NEW DECAHYDROACRIDINE-1,8-DIONES DERIVED FROM 3-AMINOCYCLOHEX-2-EN-1-ONE: SYNTHESIS, CHARACTERIZATION, ANTIOXIDANT, In-vitro, AND In-silico ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT
New decahydroacridine-1,8-dione derivatives have been synthesized from 3-aminocyclohex-2-en-1-one. The compounds were characterized based on spectroscopic data and assessed their antioxidant and anti-inflammatory properties both in-vitro and in-silico. All the compounds exhibited poor in-vitro antioxidant activity but high in-vitro anti-inflammatory activity. An in-silico study using Autodock v.4.2 integrated LigandScout software 4.4.3. against the COX-1 (PBD ID: 1CQE) and COX-2 (PBD ID: 6COX) enzymes indicated that all the compounds could interact with both enzymes. Except for 3c against COX-1, the compounds’ interaction with both enzymes exhibited binding energy lower than diclofenac and curcumin. The results confirm that the compounds have a high potential to be anti-inflammatory agents and deserve further development.

Keywords: Synthesis, Decahydroacridine-1,8-diones, Acridine-1,8-dione, Antioxidant, Anti-inflammatory, In-vitro, In-silico.

INTRODUCTION
Nonsteroidal anti-inflammatory drugs (NSAIDs) are pain reliever drugs that are most frequently applied in adults to treat chronic health problems, such as arthritis and lupus. However, these drugs often exhibit adverse side effects, especially gastric irritation.¹-³ The carboxylic functional group in their molecular structures would enable gastric cyclooxygenase-1 (COX-1) inhibition and direct gastric mucosal irritation associated with oral administration of the drugs.⁴ So, it is attractive to discover novel NSAIDs compounds that do not have the carboxylic functional group. The acridine-1,8-dione derivatives exhibit various biological activities such as anticancer, antimarial, antimicrobial, antiglaucoma, carbonic anhydrase inhibitor, antioxidant, and anti-inflammatory.⁵-¹² The compounds are typically prepared using the Hantzsch procedure by reacting 5,5-dimethyl-1,3-cyclohexanedione, aldehydes, and nitrogen-containing compounds such as ammonium acetate, amines, aldehydes, or other primary amines.⁵-¹² Various methods have been applied to synthesize the acridine-1,8-diones, but all the methods utilized 5,5-dimethyl-1,3-cyclohexanedione as starting material and resulted 3,3,6,6-tetramethyl-9-phenyl-3,4,6,7,9,10-hexahydroacridine-1,8-dione.⁵-¹¹,¹３-¹⁴ To further explore the acridine-1,8-diones as an antioxidant and anti-inflammatory, we have synthesized new decahydroacridine-1,8-dione derivatives (without four methyl substitutions at positions 3, 3, 6, and 6) (Fig.-1) using 3-aminocyclohex-2-en-1-one as starting material. In addition, we report the molecular docking study to predict the compounds’ binding affinity to cyclooxygenase enzymes.

EXPERIMENTAL

General Procedure
The chemicals we used were bought from Sigma/Merck with no purification further. Melting point measurements were carried out using the melting point device (Bibby Sterlin, UK). The compound's purity and reaction rates were observed by thin-layer chromatography (TLC). Infrared spectra measurements were performed on an FTIR-8400 Spectrometer (Shimadzu, Japan). Agilent Nuclear Magnetic Resonance

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Spectromer was utilized to obtain the compound's NMR spectra. LC-MS/MS with electrospray ionization (+) mode (UNIFI-Waters, Milford, MA, USA) was used to analyze the compound's mass spectra (MS).

**Synthesis of 9-phenyl-decahydroacridine-1,8-diones (3a-f).**

A solution of equimolar of 3-aminocyclohex-2-en-1-one (1) and aromatic aldehydes (2a-f) in anhydrous acetic acid was heated under reflux for 2–5 h until completed reaction. Then the solution volume was reduced by evaporation to about one-third, cooled, and the precipitate formed was filtered off, washed up by cold distilled water, and dried. The purification by recrystallization from ethanol or column chromatography afforded pure compounds 3a-f. Replacement of 2a-f with aromatic aldehydes (2g-i) did not produce the target compounds 3g-i. Compound 3a. Yield 70%, m.p. 250-251°C, FT-IR (υ/cm⁻¹): 3194, 3050, 2980, 1641, 1606, 1498, 1355, 1230, 1180, and 1130. ¹H-NMR (δ/ppm, J/Hz): 9.44 (s, 1H), 7.03 (m, J=3, 1H), 7.13 (7, J=3, 2H), 7.15 (d, J=3, 2H), 4.90 (s, 1H), 2.50 (m, overlap with DMSO, 4H), 1.91 (m, J=6, 2H), 12.17 (m, J=5, 4H), 1.79 (m, J=4, 2H). ¹³C-NMR (δ/ppm): 194.9 (2C=O), 152.2 (1C=C-N), 147.4 (1C=C-C), 127.8 (2CAH), 127.5 (2CAH), 125.4 (1CAH), 121.4 (2C=C-C), 36.8 (2CAI), 32.1 (1CAI), 26.3 (2CAI), 20.8 (2CAI). MS: m/z obtained 328.10874 [M+H]⁺; M= 327.10078, calculated mass for C₂₀H₁₉NO₂ = 328.10877, mass error = -0.002 ppm. Compound 3b. Yield 84%, m.p. 241-242°C, FT-IR (υ/cm⁻¹): 3200, 3050, 2960, 1690, 1610, 1530 (NO₂), 1480, 1300, 1240, 1175, 1125. ¹H-NMR (δ/ppm, J/Hz): 9.80 (s, 1H), 7.49 (t, J=8, 1H), 7.59 (d, J=8, 1H), 7.94 (d, J=6, 1H), 7.97 (s, 1H), 4.99 (s, 1H), 2.56 (m, J=6, 4H), 2.22 (m, J=5, 4H), 1.91 (m, J=4, 2H), 1.79 (m, J=3, 2H). ¹³C-NMR (δ/ppm): 194.9 (2C=O), 152.2 (1C=C-N), 149.4 (2C=C-N), 147.5 (1C=C-C), 134.4 (1CA), 129.5 (1CAH), 122.1 (1CAH), 111.5 (2C=C-C), 36.7 (2CAI), 32.9 (1CAI), 26.3 (2CAI), 20.8 (2CAI). MS: m/z obtained 338.13412 [M+H]⁺; M= 338.12666, calculated mass for C₂₁H₂₀NO₄ = 338.12667, mass error = 0.009 ppm. Compound 3c. Yield 52%, m.p. 281-282°C, FT-IR (υ/cm⁻¹): 3205, 3040, 2950, 1650, 1595, 1490, 1240, 1150, 1100, 1185, and 775 (CF₃). ¹H-NMR (δ/ppm, J/Hz): 9.39 (s, 1H), 7.22 (t, J=8, 1H), 7.30 (d, J=8, 1H), 7.39 (d, J=7, 1H), 7.43 (t, J=8, 1H), 5.35 (s, 1H), 2.50 (m, overlap with DMSO, 4H), 2.16 (m, J=6, 2H), 2.04 (t, J=5, 1H), 2.09 (t, J=5, 1H), 1.73 (m, J=6, 2H), 1.87 (m, J=6, 2H). ¹³C-NMR (δ/ppm): 194.4 (2C=O), 151.1 (2C=C-N), 147.2 (1C=C-C), 131.7 (1CAH), 130.6 (1CA-CF₃), 127.2 (1CAH), 126.8 (1CAH), 125.9 (2CAH-CF₃), 113.6 (2C=C-C), 36.7 (2CAI), 30.4 (1CAI), 26.4 (2CAI), 20.8 (2CAI). MS: m/z obtained 362.13620 [M+H]⁺; M= 361.12782, calculated mass for C₂₁H₁₉F₃NO₂ = 361.12896, mass error = 3.2 ppm. Compound 3d. Yield 30%, m.p. 250-254°C, FT-IR (υ/cm⁻¹): 3205, 3045, 2989, 1645, 1597, 1490, 1355, 1242, 1178, and 1120. ¹H-NMR (δ/ppm, J/Hz): 9.40 (s, 1H), 6.71 (d, J=9, 2H), 7.04 (d, J=8, 2H), 3.65 (s, 3H), 4.84 (s, 1H), 2.48 (m, overlap with DMSO, 4H), 1.90 (m, J=4, 2H), 1.76 (m, J=3, 2H). ¹³C-NMR (δ/ppm): 194.8 (2C=O), 157.1 (1CAO), 151.0 (2C=C-N), 139.7 (1CA-C), 128.4 (2CAH), 113.1 (2CAH), 112.7 (2C=C-C), 54.9 (1CH₂O), 36.8 (2CAI), 31.1 (1CAI), 26.3 (2CAI), 20.8 (2CAI). MS: m/z obtained 324.15850 [M+H]⁺; M= 323.15224, calculated mass for C₂₀H₁₉F₃NO₂ = 323.1521435, mass error = -0.2 ppm. Compound 3e. Yield 78%, m.p. 240-241°C, FT-IR (υ/cm⁻¹): 3235, 3060, 2920, 2890, 1706, 1645, 1592, 1473, 1360, 1241, 1172, 1162, 1080 (C-Cl). ¹H-NMR (δ/ppm, J/Hz): 9.50 (s, 1H), 7.15 (d, J=7, 1H), 7.20 (t, J=8, 2H), 4.87 (s, 1H), 2.50 overlap DMSO, 4H), 2.20 (m, J=3, 4H), 1.91 (m, J=5, 2H), 1.78 (m, J=5, 2H). ¹³C-NMR (δ/ppm): 194.8 (2C=O), 151.5 (2C=C-N), 146.3 (1CA-C), 130.0 (1CA-C), 129.4 (2CAH), 127.7 (2CAH), 112.1 (2C=C-C), 36.4 (2CAI), 31.9 (1CAI), 26.3 (2CAI), 20.8 (2CAI). MS: m/z obtained 328.10874 (M+H)⁺; M= 327.10078, calculated mass for C₁₉H₁₈ClNO₂ =
327.1026065, mass error = -3.6 ppm. Compound 3f. Yield 22%, m.p. 249-250°C, FT-IR (υ/cm⁻¹): 3230, 3005, 2963, 2915, 1665, 1646, 1605, 1460, 1355, 1240, 1178, and 1125. ¹H-NMR (δ/ppm, J/Hz): 9.40 (s, 1H), 6.94 (d, J=8, 2H), 7.05 (d, J=8, 2H), 4.85 (s, 1H), 2.62, m, J=6, 4H), 2.28 (m, J=6, f4H), 2.22 (s, 3H), 1.76 (m, J=5, 2H), 1.92 (m, J=5, 2H). ¹³C-NMR (δ/ppm): 194.8 (2C=O), 151.1 (2C=C-N), 144.5 (1C=Ar-C), 134.3 (1C=Ar-C), 128.3 (2C=ArH), 127.4 (2C=ArH), 112.6 (2C=ArH), 36.8 (2C), 31.7 (1C=Al), 30.4 (1C=Al), 26.3 (2C=Al), 20.8 (2C=Al). MS: m/z obtained 308.16326 (M+H)+; M= 307.155984, calculated mass for C₂₀H₂₁NO₂ = 307.1572289, mass error = -4.05 ppm.

**Anti-inflammatory Assay**
The title compounds (3a-f) were assessed at 15 μM in methanol (for preliminary) and at various concentrations (0.15 to 6 μM) (for IC₅₀ determination) as anti-inflammatory agents by a procedure of thermal-induced denaturation of protein as previously reported.¹⁵-¹⁶ The mixture of standard diclofenac sodium or test solutions (0.5 mL) and BSA solution 0.5% (w/v) in tris-buffer saline (pH to 6.3) (4.5 mL) was kept at 37°C for 15 min, heated at 70°C for 10 min, cooled, and the mixture's turbidity measured at 660 nm. To calculate the inhibition (%), we used the equation:

\[
\text{Inhibition} (%) = \left[\frac{(A_{cs} - A_{ts})}{A_{cs}}\right] \times 100
\]

\(A_{cs}\) = control solution absorbance; \(A_{ts}\) = test solution absorbance.

**Antioxidant Assay**
The title compounds (3a-f) were screened as antioxidants using DPPH radical scavenging technique and the Ferric Reducing Ability Potential (FRAP) method with curcumin as comparable, as earlier reported.¹⁶-¹⁸

**DPPH Radical**
The mixture of the title compounds (3a-f) or curcumin solution at 100 μg/mL (0.5 mL) and DPPH solution at 50 μg/ml (2 mL), both in methanol, was kept in the darkroom at r.t. for 30 min, then measured spectrometrically at 517 nm. To calculate the inhibition (%), we used the equation:

\[
\text{Inhibition} (%) = \left[\frac{(A_{cs} - A_{ts})}{A_{cs}}\right] \times 100
\]

\(A_{cs}\) = control solution absorbance; \(A_{ts}\) = test solution absorbance.

**FRAP**
The mixture of 0.1 mL of the title compounds (3a-f) or curcumin solution at 100 μg/mL in methanol and 0.9 mL of the fresh FRAP reagents was mixed and kept at 37°C for 6 min, then measured spectrometrically at 595 nm. To calculate the Fe²⁺ equivalent (%), we used the equation:

\[
\text{Fe}^{2+} \text{equivalent} (%) = \left(\frac{A_x}{A_y}\right) \times 100
\]

\(A_x\) = sample’s absorbance; \(A_y\) = ferrous sulfate (1000 μM) solution’s absorbance.

**Molecular Docking Study**
The molecular docking study was performed utilizing Autodock v4.2 (http://autodock.scripps.edu/) integrated LigandScout software 4.4.3.¹⁹ The compounds were drawn using MarvinSketch (www.chemaxon.com), saved as smile format (.smi), and submitted to energy minimization according to the previous procedure.²⁰-²¹ The COX-1 and COX-2 structures co-crystallized with FLP1650 and S58701, respectively (PDB ID: 1CQE and 6COX), were used as the protein targets.²²-²³ The protein targets were downloaded and run the energy minimization according to the previous procedure.²⁰-²¹ For the protocol's validation, the root-mean-square deviation (RMSD) values obtained by re-docking the native ligands should be not more than 2.0 Å.²⁴-²⁵

**RESULTS AND DISCUSSION**

**Chemistry**
The condensation reaction between 3-aminocyclohex-2-en-1-one (1) and nine aromatic aldehyde compounds (2a-i) in anhydrous acetic acid under reflux conditions obtained six decahydroacridine-1,8-dione compounds (3a-f) with low to high yields (Scheme-1). The results indicated that the electron-
withdrawing groups at the para or meta position in the aromatic aldehyde ring increased the aldehyde reactivity and enhanced the yields and vice versa for the presence of electron-donating groups.

The FTIR spectrum of compound 3a exhibited peaks at 3194, 3050, 2980, 1641, 1606, 1498, 1180, and 1130 cm\(^{-1}\) indicating amine, aromatic, aliphatic, carbonyl, ethylenic, CH\(_2\), and C-N bonds. The \(^1\)H-NMR spectrum showed the presence of N-H amine (s, 1H) at 9.44 ppm, five aromatic protons at 7.15 ppm (2H), 7.13 ppm (2H) and 7.03 ppm (1H), one proton of CH at 4.99 ppm, and aliphatic protons at 2.50 (overlap with DMSO, 4H), 2.17 (4H), 1.91 (2H), and 1.79 (2H). The \(^13\)C-NMR spectrum exhibited the presence of two carbon of carbonyl at 194.8 ppm, ten double bond and aromatic carbon at 151.3-112.4 ppm, and seven carbon aliphatic at 36.8-20.8 ppm.\(^{26-27}\) In the 2D Heteronuclear Single-Quantum Correlation (HSQC) spectra (Fig.-2a), the methylene group protons (CH\(_2\)) appear as two peaks at 1.79 ppm (2H) and 1.91 ppm (2H), were correlated with a peak of two carbons at 20.8 ppm, while the methylene group protons (CH\(_2\)) appearing at 2.17 ppm (m, 4H) were correlated with a peak of two carbons at 36.8 ppm. The methylene group protons (CH\(_2\)) appearing at 2.50 ppm (m, 4H, overlap with DMSO) were correlated with a peak of two carbons at 36.8 ppm. Protons of CH of the dihydropyridine ring connected to an aromatic ring, appearing as a single peak at 4.90 ppm (1H), were correlated with a carbon peak at 32.1 ppm. Four protons of an aromatic ring appearing at 7.13 ppm and 7.15 ppm were correlated with two carbon peaks at 127.5 and 127.8 ppm, while one proton of an aromatic ring appearing as a multiplet peak at 7.03 ppm was correlated with one carbon peak at 125.4 ppm. In the HMBC (Heteronuclear Multiple Bond Correlation) spectra (Fig.-2b), the methylene group protons (CH\(_2\)) appearing as two peaks at 1.79 and 1.91 ppm were correlated with carbon peaks at 26.3, 36.8, 151.3 and 194.8 ppm. The methylene group protons (CH\(_2\)) appearing at 2.17 ppm (m, 4H) were correlated with carbon peaks at 20.8, 26.3, and 194.8 ppm, while the methylene group protons (CH\(_2\)) appearing at 2.50 ppm (m, 4H, overlap with DMSO) were correlated with carbon peaks at 20.8, 36.8, 112.4, and 151.3 ppm. Protons of CH of the dihydropyridine ring connected to the aromatic ring appearing as a single peak at 4.90 ppm were correlated with carbon peaks at 112.4, 127.5, 147.4, 151.3, and 194.8 ppm. One proton of an aromatic ring appearing as a multiplet peak at 7.03 ppm was correlated with carbon peaks at 127.5 and 127.8 ppm; two protons of an aromatic ring appearing as triplet peaks at 7.13 ppm were correlated with carbon peaks at 125.4, 127.8, and 147.4 ppm; and two protons of an aromatic ring appearing as a doublet peak at 7.15 ppm were correlated with carbon peaks at 32.1, 125.4, 127.5, and 147.4 ppm. Meanwhile, the proton of amine (NH) was correlated with carbon peaks at 26.3,
The m/z in the mass spectrum obtained ultimately confirmed the expected structure of compound 3a. For elucidation of other prepared compounds' structures (3b-f) was done based on FTIR, $^1$H-NMR, $^{13}$C-NMR, and MS data. Those also confirmed the expected structures.

![Fig.-2: The HSQC (a) and HMBC (b) spectra of compound 3a.](image)

**Antioxidant and Anti-inflammatory Properties**

The antioxidant property of all prepared compounds was done using DPPH radical scavenging and FRAPimethods. In contrast, the anti-inflammatory activity was performed by a procedure of thermal-induced denaturation of protein. In a preliminary study, we observed that all prepared compounds had lower antioxidant activity than curcumin but higher anti-inflammatory activity than sodium diclofenac (Table-1). Therefore, we continued the anti-inflammatory activity test to determine the IC$_{50}$. The test compounds showed a dose-dependent relationship (Fig.-3) and compound 3a-d exhibited higher activity (IC$_{50}$ = 0.125 - 0.316 μM) than diclofenac sodium (IC$_{50}$ = 0.563 μM) (Table-2). Compound 3d having a 4-methoxy group at phenyl ring had the highest activity. It’s in line with reported earlier on asymmetrical mono-carbonyl analogs of curcumin.\(^{16}\) Inflammation has been involved in developing many diseases such as arthritis, lupus, and others. Protein denaturation produces auto antigens stimulating inflammatory response. NSAIDs are frequently applied to treat inflammatory conditions. They can bind with plasma protein and have shown a significant ability to inhibit protein denaturation.\(^{28-32}\) The compound's ability to inhibit protein denaturation higher than diclofenac sodium implies an evident prospect as a potent anti-inflammation agent.

**Molecular Docking Study**

To predict the ligand-target and possible mechanism of action of the compounds at a molecular level, the molecular docking of title compounds has been done. Most NSAIDs inhibit inflammation by inhibiting the COX enzyme's catalytic activity, so we dock the compounds to that enzymes. The RMSD value obtained by re-docking native ligands to 1CQE and 6COX binding site for protocol’s validation were 1.90 Å and 0.22 Å, confirming that the protocol has good performance for the docking (Fig.-4).\(^{24-25}\) The results of the docking stated that all the compounds could interact with both enzymes. Almost all the compounds to both enzymes exhibited binding energy lower than diclofenac and curcumin (Table-2). Except for compounds 3e and 3f, this in-silico affinity was in line with their in-vitro activity. The ligand-amino acid interactions of the protein target are presented in Tables-3 and 4. Diclofenac forms hydrogen bonding with Tyr385 residue of COX-1 binding site. While in the COX-2 binding site, it creates hydrogen bonding to Ser530 and Tyr385. It also forms several hydrophobic interactions with hydrophobic residues. The results are in line with previous research.\(^{33}\) The interaction between the ligand with Ser530 is essential for several compounds' inhibitory COX-2 activities.\(^{33-34}\) The compound 3d forms hydrogen bonding with Tyr385 in the COX-1 binding site, and 3c forms hydrogen bonding with Ser530 in the COX-2 binding site. Except for 3b against COX-1, all the synthesized compounds and diclofenac form hydrophobic interaction with several identical residues.
### Tabel-1: Preliminary Anti-inflammatory and Antioxidant Properties of the Title Compounds

| Compds | Antioxidant | Anti-inflammatory |
|--------|-------------|------------------|
|        | 25 ppm      | 3.33 ppm         | 15 μM            |
| DPPH   | % Scavenge  | % Scavenge       | % Inhibition     |
|        | ± SD        | ± SD equivalent  | ± SD             |
| 3a     | 14.08 ± 0.96| 30.16 ± 1.19     | 96.04 ± 0.006    |
| 3b     | 11.07 ± 0.23| 26.92 ± 0.59     | 96.04 ± 0.004    |
| 3c     | 7.32 ± 0.29 | 26.27 ± 0.59     | 98.70 ± 0.001    |
| 3d     | 7.13 ± 0.62 | 29.64 ± 0.45     | 98.85 ± 0.001    |
| 3e     | 11.04 ± 0.56| 27.57 ± 1.03     | nd               |
| 3f     | 5.97 ± 0.23 | 30.29 ± 0.78     | nd               |
| Curcumin | 78.38 ± 0.23| 402.28 ± 3.32   | 97.64 ± 0.002    |
| Diclofenac Sodium | nd   | nd               | 69.79 ± 0.008    |
| nd = no data

### Table-2: The Title Compound’s Anti-Inflammatory Activity and Binding Energy Interaction to COX-1 (1CQE) and COX-2 (6COX)

| Compounds (Ligands) | IC<sub>50</sub>(μM) | Binding Energy (Kcal/mol) |
|---------------------|----------------------|---------------------------|
|                     | COX-1                | COX-2                     |
| 3a                  | 0.316 ± 0.015        | -8.83                     |
| 3b                  | 0.258 ± 0.006        | -10.32                    |
| 3c                  | 0.203 ± 0.009        | -7.55                     |
| 3d                  | 0.125 ± 0.011        | -8.32                     |
| 3e                  | 0.942 ± 0.025        | -8.46                     |
| 3f                  | 0.811 ± 0.031        | -9.79                     |
| Diclofenac          | 0.706 ± 0.021        | -7.70                     |
| Curcumin            | 0.563 ± 0.017        | -8.25                     |
| FLP1650             | -                    | -                         |
| S58701              | -                    | -10.6                     |

*Inhibition of protein denaturation

### Table-3: Ligand-Amino Acid Interactions in the COX-1 Binding Site and Their Bonds Type

| Ligands | Amino Acids Residues/Interactions | Hydrogen Bond |
|---------|----------------------------------|---------------|
| FLP1650 | Val116, Leu359, Leu531, Ile523, Val349, Leu352, Phe381, Tyr385, Ala527 | Arg120, Tyr355 |
|         | Leu531, Val349, Leu352, Phe381, Ala527, Leu384 | Tyr385 |
| Diclofenac | Val116, Leu359, Leu531, Val349 Ala527, Met113, Leu357 | - |
| Curcumin | Ala527, Val349 | - |
| 3a      | Val349, Leu352, Tyr385, Tyr348 | - |
| 3b      | Tyr355, Bog1702, Ile89 | - |
| 3c      | Val349, Leu352, Phe381, Tyr385, Ala527, Leu384, Tyr348 | - |
| 3d      | Val349, Leu352, Tyr348 | Tyr385 |
| 3e      | Val349, Leu352, Phe381, Tyr385, Ty348, Phe205, Val344 | - |
| 3f      | Val349, Leu352, Phe381, Tyr385, Ala527, Tyr348 | - |

### Table-4: Ligand-Amino Acid Interactions in the COX-2 Binding Site and Their Bonds Type

| Ligands | Amino Acids Residues/Interaction | Hydrogen Bond |
|---------|---------------------------------|---------------|
| S58701  | Leu359, Val116, Leu531, Ala527, Val349, Leu384, Tyr385, Phe381, Leu532, Val523, Tyr355 | Phe518, Gln192, Ser353 |
| Diclofenac | Leu531, Ala527, Val349, Phe518, Leu352, Val523 | Tyr385, Ser350 |
| Curcumin | Ala527, Val349, Phe518, Leu352, Val523, Tyr355, Arg120 | - |
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|   |   |   |
|---|---|---|
| 3a | Phe518, Leu352, Val523 | - |
| 3b | Phe518, Leu352, Val523 | Tyr355 |
| 3c | Ala527, Val349, Phe518, Leu352, Val523, Tyr348 | Ser530 |
| 3d | Phe518, Leu352, Val523 | - |
| 3e | Phe518, Leu352, Val523, Ala516, Ile517 | - |
| 3f | Phe518, Leu352, Val523, Ala516, Ile517 | - |

![Fig.-4: Superpose Visualization of Co-Crystalline Ligand (blue) with Copy Ligand FLP1650 to COX-1 (1CQE) (a) and S58701 to COX-2 (6COX)](image)

**CONCLUSION**

New decahydroacridine-1,8-dione derivatives have been successfully synthesized. All the compounds demonstrated higher activity to inhibit thermal-induced protein denaturation than diclofenac sodium but lower antioxidant activity than curcumin. Docking’s study showed that all the prepared compounds could interact well with COX-1 and COX-2, and the majority showed lower binding energies than diclofenac and curcumin. The compounds promise to be a potent anti-inflammatory agent. However, the compounds should be further investigated to search for their in vivo activity and their toxicity.

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