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Original Article

# MOLECULAR DOCKING, DESIGN, SYNTHESIS, *IN VITRO* ANTIOXIDANT AND ANTI-INFLAMMATORY EVALUATIONS OF NEW ISOQUINOLINE DERIVATIVES

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**ABSTRACT**

**Objectives:** To design and synthesis N-substituted (E)-4-arylidene-isoquinoline-1,3-dione derivatives as anti-inflammatory and antioxidant drug moieties using molecular docking as a tool.

**Methods:** The structure of compounds (5a-h) was elucidated by means of FT-IR, GC-MS and NMR spectroscopy. Molecular docking was carried out to clarify the molecular aspects of the observed COX-inhibitory activities of the investigated compounds. DPPH radical scavenging analysis method was used to determine antioxidant activity and i*n-vitro* anti-Inflammatory activity was conducted by Human Red Blood Cell (HRBC) membrane stabilization method utilizing Diclofenac sodium as standard.

**Results:** Isoquinoline (N-substituted (E)-4-arylidene-isoquinoline-1,3-dione) derivatives were achieved using oxalic acid as the catalyst, by aldol condensation of the corresponding aldehydes and the corresponding N-substituted homophthalimides with a maximum yield of 92%. Ligand efficiency (LE) consequences being a clear indication that the action potential of the compounds 5e (-0.72) and 5d (-0.64) is high when compare with the standard (-0.63) for COX-1. While for COX-2, compounds 5e (-0.81) 5d (-0.79) and 5h (-0.98) were shown a remarkable ligand efficiency than the standard (-0.65). Anti-inflammatory and antioxidant studies on the compounds 5h<5d<5g was found best activity results.

**Conclusion:** From our overall studies, it was understood that the activities of both *in silico* and *in vitro* anti-inflammatory results are coincide together. The *p*-values were significant for all the compounds 5(a-h) in both COX-1 and COX-2 activities which indicate that all the compounds have 'competency' towards druggability for both anti-inflammatory and antioxidant, especially the compounds 5h<5d<5g<5e can be suggested for *in vivo*.

**Keywords:** Antioxidant, N-arylhomophthalimides, Anti-inflammation, (E)-4arylidne-2-(4 chlorophenyl) isoquinoline 1,3-dione, Molecular Docking.

## INTRODUCTION

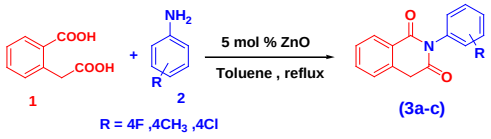
Isoquinolines have established widespread biological activities and constitute a large number of naturally occurring alkaloids. Several isoquinoline alkaloids (berbamine, berberine, cepharanthine and tetrandine) were examined for anti-inflammatory activity [1, 2]. The presence of nitrogen bearing side chain(s) in specific positions of their skeletons improves binding affinity and enhances solubility under physiological conditions [3]. An antioxidant is a chemical that prevents juvenile oxidation of other chemicals. They protect key cell components by neutralizing the damaging effects of free radicals, which are normal by-products of cell metabolism [4, 5]. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol [6]. Inflammation is a complicated biological response of vascular tissue to harmful stimuli caused by injury, infection, environmental agents, malignancy and cellular changes [7]. The inflammatory process deals with the complex interplay between cells of the blood, the blood vessels themselves and the cells of the involved tissue. The process can be seen as a coordinated response to a large number of cells to an initial stimulus [8]. The cyclooxygenase-1 (COX-1) active site is prepared by a long, hydrophobic channel that is the site of non-steroidal anti-inflammatory drug binding [9]. COX exists in two isotherms, COX-1 and COX-2, which are regulated and expressed differently [10-12]. Rowlinson, S. W and group (2003) tested the generality of this binding mode by analyzing the action of a series of COX inhibitors against site-directed mutants of cyclooxygenase-2 (COX-2) bearing changes in Arg-120, Tyr-355, Tyr-348, and Ser-530 [13]. Determination of the crystal structure of a complex of Diclofenac with murine COX-2 demonstrates that Diclofenac binds to COX-2 in an inverted conformation with its carboxylate group hydrogen-bonded to Tyr-385 and Ser-530. AUTODOCK predicts the binding affinity using one conformation of the ligand-protein complex. The primary method for conformations searching is a Lamarckian Genetic Algorithm [14] and AutoDock is shown to be an effective tool capable of quickly and accurately predicting binding conformations and binding energies of ligands with macromolecular targets [15, 16]. On the whole above referred basis, we directed our attention to the synthesis of new isoquinoline (N-substituted (E)-4-arylideneisoquinoline-1,3-dione) derivatives 5(a–h) and biological studies through *in silico* and *in vivo* evaluations.

## MATERIALS AND METHODS

All the chemicals used in the synthesis were of laboratory grade. Solvents and reagents were commercially available and purchased from sigma Aldrich and Avra synthesis. The melting points were observed on an Elchem digital melting point apparatus. FT-IR spectra were issued by SHIMADZU IR affinity 1 spectrometer with anhydrous KBr pellets in the range of 4000–400 $cm^{-1}$. $^1H$ NMR and $^{13}C$ NMR spectra were registered in a Bruker ADVANCE III 400 spectrometer in $CDCl_3$ solution using tetramethylsilane (TMS) as an internal standard. GC-MS was analyzed in GC model Clarus 680 and Mass Spectrometer Clarus 600 (EI); Perkin Elmer, Inc., USA.

### Synthesis of N-aryl homo phthalimides (3a-c)

A mixture of homo phthalic acid (1) and substituted anilines (2) (1:1 ratio) in toluene and 5 mol% ZnO were amended to the suspension. The reaction mixture was heated under reflux condition. The progress of the reaction was monitored by Thin Layer Chromatography (TLC). After the completion of the reaction, the catalyst was separated by filtration. The solvent was removed under vacuum, and then a crude sample was purified by silica gel column chromatography using ethyl acetate and n-hexane mixture as an eluant. The obtained pale yellow solid compounds (3a-c) were characterized by FT-IR, GC-MS, $^1H$ NMR and $^{13}C$ NMR.




**Scheme 1: General synthesis of 2-(4-flurophenyl) isoquinoline-1, 3(2H, 4H)-dione**

**The spectral data of compounds**

**3a; (2-(4-flurophenyl) isoquinoline-1,3 (2H,4H)-dione)**

Melting Point 575–577°C; IR (KBr, $cm^{-1}$) 3061, 1714, 1598, 1510, 1462, 1396, 1259, 1236, 1224, 1207, 1192, 1168, 1139, 1097, 991, 970, 933, 840; $^1H$ NMR (400MHz, $CDCl_3$) 8.277-8.258 (d, J=7.6 Hz, 1H), 7.698-7.661 (t, J=7.4Hz, 1H), 7.533-7.495 (t, J=7.6 Hz, 1H), 7.386-7.367, (t, J=7.6 Hz, 1H), 7.218-7.202 (d, J=6.4 Hz, 1H), 4.242 (s, 2H), $^{13}C$ NMR (400 MHz, $CDCl_3$) 169.9, 165.1, 163.7, 134.2, 134.1, 130.4, 130.3, 129.6, 127.9, 127.4, 116.5, 116.2; GCMS for $C_{15}H_{10}FNO$, calculated (M+) m/z 255.24, found 255.1507.

**3b; (2-(4-methylphenyl) isoquinoline-1,3 (2H,4H)-dione)**

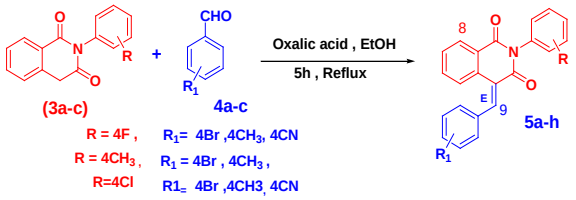
Melting Point 313–315°C, Obtained 314.24°C; IR (KBr, $cm^{-1}$) 2924, 1716, 1668, 1512, 1462, 1369, 1234, 1203, 1138, 1107, 993, 929, 862; $^1H$ NMR (400MHz, $CDCl_3$) 8.289-8.269 (d, J=8.0 Hz, 1H), 7.686-7.649 (t, J=7.4Hz, 1H), 7.525-7.487 (t, J=7.6 Hz, 1H), 7.381-7.333, (t, J=9.6 Hz, 3H), 7.132-7.114 (d, J=7.2 Hz, 2H), 4.244 (s, 2H), 2.449 (s, 3H); $^{13}C$ NMR (400 MHz, $CDCl_3$) 170.0, 165.1, 138.7, 134.2, 133.9, 132.3, 130.0, 129.5, 129.5, 128.1, 127.8, 127.3, 125.5; GCMS for $C_{16}H_{13}NO_2$, calculated (M+) m/z 251.28, found 255.1942.

**3c; (2-(4-chlorophenyl) isoquinoline-1,3 (2H,4H)-dione)**

Melting Point 331–333°C, Obtained 332.89°C; IR (KBr, $cm^{-1}$) 3120, 2924, 1716, 1668, 1602, 1487, 1462, 1365, 1261, 1234, 1195, 1136, 1085, 1012, 991, 931, 860; $^1H$ NMR (400MHz, $CDCl_3$) 8.260-8.241 (d, J=7.6 Hz, 1H), 7.694-7.657 (m, 1H), 7.525-7.477 (m, 3H), 7.372-7.353, (m, J=7.6 Hz, 1H), 7.182-7.160 (d, J=8.8 Hz, 2H); $^{13}C$ NMR (400 MHz, $CDCl_3$) 169.8, 164.9, 134.6, 134.1, 133.9, 133.5, 130.1, 129.9, 129.6, 129.5, 127.9, 127.4, 125.3; GCMS for $C_{15}H_{10}ClNO$, calculated (M+) m/z 271.7, found 271.1232.

**Synthesis of (E)-4-arylidene isoquinoline-1,3-diones derivative (5a-h)**

The general synthesis of compound 5a-he was achieved as shown in the scheme 2. N-Substituted homo phthalimide (3a-c) (0.001 mol) was dissolved in ethanol (10 mL) and the aromatic aldehyde (4a-c) (0.001 mol) followed by oxalic acid (5 mmol) was added and the reaction mixture refluxed for 5 h. After cooling to room temperature, the solid, settled on the bottom was filtered and washed with 10 ml of ethanol and the solvent was evaporated in a vacuum. The obtained product was crystallized in ethanol and the purity was tested by TLC. The obtained pale yellow solid compounds were characterized by FT-IR, GC-MS, $^1H$ NMR, $^{13}C$ NMR.




**Scheme 2: General Synthesis of (E)-4-arylidene isoquinoline-1,3-diones derivative (5a-h)**

**The spectral data of compounds (5a-h)**

**(E)-4-(4-bromobenzylidene)-2-(4-fluorophenyl) isoquinoline-1,3(2H,4H)-dione (5a)**

Melting Point 490-492°C, Obtained 491.55°C; IR (KBr, $cm^{-1}$) 1712, 1668, 1600, 1506, 1402, 1357, 1292, 1240, 1190,1070, 1008, 927, 867; $^1H$ NMR (400MHz, $CDCl_3$) 8.299-8.279 (d, J=8.0 Hz, 1H), 8.136 (s, 1H) 7.640-7.620 (d, J=8.0 Hz, 1H), 7.577-7.558, (d, J=7.6 Hz, 2H), 7.514-7.477 (t, J=7.4 Hz, 1H) 7.438-7.370 (m, 3H), 7.289-7.207 (m, 4H); $^{13}C$ NMR (400 MHz, $CDCl_3$) 165.8, 164.20, 161.3, 143.2, 134.1, 133.8, 132.4, 131.9, 130.4, 130.4, 130.2, 129.6, 129.2, 127.2, 126.2, 125.9, 124.1, 116.2; GCMS for $C_{22}H_{13}BrFNO_2$, calculated (M+) m/z 422.25, found 422.1040.

**(E)-4-(4-methylbenzylidene)-2-(4-fluorophenyl) isoquinoline-1,3 (2H,4H)-dione (5b)**

Melting Point 442-444°C, Obtained 443.02°C; IR (KBr, $cm^{-1}$) 3086, 1710, 1666, 1593, 1508, 1458, 1359, 1240, 1217, 1190,1153, 1126, 1091, 1002, 813; $^1H$ NMR (400MHz, $CDCl_3$) 8.291-8.272 (d, J=7.6 Hz, 1H), 8.234 (s, 1H) 7.776-7.756 (d, J=8.0 Hz, 1H), 7.484-7.397 (m, 4H), 7.378-7.213 (m, 6H), 2.444 (s, 3H); $^{13}C$ NMR (400 MHz, $CDCl_3$) 166.1, 164.4, 163.7, 145.2, 140.3, 132.5, 132.2, 130.4, 130.3, 129.7, 129.3, 129.3, 128.9, 128.9, 127.1, 125.8, 124.9, 116.4, 116.1; GCMS for $C_{23}H_{16}FNO_2$, calculated (M+) m/z 357.38, found 357.1517.

**4-((E)-(2-(4-fluorophenyl)-2,3-dihydro-1,3-dioxoisoquinolin-4(1H)-ylidene)methyl)benzonitrile (5c)**

Melting Point 507-509°C, Obtained 508.01°C; IR (KBr, $cm^{-1}$) 3027, 2211, 1714, 1586, 1475, 1375, 1351, 1251, 121-47, 1186,1174, 1075, 1011, 986, 862; $^1H$ NMR (400MHz, $CDCl_3$) 8.325-8.306 (d, J=7.6 Hz, 1H), 8.182 (s, 1H) 7.746-7.727 (d, J=7.6 Hz, 2H), 7.606-7.588 (d, J=7.2 Hz, 2H) 7.546-7.515 (t, J=6.2 Hz, 1H), 7.427 (s, 2H), 7.284-7.219 (m, 4H); $^{13}C$ NMR (400 MHz, $CDCl_3$) 165.5, 163.9, 163.7, 141.5, 140.2, 132.8, 131.2, 130.3, 130.2, 129.8, 129.3, 128.1, 127.2, 126.1, 118.2, 116.54, 116.3, 113.0; GCMS for $C_{23}H_{13}FN_2O_2$, calculated (M+) m/z 368.36, found 368.0132.

**(E)-4-(4-bromobenzylidene)-2-(4-methylphenyl) isoquinoline-1,3 (2H,4H)-dione (5d)**

Melting Point 501-503°C, Obtained 502.23°C; IR (KBr, $cm^{-1}$) 3078, 1710, 1668, 1602, 1446, 1349, 1227, 1182, 1127, 966 812; $^1H$ NMR (400MHz, $CDCl_3$) 8.306-8.287 (d, J=7.6 Hz, 1H), 8.135 (s, 1H) 7.620-7.558 (m, 3H), 7.502-7.468 (t, J=6.8 Hz, 1H), 7.424-7.348 (m, 5H), 7.182-7.164 (d, J=7.2 Hz, 2H), 2.444 (s, 3H); $^{13}C$ NMR (400 MHz, $CDCl_3$) 165.9, 164.3, 142.8, 138.6, 134.2, 132.6, 132.3, 131.9, 131.1, 130.3, 1301, 129.6, 129.3, 128.1, 127.1, 126.4, 126.1, 123.8; GCMS for $C_{23}H_{16}BrNO_2$, calculated (M+) m/z 418.28, found 418.1310.

**(E)-4-(4-methylbenzylidene)-2-(4-methylphenyl) isoquinoline-1,3(2H,4H)-dione (5e)**

Melting Point 442–444 °C, Obtained 453.7 °C; IR (KBr, $cm^{-1}$) 2970, 1735, 1707, 1664, 1595, 1510, 1446, 1357, 1236, 1217, 1193,1178, 1091, 1002, 817; $^1H$ NMR (400MHz, $CDCl_3$) 8.302-8.283 (d, J=7.6 Hz, 1H), 8.236 (s, 1H) 7.761-7.742 (d, J=7.6 Hz, 1H), 7.476-7.354 (m, 6H), 7.246-7.181 (m, 4H), 2.466 (s, 3H), 2.446 (s, 3H); $^{13}C$ NMR (400 MHz, $CDCl_3$) 166.23, 164.5, 144.9, 140.1, 138.5, 132.9, 132.5, 132.4, 130.0, 129.7, 129.3, 128.9, 128.8, 128.3, 128.2, 127.1, 126.0, 125.1; GCMS for $C_{23}H_{16}FNO_2$, calculated (M+) m/z 353.41, found 353.1938.

**(E)-4-(4-bromobenzylidene)-2-(4-chlorophenyl) isoquinoline-1,3(2H,4H)-dione (5f)**

Melting Point 520–522 °C, Obtained 520.88 °C; IR (KBr, $cm^{-1}$) 3095, 1710, 1668, 1602, 1583, 1460, 1355, 1234, 1182,1134, 1006, 827; $^1H$ NMR (400MHz, $CDCl_3$) 8.295-8.275 (d, J=8.0 Hz, 1H), 8.135 (s, 1H) 7.637-7.617 (d, J=8.0 Hz, 1H), 7.579-7.559 (d, J=8.0 Hz, 2H), 7.529-7.478 (m, 3H), 7.441-7.369 (m, 3H), 7.245-7.224 (d, J=8.0 Hz, 2H); $^{13}C$ NMR (400 MHz, $CDCl_3$) 165.6, 164.0, 143.2, 134.7, 134.1, 133.7, 132.8, 132.3, 131.8, 130.3, 129.9, 129.6, 129.6, 129.4, 127.2, 126.1, 125.9, 124.0; GCMS for $C_{23}H_{16}FNO_2$, calculated (M+) m/z 438.7, found 439.1080.

**(E)-4-(4-methylbenzylidene)-2-(4-chlorophenyl) isoquinoline-1,3(2H,4H)-dione (5g)**

Melting Point 471–473°C, Obtained 472.35°C; IR (KBr, $cm^{-1}$) 2970, 1737, 1710, 1664, 1593, 1490, 1446, 1357, 1274, 1234, 1193, 1178, 1085, 1001, 923, 923, 817; $^1H$ NMR (400MHz, $CDCl_3$) 8.281-8.262 (d, J=7.6 Hz, 1H), 8.227 (s, 1H) 7.771-7.752 (d, J=7.6Hz, 1H), 7.529-7.376 (m, 6H), 7.289-7.223, (m, 4H); $^{13}C$ NMR (400 MHz, $CDCl_3$) 165.9, 164.2, 145.3, 140.3, 134.3, 134.5, 133.9, 132.6, 132.5, 132.1, 130.1, 129.7, 129.5, 129.3, 129.0, 128.9, 127.2, 125.7, 124.8; GCMS for $C_{16}H_{13}NO_2$, calculated (M+) m/z 373.83, found 373.1310.

**4-((E)-(2-(4-chlorophenyl)-2,3-dihydro-1,3-dioxoisoquinolin-4(1H)-ylidene)methyl)benzonitrile (5h)**

Melting Point 536–538°C, Obtained 537.34°C; IR (KBr, $cm^{-1}$) 3074, 2227, 1714, 1666, 1597, 1490, 1375, 1251, 1236, 1186, 1174, 1087,

1014, 974, 862; $^1$H NMR (400MHz, $CDCl_3$) 8.312-8.293 (d, J=7.6 Hz, 1H), 8.175 (s, 1H) 7.598-7.578 (d, J=8.0Hz, 2H), 7.547-7.494 (m, 3H), 7.445-7.412 (m, 2H), 7.251-7.216 (m, 2H); $^{13}$C NMR (400 MHz, $CDCl_3$) 165.3, 163.8, 141.6, 140.2, 134.8, 133.0, 132.8, 131.2, 129.9, 129.9, 129.8, 129.3, 127.9, 127., 126.1, 118.2, 113.1; GCMS for $C_{23}H_{13}ClN_2O_2$, calculated (M+) m/z 384.81, found 384.2451.

**Statistical analysis**

All biological *in vitro* and *in silico* experiments results were expressed as percentage decrease with respect to control values and compared by one-way ANOVA with Dunnett's post test was performed. GraphPad Prism version 6.07 for Windows, GraphPad Software, San Diego California USA, www. graphpad. com was used for statistical analysis. A difference was considered statistically significant if $p \leq 0.05$. The 50% inhibitory concentration ($IC_{50}$) was calculated from the dose-response curve obtained by plotting percentage inhibition versus concentrations.

**Molecular docking studies**

Docking studies were performed in order to get more insight into the binding mode of the compounds. Docking studies were conducted by using Autodock version 4.2.6 and Autodock Tools (ADT) version 1.5.6. and the Arguslab version 4.0.1. The structures of compounds 5(a-h) and standard (Diclofenac–Fig.1) were generated as ligands using Chemdraw ultra 10.0 version of Cambridge University. Their 3D atomic coordinates were created utilizing the ACD/labs–Chemsketch 12.0 software. Compound geometries were cleaned and generated as the corresponding *pdb.* files using the Argus lab software. The three-dimensional structure of human COX–1 (PDB id: 1CQE) and COX-2 (PDB id: 6COX) enzymes were retrieved from the protein data bank (PDB) (Source: www. rcsb. org/pdb/). The proteins and ligands in the docking tests were treated using the united-atom approximation and only polar hydrogens were added to the protein, and Kollman united atom partial charges were assigned. Unless stated otherwise, all waters were removed [17]. The *pdbqt* files for protein and ligands preparation and grid box creation were completed using Graphical User Interface program AutoDock Tools (ADT). AutoGrid was used for the preparation of the grid map using a grid box. The grid size was set to 66 × 66 × 66 xyz points with grid spacing of 0.385 Å and grid center was designated at dimensions (x, y, and z): 1.085, 0.864 and 2.564. The ligands 5(a-h) were docked into the active sites of COX-1 and COX-2. The results less than 2.0 Å in positional root-mean-square deviation (RMSD) was clustered together and represented by the result with the most favorable free energy of binding. The docked poses with Lowest Binding Energy (LBE), Hydrogen bond, π-π interaction and π-cation interaction results were recorded (table 1&2) and validated.

***In-vitro* anti-inflammatory activity**

*In-vitro* Anti-Inflammatory activity was carried out by Human Red Blood Cell (HRBC) membrane stabilization method [18] using Diclofenac sodium as standard. 5 ml of human blood was collected from a healthy volunteer and mixed with equal volume of sterilized Alsever solution. The blood was subsequently centrifuged at 3000 rpm and packed cells were washed with isosaline 0.85% (pH 7.21) and a 10% v/v suspension was made with isosaline. Drug concentrations of 50, 100, 150, 200 µg/ml were prepared. The desired concentration of the drug was combined with 1 ml phosphate buffer (0.15 M, pH 7.4), 2 ml hypo saline (0.36%) and 0.5 ml HRBC suspension. Instead of hypo saline 2 ml distilled water served as the control. Hemoglobin content in the supernatant solution obtained after centrifugation was estimated at 560 nm. The percentage haemolysis was calculated by assuming the haemolysis produced in the presence of distilled water at 100%. The percentage of HRBC membrane stabilization was calculated and tabulated (table 4) using the following formula:

$$\% \text{ Inhibition of haemolysis} = 100 \times [(OD1-OD2)/OD1]$$

Where OD2 = optical density of sample OD1 = optical density of control.

**DPPH radical scavenging analysis of antioxidant activity**

The antioxidant activity was evaluated in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. Experiments were performed according to the method of Gardeli, C [19]. 4.3 mg of DPPH (1, 1-Diphenyl–2-picrylhydrazyl) is mixed with 3.3 ml methanol. It is shielded from light by covering the test tubes with aluminum foil. 150 µl of DPPH solution were added to 3 ml methanol and absorbance was noted at 517 nm as control. 10-200 µl of different concentrations of test compounds and standard compound were taken and the volume was made up to 200 µl using methanol. The samples were then diluted with methanol up to 3 ml and to each 150 µl DPPH was added. Absorbance was taken after 15 min at 517 nm using methanol as blank on the UV-visible spectrometer. The scavenging activity percentage (AA %) was determined according to Mensor [20]. The amount of sample necessary to decrease the observance of DPPH by 50% ($IC_{50}$) was calculated graphically for the test compounds in five different concentrations. The percentage inhibition of the DPPH radical was calculated and tabulated (table 5) using the following formula:

$$AA\% = 100-[(A_2-A_1) \times 100]/A_0$$

Where A2 = absorbance of the sample, the A1= absorbance of blank and A0= absorbance of control

**Table 1: Chemical yield of the new compounds 5(a-h) via Scheme 1 and 2**

| Compound *3 (a-c)* | Compound *4 (a-c)* Conditions | Reaction time | Compounds | Yield % |
|---|---|---|---|---|
| $C_{15}H_{10}FNO$ (0.001 mol) | 4-bromo benzaldehyde (0.001 mol), Ethanol (10 mL), Oxalic acid (5 mmol) | 5 H | 5a, 5d, 5f | 92, 89, 90 |
| $C_{16}H_{13}NO_2$ (0.001 mol) | 4-Methyl benzaldehyde (0.001 mol), Ethanol (10 mL), Oxalic acid (5 mmol) | 5 H | 5b, 5e, 5g | 90, 91, 90 |
| $C_{15}H_{10}ClNO$ (0.001 mol) | 4-formylbenzonitrile (0.001 mol), Ethanol (10 mL), Oxalic acid (5 mmol) | 5 H | 5c, 5h | 88, 86 |

**RESULTS AND DISCUSSION**

**Chemistry**

Synthesis of intermediate and target compounds was accomplished according to the steps depicted in scheme 1 and scheme 2. The (E)-4-arylidene isoquinoline-1,3-diones derivatives 5(a–h) were obtained by condensation of aromatic aldehydes 3(a-c) with N-substituted homo phthalimides 4(a-c) in ethanol using oxalic acid as catalyst. All synthesized derivatives appeared as yellow solid and the chemical yield of the new compounds 5(a-h) was calculated and tabulated (table 1). (See figure-1 in supplementary file for synthesized structures of the compounds 5 (a-h))

**Molecular docking analysis**

Dog site [21] web server was employed to detect the binding pocket of 1CQE and 6COX. Diclofenac was used as the standard (fig. 1) for docking analysis. The results of the molecular docking analysis indicate that all the compounds were more selective towards COX-2 than COX-1. The expected binding free energy for COX-2 was found between-31.94 and-6.03 kcal/mol and the COX-1 showed the binding free energy between-18.77 and-0.56 kcal/mol. These free energy values indicating that the newly synthesized compounds had shown a fortunate selectivity towards COX-2 instead of COX-1. The best-squared correlation coefficient was observed ($r^2$=-0. 91) between binding affinities (Y-axis) and experimental values for COX-2, 6COX (fig. 2).

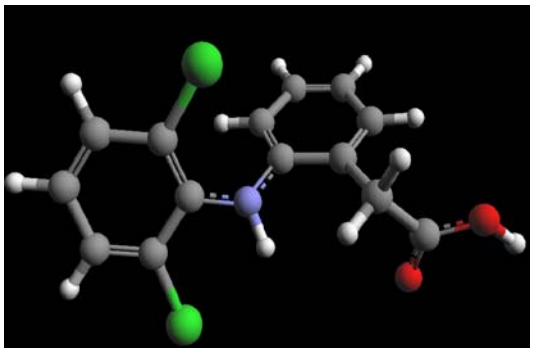


**Fig. 1: Molecular structure of the standard (Diclofenac)**

The 2D view of protein–ligand interactions of the best poses generated by COX-1 and COX-2 studied routines are shown in fig. 3. All the top docked poses generated (table 1&2) by each docking routine exhibited well-established bonds with one or more amino acids in the binding pocket of 1CQE and 6COX. Especially with three hydrogen bonds were evolved with HIS207 for 1CQE and four hydrogen bonds evolved with HIS388 for 6COX. Different sets of hydrogen bonding interactions with polar side chain residues of ASN515, ARG83, GLY289, HIS207, ILE137, SER579, THR94 and TYR136 were observed at distances within 2.9 Å. For 1CQE and 6COX almost all ligands (5a-h) were shown π-π interactions show the drug efficiency of the compounds.

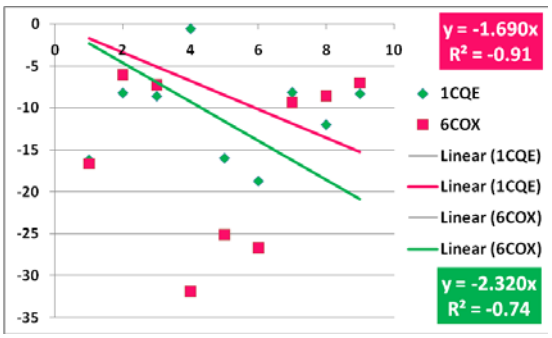



**Fig. 2: Squared correlation coefficient comparison for COX1 (1CQE) and COX2 (6COX)**

**Table 2: Molecular docking scores of 5(a-h) with COX-1 (1CQE)**

| Compounds | LBE (kcal/mol) | LE | AA# residue H-bond Å | | π-π interaction | π-cation interaction |
|---|---|---|---|---|---|---|
| 5a | -8.23 | -0.3 | ASN515: HN | 2.22Å | HIS207, HIS214, PHE198, THR383 | NF** |
| 5b | -8.62 | -0.32 | ARG83: H H12 | 1.826Å | HIS90, PHE381 | ARG120 |
| 5c | -0.56 | -0.02 | HIS386: HE21 HIS388: HE2 1 | 2.102Å, Invisible | TRP387, TYR348, TYR385 | NF |
| 5d | -16.03 | -0.64 | ILE137: HN 1 | 2.159Å, | PHE210 | NF |
| 5e | -18.77 | -0.72 | HIS207: HE2 1 | 2.159Å | PHE210 | NF |
| 5f | -8.16 | -0.29 | THR94: HG1 1 | 1.937Å | HIS207 | NF |
| 5g | -12.02 | -0.42 | PHE580: HN1 | 2.158Å | HIS388 | NF |
| 5h | -8.32 | -0.31 | HIS207: HE2 1, GLN289: HE22 1 | 1.856Å, 1.855Å | HIS388, PHE404 | NF |
| Std* | -16.23 | -0.63 | ASN382: HD21 1, THR212: HN1 1, THR212: HG1 1 | 2.114Å, 1.826Å, 2.044Å | PHE209, PHE381 | NF |

#Amino Acid, * Std-Standard (Diclofenac), **NF-Not Formed

**Table 3: Molecular docking scores of 5(a-h) with COX-2 (6COX)**

| Compounds | LBE (Kcal/mol) | LE | AA# residue H-bond Å | | π-π interaction | π-cation interaction |
|---|---|---|---|---|---|---|
| 5a | -6.03 | -0.22 | HIS388: HE2 1 | 2.215Å | HIS207, HIS214, PHE198, THR383 | ARG120 |
| 5b | -7.25 | -0.25 | NF | NF | HOS90, PHE381 | ARG120 |
| 5c | -7.03 | -0.28 | HIS388: HE2 1 | 1.593Å | HIS388, PHE404 | NF** |
| 5d | -25.14 | -0.79 | TYR385:O 1, GLN203:O 1 | 2.677Å, Invisible | PHE210 | NF |
| 5e | -26.69 | -0.81 | HIS388: HE2 1 | 2.146Å | PHE210 | NF |
| 5f | -9.35 | -0.42 | HIS388: HE2 1 | 1.745Å | HIS207 | NF |
| 5g | -8.56 | -0.34 | HIS388: HE2 1 | 2.129Å | HIS388 | NF |
| 5h | -31.94 | -0.98 | MET522:O | 2.433Å | TRP387, TYR348, TYR385 | NF |
| Std* | -18.69 | -0.71 | HIS386: HE2 1 | 2.176Å | PHE209, PHE381 | NF |

#Amino Acid, * Std-Standard (Diclofenac), **NF-Not Formed

An ionic interaction with the side chain residue Arg120 was exposed in 1CQE for 5b and in 6COX for 5a and 5b (fig. 4a). Interestingly 5b showed both π–cation (ARG120) and π-π interactions (HIS90, PHE381 and TYR355) (fig. 4b). Compounds 5a-h formed strong hydrophobic interactions with nonpolar residues in 1CQE like GLN289, ILE523, ILE89, LEU390, and LYS211 and in 6COX like ALA516, ILE517, PHE518, VAL349 and VAL349 are suggested to increase the binding affinity (fig. 5).

The results of the ligand docking showed that the binding pocket involves the amino acid residues of 1CQE like ASN382, 515; ARG49, 83, 120, 433; Asp145; GLU290, 346, 347, 524; PHE91, 210, 356, 580; GLY214, 354; HIS90, 207, 274, 386, 388, 513; ILE46, 89, 137; LYS211, 222, 565; LEU92, 93, 115, 294, 295, 584; PRO86, 191, 514; GLN289, 350, 351, 358; SER85, 87, 138, 213, 516, 579; THR94, 212; VAL 116, 291, 578; TRP100; TYR130, 136, 385; CYS12, 512;

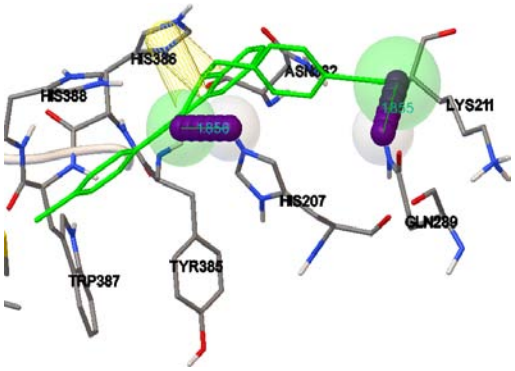



**Fig. 3a: The proposed binding mode of *5e* into the active binding site of 1CQE with a lowest binding energy of-8.32. Two hydrogen bonds (1.855Å&1.856Å) with HIS207 and LYS211 formed with π-π (Non-covalent) interaction**

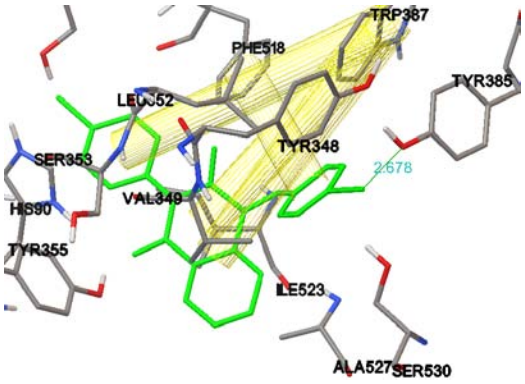



**Fig. 3b: The proposed binding mode of *5h* into the active binding site of 6COX with a lowest binding energy of-19.62. A hydrogen bond (2.678Å) with one of a key residue TYR385 formed with π-π (non-covalent) interaction**

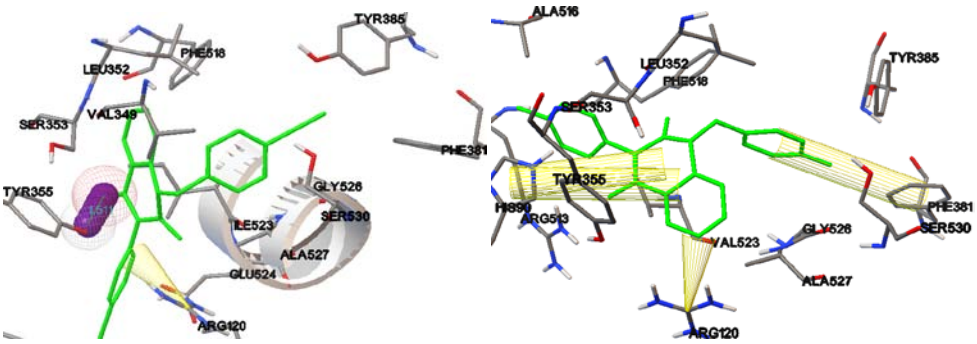



**Fig. 4(a, b): Result shows π-cation interaction between the residue AR120 and ligand *5a* (1CQE) and *5b* (6COX)**

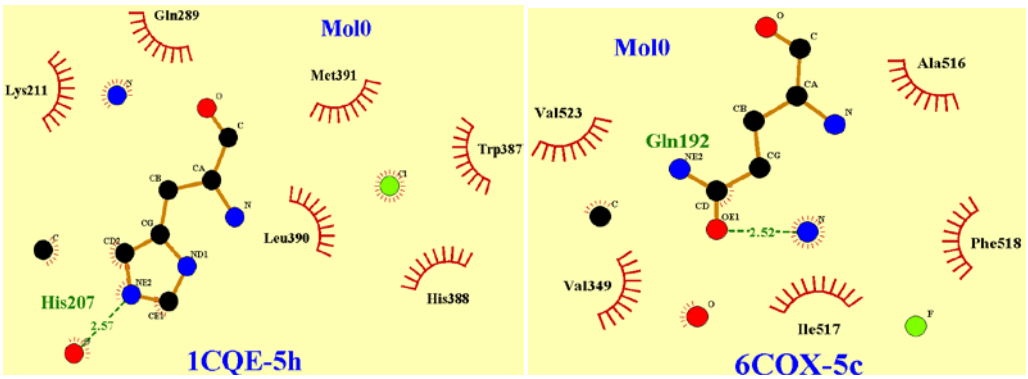



**Fig. 5(a, b): Ligands showing the interaction with the crucial residues in the active site cleft**

And with the key residues of 6COX like ALA 199, 202, 517, 527; ARG120, 513, 222; ASN104, 382; GLN192, 203, 289, 350; GLU346; GLY354, 526; HIS90, 207, 366, 386, 388; ILE89, 200, 517, 523; LEU531, 350-51, 359, 384, 390-91; LYS211; PHE210, 381, 210, 404, 518; SER353, 516, 530; THR206, 212; TRP387; TYR348, 355, 385; VAL116, 295, 349, 444, 447, 523 as shown in the fig. 5-7. The important hydrogen bond forming amino acid residues (table 1&2) for 1CQE was ASN515; PHE580; HIS207, 386, 388; ILE137; GLN289; THR94; VAL578; TYR 385 and for 6COX it was GLN230; HIS386, 388; MET522 and TYR355, 385.

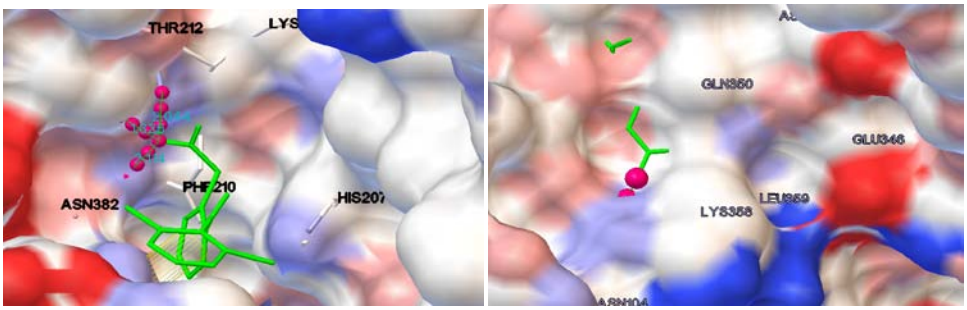



**Fig. 5(a, b): Diclofenac in the binding pocket of 1CQE & 6COX**

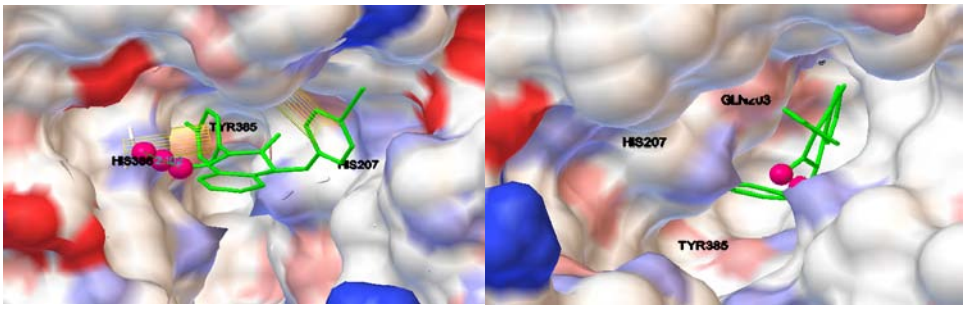



**Fig. 6(a, b): Compound *5c* in 1CQE and *5e* in 6COX binding pockets**

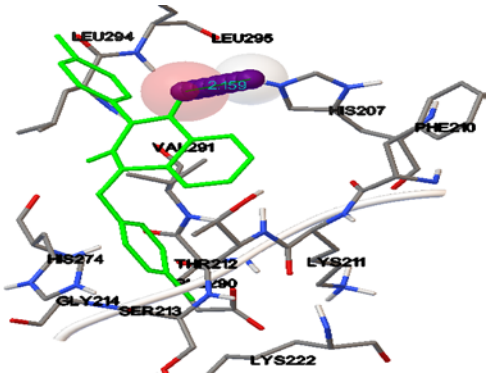



**Fig. 7a: Ribbon shows close residues for the ligands (*5e*-1CQE)**

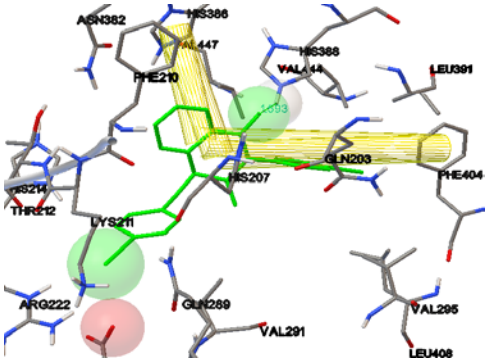



**Fig. 7b: Close residues along with one invisible H-bond and π-π (Non-covalent) interaction (*5h*-6COX)**

Ligand efficiency (LE) results (table 1&2) clearly showing that the action potential of the compounds 5e (-0.72) and 5d (-0.64) is high when compare with the standard (-0.63) for COX-1. While for COX-2, compounds 5e (-0.81) 5d (-0.79) and 5h (0.98) were shown a remarkable Ligand efficiency than the standard (-0.65). The RMSD (Root-Mean-Square Deviation) (table 3) was found at 0.28Å with a low Estimated Inhibition Constant (EIC)–(*ki)* value of 354.99 uM for 5e was the best among all analyzed ligands of COX-1 (1CQE). The lowest LBE (least binding energy) was-18.77 kcal/mol. While the COX-2 (6COX) had an RMSD value of 0.81Å, *ki* value as slow as 389.12 mM for the compound 5h with a LBE of-31.94 kcal/mol. The standard Diclofenac had an average RMSD of 0.7, *ki* as low as 20.05 uM and the LBE was-17.46 kcal/mol.

**Table 4: Results of statistical mechanical analysis for best-docked poses in 5(a-h)**

| Compounds | Binding Energy (kcal/mol) | | RMSD | | Inhibition Constant (*ki*) | |
|---|---|---|---|---|---|---|
| | COX-1 | COX-2 | COX-1 | COX-2 | COX-1 | COX-2 |
| 5a | -8.23 | -6.03 | 0.88 | 0.59 | 46.54 uM | 925.51 nM |
| 5b | -8.62 | -7.25 | 0.59 | 0.3 | 4.85 uM | 480.77 nM |
| 5c | -0.56 | -7.03 | 0.32 | 0.68 | 197.20 aM | 68.15nM |
| 5d | -16.03 | -25.14 | 0.94 | 0.62 | 20.05 uM | 44.29 nM |
| 5e | -18.77 | -26.69 | 0.28 | 0.78 | 354.99 uM | 48.52 nM |
| 5f | -8.16 | -9.35 | 0.59 | 0.62 | 139 nM | 244.95 nM |
| 5g | -12.02 | -8.56 | 0.81 | 0.86 | 529.95 nM | 793.99 nM |
| 5h | -8.32 | -31.94 | 0.71 | 0.81 | 7.06 uM | 389.12 mM |
| Std* | -16.23 | -18.69 | 0.69 | 0.71 | 19.95 uM | 20.15 uM |

* Std-Standard (Diclofenac)

***In-vitro* anti-Inflammatory activity**

As per the results stated in table 4, the maximum values were exposed only at the higher concentration. From this, it was understood that the anti-inflammatory activity of compounds 5(a-h) is dose depended.

The calculated % inhibition indicates that the compounds 5d and 5h showing best activity when is compared to rest compounds while compounds 5a and 5e showing a moderate activity. Also the $IC_{50}$ values almost equal for 5h with standard Diclofenac. Surprisingly, $IC_{50}$ value was a little bit less than the standard for the compound 5d indicate its drug efficiency.

**Antioxidant activity**

As per the results depicted in Table-5, it was important to understand that the antioxidant activity of the compounds 5(a-h) is also dose depended. The calculated % inhibition indicates that the compounds 5b and 5e showing less activity when is compared to rest compounds while the compounds 5d, 5g and 5h showing an equal activity when compared to the standard ascorbic acid.

**Table 5: *In-vitro* anti-inflammatory activity of synthesized analogues***

| Compounds | % Inhibition mean±SEM | R square | *p*-value | $IC_{50}$ |
|---|---|---|---|---|
| 5a | 75.98±7.59** | 0.9794 | 0.0103 | 53.823 |
| 5b | 65.31±5.43** | 0.9449 | 0.028 | 96.331 |
| 5c | 63.24±7.41** | 0.9237 | 0.0389 | 94.385 |
| 5d | 82.21±8.33** | 0.9836 | 0.0082 | 35.429 |
| 5e | 80.35±9.23** | 0.9461 | 0.0273 | 49.983 |
| 5f | 73.03±6.46** | 0.949 | 0.0258 | 67.066 |
| 5g | 73.98±7.15** | 0.9827 | 0.0087 | 63.579 |
| 5h | 81.72±7.84** | 0.9631 | 0.0186 | 35.897 |
| Diclofenac | 87.93±3.59** | 0.9752 | 0.0125 | 36.363 |
| Control[#] | No inhibition | -- | -- | -- |

[#]Distilled water, *Data are expressed as mean±SEM. (N = 3) and the results considered significant when P<0.05

**Table 6: Results of antioxidant activity of synthesized analogues***

| Compounds | % inhibition mean±SEM | R square | *p*-value | IC50 Value |
|---|---|---|---|---|
| 5a | 63.16±09.46** | 0.9802 | 0.0012 | 75.1634 |
| 5b | 52.06±11.88** | 0.9009 | 0.0137 | 122.972 |
| 5c | 63.79±09.27** | 0.8624 | 0.0226 | 89.6115 |
| 5d | 68.92±10.29** | 0.9436 | 0.0058 | 68.323 |
| 5e | 42.65±06.30** | 0.9193 | 0.01 | 187.274 |
| 5f | 62.36±09.81** | 0.9802 | 0.0012 | 88.575 |
| 5g | 68.01±11.42** | 0.938 | 0.0067 | 69.216 |
| 5h | 67.38±08.11** | 0.8973 | 0.0144 | 56.9444 |
| Ascorbic acid | 70.24±10.54** | 0.9526 | 0.0044 | 67.9058 |
| Control[#] | No inhibition | -- | -- | -- |

[#]Methanol+DPPH, * Data is expressed as mean±SEM. (N = 4) and the results considered significant when P<0.05

Also the $IC_{50}$ value found for 5h was lower than the standard, but it was almost equal for the compounds 5d and 5f, only a negligible variation found for 5a with the standard.

From all these analyses, except the compounds 5b and 5e rest all compounds were shown best antioxidant activities.

**Statistical results**

As mentioned, statistical analyzes were performed on all the part of this study. $R^2$ values and the *p*-values were calculated and used to identify the final results. The value $R^2$ quantifies the goodness of fit while a *p*-value helps to determine the significance of the

consequences. In the molecular docking studies, comparing the RMSD values, both COX-1 and COX-2 were shown a better result than the standard and the compounds are more selective for COX-2. For anti-inflammatory the calculated $R^2$ values of the standard were 0.9835 which was coinciding with the $R^2$ value of compound 5d (0.9836), but compound 5g and 5h had better $R^2$ values as, 0.9827 and 0.9534. The *p*-values were significant for all the compounds 5(a-h). When comparing the $IC_{50}$ values for anti-inflammatory studies with the standard ($IC_{50}$-36.36), the compound 5d and 5h had a lesser $IC_{50}$ value of 35.43 and 35.89. It is a remarkable indication that these compounds have the capability to be a good drug like the standard. For the antioxidant results, the *p*-values were significant for all the compounds 5(a-h). The $R^2$ values of standard (0.9526) closely coincided with the compounds 5d (0.9436) and 5g (0.9380), but compound 5h had better $R^2$ value (0.8973). $IC_{50}$ value was very less for the compound 5h (56.944) when compare the standard (67.905) and compounds 5d (68.322) and 5g (69.215).

## DISCUSSION

(E)-4-arylideneisoquinoline-1,3-diones derivatives obtained by condensation of aromatic aldehydes with N-methyl homo phthalimide or N-phenyl homo phthalimide in dry chloroform using piperidine as a catalyst [22]. Comparing [22] the targeted compounds were obtained in this study using an Eco-friendly catalyst (Ethanol) with a remarkable purity and high yield when compared with the reported. COX-2 inhibition reported by hydrogen bonds formed in Arg 120 and Tyr 385, with the free binding energy of–1.73 kcal/mol [23]. In this study, ionic interaction with the side chain residue Arg120 was found in 1CQE for 5b and in 6COX for 5a and 5b. Interestingly 5b showed both π–cation (ARG120) and π-π interactions (HIS90, PHE381 and TYR355) with a lowest free binding energy of-26.69 kcal/mol. COX (Cyclooxygenase) was believed to be expressed constitutively with constant levels in individual tissues [24]. Prostaglandin synthesis was believed to increase in inflammation because of increased release of precursor [25]. COX activity increases in inflammation and this increase can be prevented by corticosteroids [26].

The increased COX2 inhibitory results of our report reflect the above-mentioned [24-26] points rightfully. This due to the compounds may show anti-corticosteroid activity. Both the COX inhibition results and *in vitro* anti-inflammatory results coincided together for the compounds 5h, 5d and 5e. Top three binding energy value for COX2 was found as for 5d-25.14 kcal/mol, 5e-26.69 kcal/mol and for 5h-31.94 kcal/mol. While the top three % inhibition mean±SEM was found for 5d 82.21±8.33, 5h 81.72±7.84, 5e 80.35±9.23. This suggests the need for further *in vivo* investigations. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), a stable free radical, accepts hydrogen or an electron to become a durable diamagnetic molecule [27]. It is commonly used to test the free radical scavenging ability of various samples. Free radical scavenging activity is gradually increased with the increase in the concentration of the compounds tested and Ascorbic acid (table 6) may be due to the dose-dependent activity variations. This is incongruent with previous *in vitro* studies [28, 29].

## CONCLUSION

Eight compounds were synthesized and screened for antioxidant and anti-inflammatory studies. Docking study of these synthesized compounds was also performed. Most of the compounds exhibited significant anti-inflammatory and antioxidant activity. Compounds 5d, 5e, 5g and 5h exhibited more prominent and constituted anti-inflammatory activity. Compounds 5d, 5g and 5h showed strong antioxidant activity in the DPPH tests. From the detailed analysis of the results of pharmacological studies, we conclude that the synthesized compounds have not only retained but showed enhanced anti-inflammatory profile. Also, all the synthesized derivatives exhibited significant antioxidant activity. The *p*-values were significant for all the compounds 5(a-h) in both COX-1 and COX-2 activities which indicate that all the compounds have 'competency' towards drug-ability for both anti-inflammatory and antioxidant especially the compounds 5h<5d<5g<5e can be suggested for *in vivo*.

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## CONFLICT OF INTERESTS

Declared None

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