(m, 4H, J = 8.53 Hz)
	NH (Amide)	10.21	(s, 1H)
2a	Ar-H (Pyr)	7.42-9.09	(m, 4H, J = 7.32 Hz)
	Ar-H (4-methoxy benzoyl)	7.20-7.99	(m, 4H, J = 7.39 Hz)
	CH3 (Methoxy)	3.85-7.83	(s, 3H, J = 8.48)
	NH (Amide)	10.11	(s, 1H)
2b	Ar-H (Pyr)	6.42-7.09	(m, 4H, J = 7.85 Hz)
	Ar-H	7.4-7.94	(m, 4H, J = 8.08 Hz)
	NH (Amide)	10.66	(s, 1H)
2c	Ar-H (Pyr)	7.42-9.09	(m, 4H, J = 8.63 Hz)
	Ar-H	7.53-7.76	(m, 4H, J = 8.46 Hz)
	NH (Amide)	9.20	(s, 1H)
2d	Ar-H (Pyr)	6.93-7.78	(m, 4H, J = 7.63 Hz)
	Ar-H	8.13-9.43	(m, 4H, J = 8.53 Hz)
	NH (Amide)	11.52	(s, 1H)

ANTI-INFLAMMATORY ACTIVITY OF SYNTHESIZED DERIVATIVES

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The anti-inflammatory potential of the newly synthesized derivatives was evaluated using the carrageenan-induced paw edema model. Each compound was administered, with diclofenac sodium serving as the standard reference. Paw volumes were measured at 0, 1, 2, 3, and 4 hours post-carrageenan injection to monitor the progression of inflammation. Among all the tested compounds, 1c and 2b exhibited the highest inhibitory effect on paw edema after 4 hours, with values of 0.233 ± 0.012 and 0.237 ± 0.003, respectively. All data are presented as mean ± SEM (n = 5). The detailed results are summarized in Table 5 and illustrated in Figure 5.

Table 5: Anti-inflammatory activity of synthesized compounds

Compound	Mean paw volume (±) SEM (ml)				
	Time after carrageenan Injection				
	0 hr	1 hr	2 hr	3 hr	4 hr
Control	1.920 ±0.015	1.023 ± 0.003	1.040 ± 0.006	0.973 ± 0.009	0.897 ± 0.003
Standard	0.483 ± 0.012	0.490 ± 0.006	0.250 ± 0.006	0.220 ± 0.006	0.137 ± 0.009
1a	0.900 ± 0.025	0.770 ± 0.006	0.483 ± 0.003	0.447 ± 0.003	0.390 ± 0.006
1b	1.053 ± 0.009	1.030 ± 0.006	0.917 ± 0.003	0.720 ± 0.006	0.490 ± 0.006
1c	0.643 ± 0.009	0.540 ± 0.006	0.463 ± 0.009	0.337 ± 0.007	0.233 ± 0.012
1d	1.903 ± 0.035	1.130 ± 0.006	1.013 ± 0.007	0.930 ± 0.006	0.623 ± 0.012
2a	1.847 ± 0.009	0.977 ± 0.009	0.850 ± 0.006	0.750 ± 0.006	0.720 ± 006
2b	0.957 ± 0.020	0.770 ± 0.006	0.440 ± 0.006	0.367 ± 0.003	0.237 ± 0.003
2c	0.810 ± 0.006	0.770 ± 0.006	0.700 ± 0.006	0.650 ± 0.006	0.490 ± 0.006
2d	1.043 ± 0.012	0.930 ± 0.006	0.830 ± 0.006	0.763 ± 0.003	0.304 ± 0.006

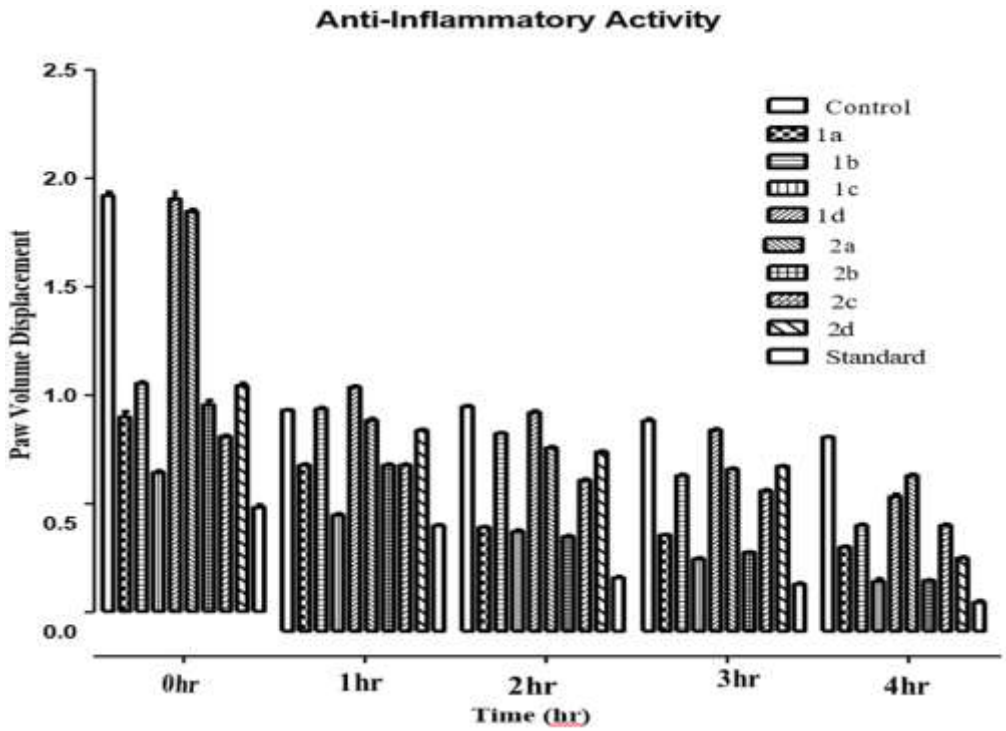

Figure 5: Anti-inflammatory effect against carrageenan paw edema method

DOCKING OF LIGANDS WITH CYCLOOXYGENASE-2 (5F1A)

Cyclooxygenase 2 was used for docking the structures of newly synthesized ligands according to the methodology discussed in chapter 2. Highest binding affinity of the distinct ligands is given in Table 6. Furthermore, Binding affinity of diclofenac is also mentioned as standard drug for reference. Compound 1c, 1d, 2b and 2c exhibited the highest binding affinity amongst the newly synthesized derivatives with a value of -9.6 Kcal/mol. While compound 2a was perceived with the lowest binding affinity of -8.6 Kcal/mol.

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Table 6: Binding affinities of ligands with 5F1A

Protein Target	Ligand	Highest binding affinity (Kcal/mol) [a]
COX 2 (PDB ID: **5F1A**)	1a	-9.5
	1b	-8.9
	1c	-9.6
	1d	-9.6
	2a	-8.6
	2b	-9.6
	2c	-9.6
	2d	-9.1
	Diclofenac	-7.7

PROTEIN LIGAND BINDING INTERACTIONS

All the residues of synthesized compounds involved in ligand protein interactions are mentioned in Table 7. Distance of ligand protein interactions ranged from 2.53 Å to 5.94 Å. Synthesized compounds were observed interacting with formerly reported amino acids of the active site. The amino acids involved are CYS 36, CYS 47, PRO 153, TYR 130, TYR 136, LEU152, VAL 46 and PRO 156. 2-D and 3-D interaction patterns of five derivatives having highest anti-inflammatory activity are given in Figure 6.

Table 7: Distances of protein ligand interactions

Compound	Amino acid involved	Distance (Å)
1a	CYS 36, CYS 47, PRO 153, HIS 39, GLY 461, CYS 41, ARG 44, GLY 45, TYR 130	(4.21, 4.60), 3.94, (4.91, 4.69), 3.16, 3.00, 2.87, 4.43, 4.18, 4.78
1b	TYR 136, PRO 156, PRO 153, CYS 36, CYS 47, VAL 46, LEU 152	3.52, (3.82, 4.99, 3.78), 6.55, 4.46, 5.00, 5.73, 5.38, 4.43
1c	TYR 136, PRO 156, CYS 36, PRO 153, HIS 39, CYS 41, VAL 46, TYR 130	5.09, 3.65, (5.74, 5.49, 5.56), (4.57, 4.60, 3.71), 3.17, 3.39, 5.47, 2.92
1d	TYR 136, PRO 156, CYS 36, CYS 47, PRO 153, GLN 461, HIS 39, VAL 46	4.89, (4.72, 5.86), 5.37, 5.46, (4.27, 4.28), 3.69, (3.23, 2.65), 5.21
2a	PRO 156, CYS 36, PRO 153, CYS 47, VAL 46, TYR 130, ARG 44	(5.03, 5.08), (5.08, 4.97), 4.63, 5.17, 5.33, 3.09, 3.43
2b	LYS 468, LEU 152, TYR 130, ARG 44	4.50, 3.77, (2.99, 5.47), 4.80
2c	HIS 386, THR 212, HIS 214, THR 206, LEU 391	(5.94, 4.51), (3.36, 2.97), 3.64, (2.71, 2.53), 3.58
2d	PRO 156, PRO 154, CYS 36, PRO 153, HIS 39, VAL 46	4.64, 3.65, 4.82, (4.20, 4.43), 3.09, 5.21
Diclofenac	LEU 145, PHE 142, TRP 139, ASN 537, VAL 228, HIS 226, GLN 225	(5.17, 4.79), (4.84, 4.70, 4.69), (5.12, 4.84), 2.91, 3.43, 2.30, 1.85

Brackets represent interaction of a single amino acid with more than one group of ligands

Reported 2-amino 1, 3, 4-oxadiazoles were modified at position 2 and 5 of oxadiazole ring [25, 30]. We investigated cyclooxygenase inhibitory potential of 2- amino 1, 3, 4-oxadiazole derivatives. Moreover, results of activity were validated by performing molecular docking studies against COX-2 enzyme. Synthesis of 2-[substituted phenyl)-1, 3, 4-oxadiazol-5-yl] benzamide derivatives was based on the following steps as indicated in Figure 1 scheme (Niu et al., 2014). Yield of these 2-amino 1, 3, 4-oxadiazole nucleus 1 and 2 range from 55 to 90 %. Expected stretches of various functional groups were observed in the FTIR spectral data. Peaks of N-H, C=O, C=C, C=N and C-X were ranged from 3000-3300 cm^{-1}, 1600-1670 cm^{-1}, 1380-1450 cm^{-1}, 1680-1710 cm^{-1}, 1280-1320 cm^{-1} and 640-767

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cm^{-1} respectively. Moreover, structures of the newly synthesized compounds were established by proton ^{1}H NMR data (Table 4). Chemical shift values of oxadiazole protons were compared with the NMR results of the formerly 5-(substituted phenyl)-1, 3, 4-oxadiazol-2-amine derivatives [30]. For derivatives 1a-1d aromatic protons peaks were observed as multiplet in the range of 6.766-7.83ppm. A singlet of N-H was observed downfield at 9.23-10.21ppm. Aromatic protons appeared as multiplet in the range of 7.20-7.99 ppm. Amide derivatives (2a-2d) aromatic pyridine peak was observed as multiplet in the range of 6.42-9.09 ppm. A singlet of amide-NH peak was observed in the range of 9.20-11.52 ppm. Aromatic protons appeared as multiplet in the range of 7.20-7.99 ppm. In comparison to the docking results, all the compounds which had more than one hydrogen bonding interaction with the amino acids of protein molecule exhibited a good binding affinity, hence better anti-inflammatory activity. Compound 1c had two hydrogen acceptors that are oxygens of para nitro group of phenyl ring which facilitate more hydrogen bond interactions with the protein molecule. All four derivatives having 4-chloro phenyl group at 5 positions of oxadiazole ring 1a, 1b, 1c and 1d showed good binding affinities as well as good anti- inflammatory activity representing the importance of chloro group and showing pi sigma interaction which is an important interaction. This activity was decreased by the modification of 4- chloro phenyl ring in to pyridine ring. All compounds (2a-2d) showed minimum anti- inflammatory activity. This was further supported by the results of docking which gave evidence that compounds 1a-1b exhibit significant binding affinities. Overall, all eight 2-[substituted phenyl)-1, 3, 4-oxadiazol-5-yl] benzamide derivatives showed significant anti-inflammatory activity in comparison to control group but less than that of standard drug. This activity is conducted on acute inflammatory condition, so there might be a possibility that by increasing the dose these compounds can show improved results in chronic inflammation model. 2-[substituted phenyl)-1, 3, 4-oxadiazol-5-yl] benzamide derivatives exhibited promising in vivo anti-inflammatory activity and binding affinities for COX 2. These compounds can become drug molecules if modified and experimented for further evaluation.

CONCLUSION

In this study, 2-[substituted phenyl)-1, 3, 4-oxadiazol-5-yl] benzamide derivatives were successfully synthesized and characterized by FTIR and 1HNMR. These compounds were investigated for their anti-inflammatory activity using carrageenan induced paw edema method. All compounds showed moderate to average anti-inflammatory activity particularly 1a, 1b, 1c, 2b and 2c showed better results as compared to other synthesized compounds. Compound 1c was found to be most potent cyclooxygenase inhibitor with maximum mean paw volume displacement volume 0.233 ± 0.012. These compounds were then further investigated by docking studies and all showed excellent binding affinities with COX-2. It is concluded that the compounds synthesized in this research work can be used as lead molecules for further developed in to more potent anti-inflammatory agents.

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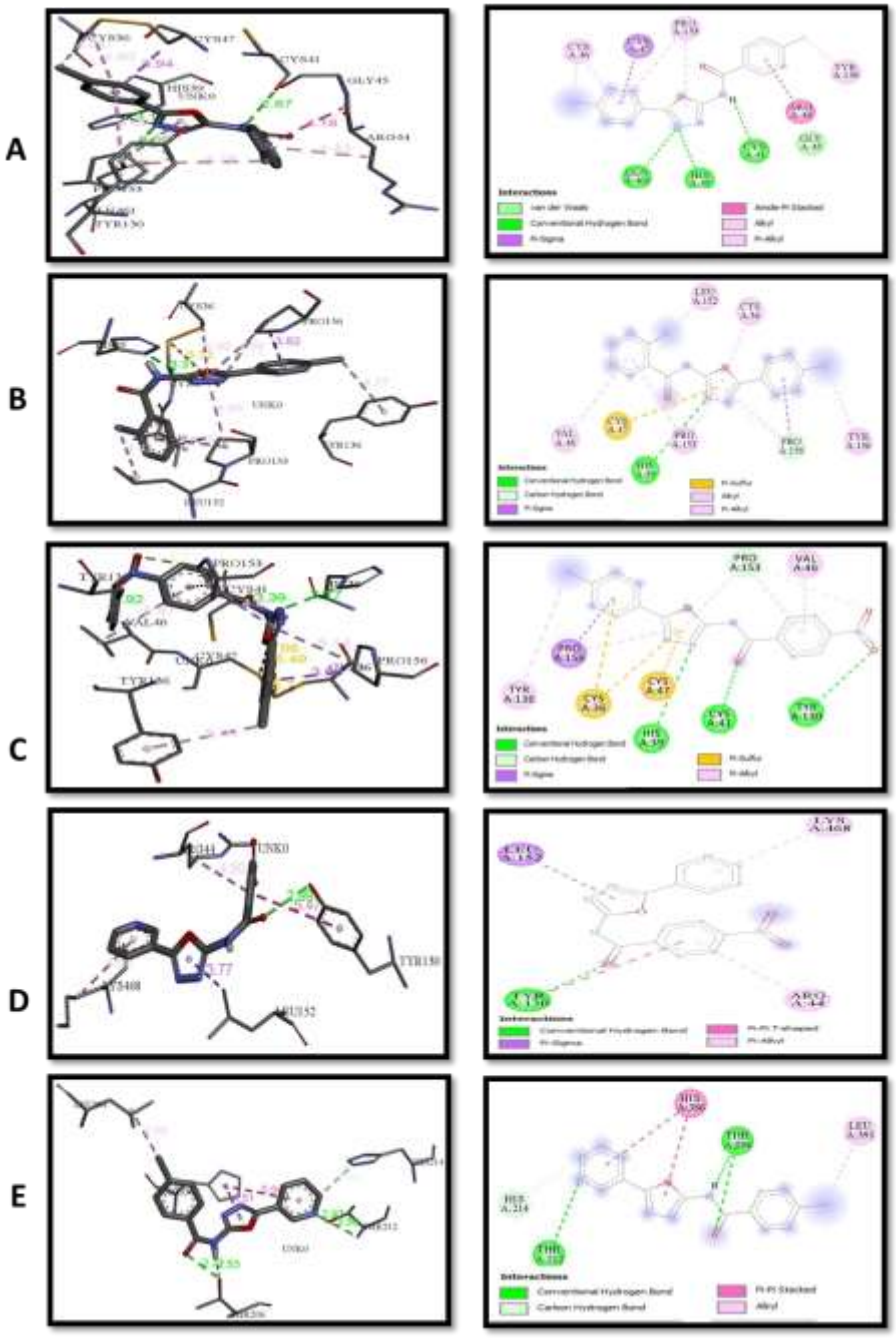

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Figure 6: Ligand–protein interactions. (A) Binding interactions of compound 1a with the active site of cyclooxygenase (PDB ID: 5F1A), (B) interactions of compound 1b, (C) interactions of compound 1c, (D) interactions of compound 2b, and (E) interactions of compound 2c, all visualized using Discovery Studio 4.1.0. The panels display both three-dimensional and two-dimensional representations of the compounds docked within the enzyme's binding pocket. Dashed lines indicate the distances of bonds between ligand functional groups and protein residues. The inset legend identifies the types of interactions between the ligand atoms and the amino acid residues of the receptor.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

AUTHOR CONTRIBUTION

All the Authors contributed in planning and the collection of data, drafting manuscript and analyzing data to be qualified for Authorship.

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