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

by Hariyanti Uploaded By Mas'ud Syaiful

RJC

RASĀYAN *J. Chem.*
Vol. 15 | No. 2 |1241-1248| April - June | 2022
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

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

I. P. Sari[1], H. Hariyanti[2], A. Yanuar[1] and H. Hayun[1,✉]

[1]Faculty of Pharmacy, Universitas Indonesia, Depok16424, West Java, Indonesia.
[2]Faculty of Pharmacy and Sciences, Universitas Muhammadiyah Prof. DR. HAMKA, Jakarta, 13460, Indonesia
✉Corresponding Author: hayun@farmasi.ui.ac.id

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.*

RASĀYAN *J. Chem.*, Vol. 15, No.2, 2022

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, antimalarial, 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, anilines, alkylamines, 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 Sterilin, 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

Spectrometer 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).

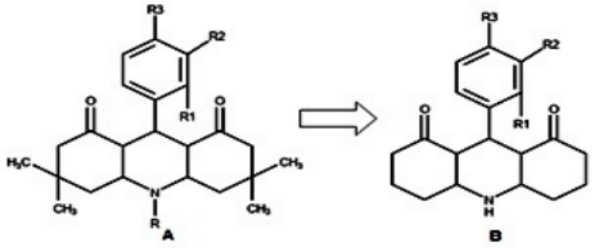


Fig.-1: The Typically Published Decahydroacridine-1,8-diones (A). The Title Compounds (B)

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. ^{1}H-NMR (δ/ppm, *J*/Hz): 9.44 (s, 1H), 7.03 (m, *J*=3, 1H), 7.33 (t, *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). ^{13}C-NMR (δ/ppm): 194.8 (2C=O), 151.3 (2C=C-N), 147.4 (1C$_{Ar-C}$), 127.8 (2C$_{ArH}$), 127.5 (2C$_{ArH}$), 125.4 (1C$_{ArH}$), 112.4 (2C=C-C), 36.8 (2C$_{Al}$), 32.1 (1C$_{Al}$), 26.3 (2C$_{Al}$), 20,8 (2C$_{Al}$). MS: m/zfobtained 294.14919 [M+H]$^{+}$, M= 293.14158, calculated mass for $C_{19}H_{19}NO_2$ = 293.14157, mass error = -0.03 ppm. The 2D-NMR (HSQC and HMBC) spectra of 3a can be seen in Fig.-2. Compound 3b. Yield 84%, m.p. 241-242°C, FT-IR (υ/cm^{-1}): 3300, 3050, 2960, 1690, 1610, 1530 (NO_2), 1480, 1300, 1240, 1175, 1125. ^{1}H-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). ^{13}C-NMR (δ/ppm): 194.9 (2C=O), 152.2 (1C$_{Ar-NO}$), 149.4 (2C=C-N), 147.5 (1C$_{Ar-C}$), 134.4 (1C$_{ArH}$), 129.5 (1C$_{ArH}$), 122.1 (1C$_{ArH}$), 120.1 (1C$_{ArH}$), 111.5 (2C=C-C), 36.7 (2C$_{Al}$), 32.9 (1C$_{Al}$), 26.3 (2C$_{Al}$), 20,8 (2C$_{Al}$). MS: m/z obtainedd339.13412 [M+H]$^{+}$; M=0338.12666, calculated mass for $C_{19}H_{18}N_2O_4$ = 338.126657, 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 (CF_3). ^{1}H-NMR (δ/ppm, *J*/Hz): 9.39 (s, 1H), 7.22 (t, *J*=8, 1H), 7.30 (t, *J*=8, 1H), 7.39 (d, *J*=8, 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). ^{13}C-NMR (δ/ppm): 194.4 (2C=O), 151.1 (2C=C-N), 147.2 (1C$_{Ar-C}$), 131.7 (1C$_{ArH}$), 130.6 (1C$_{Ar-CF}$), 127.2 (1C$_{ArH}$), 126.8 (1C$_{ArH}$), 125.9 (2C$_{ArH, CF3}$), 113.6 (2C=C-C), 36.7 (2C$_{Al}$), 30.4 (1C$_{Al}$), 26.4 (2C$_{Al}$), 20.8 (2C$_{Al}$). MS: m/z obtained 362.13620 (M+H)$^{+}$; M= 361.12782, calculated mass for $C_{20}H_{19}F_3NO_2$ = 361.12896, mass error = 3.2 ppm. Compound 3d. Yield 30%, m.p. 253-254°C, FT-IR (υ/cm^{-1}): 3205, 3045, 2989, 1645, 1597, 1490, 1355, 1242, 1178, and 1120. ^{1}H-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), 2.20 (m, *J*=5, 2H), 1.90 (m, *J*=4, 2H), 1.76 (m, *J*=3, 2H). ^{13}C-NMR (δ/ppm): 194.8 (2C=O), 157.1 (1C$_{ArO}$), 151.0 (2C=C-N), 139.7 (1C$_{Ar-C}$), 128.4 (2C$_{ArH}$), 113.1 (2C$_{ArH}$), 112.7 (2C=C-C), 54.9 (1CH$_3$O), 36.8 (2C$_{Al}$), 31.1 (1C$_{Al}$), 26.3 (2C$_{Al}$), 20, 8 (2C$_{Al}$). MS: m/z obtained 324.15850 (M+H)$^{+}$; M= 323.151224, calculated mass for $C_{20}H_{21}NO_3$ = 323.1521435, mass error = -2.8 ppm. Compound 3e. Yield 78%, m.p. 240-241°C, FT-IR (υ/cm^{-1}): 3235, 3060, 2930, 2890, 1706, 1645, 1592, 1473, 1360, 1241, 1172, 1162, 1080 (C-Cl). ^{1}H-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). ^{13}C-NMR (δ/ppm): 194.8 (2C=O), 151.5 (2C=C-N), 146.3 (1C$_{ArCl}$), 130.0 (1C$_{Ar-C}$), 129.4 (2C$_{ArH}$), 127.7 (2C$_{ArH}$), 112.1 (2C=C-C), 36.4 (2C$_{Al}$), 31.9 (1C$_{Al}$), 26.3 (2C$_{Al}$), 20.8 (2C$_{Al}$). MS: m/z obtained 328.10874 (M+H) $^{+}$; M= 327.10078, calculated mass for $C_{19}H_{18}ClNO_2$ =

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. ^{1}H-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*=5, 4H), 2.28 (m, *J*=6, f4H), 2.22 (s, 3H), 1.76 (m, *J*=5, 2H), 1.92 (m, *J*=5, 2H). ^{13}C-NMR (δ/ppm): 194.8 (2C=O), 151.1 (2C=C-N), 144.5 (1$C_{Ar\text{-}C}$), 134.3 (1$C_{Ar\text{-}C}$), 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 $C_{20}H_{21}NO_2$ = 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 IC_{50} 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:

$$\text{Inhibition (\%)} = [(\text{Acs} - \text{Ats})/\text{Acs}] \times 100$$

Acs = control solution absorbance; Ats = 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:

$$\text{Inhibition (\%)} = [(\text{Acs} - \text{Ats})/\text{Acs}] \times 100$$

Acs = control solution absorbance; Ats = 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 Fe^{2+} equivalent (%), we used the equation:

$$Fe^{2+}\text{ equivalent (\%)} = (\text{Ax})/(\text{Ay}) \times 100$$

Ax = sample's absorbance; Ay = ferrous sulfate (1000 µM) solution's absorbance.

Molecular Docking Study

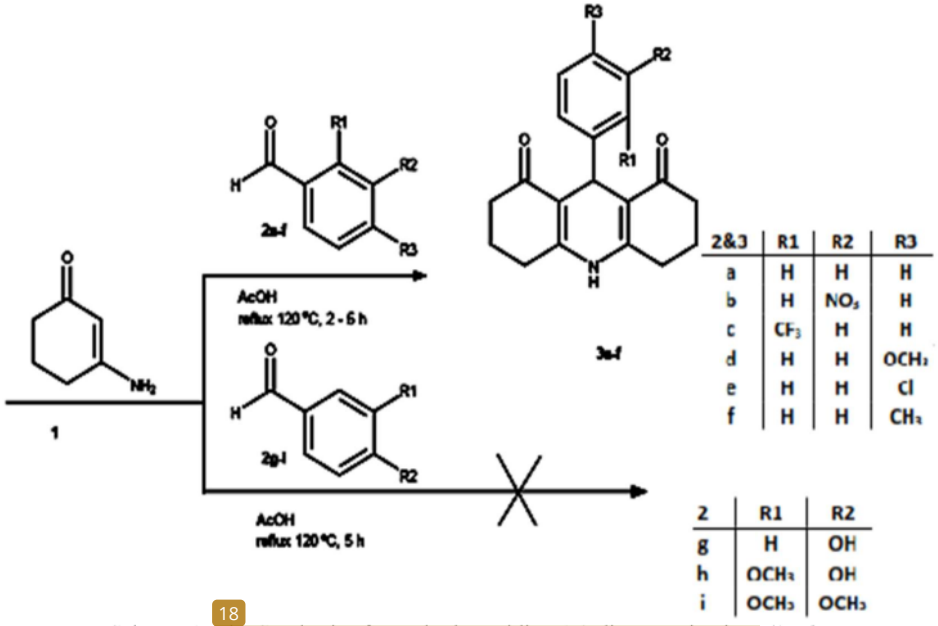
The molecular docking study was performed utilizing Autodock v4.2 (http://autodock.scripps.edu/) integrated LigandScout software 4.4.3.[19] The compounds were drawn using Marvinsketch (www.chemaxon.com), saved as smile format (.smi), and submitted to energy minimization according to the previous procedure.[20-21] 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.



2&3	R1	R2	R3
a	H	H	H
b	H	NO_2	H
c	CF_3	H	H
d	H	H	OCH_3
e	H	H	Cl
f	H	H	CH_3

2	R1	R2
g	H	OH
h	OCH_3	OH
i	OCH_3	OCH_3

Scheme-1: The Synthesis of Decahydroacridine-1,8-dione Derivatives (**3a-f**).

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 26.3 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 anda7.15 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,

112.4, 151.3, and 194.8 ppm.[26] 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.

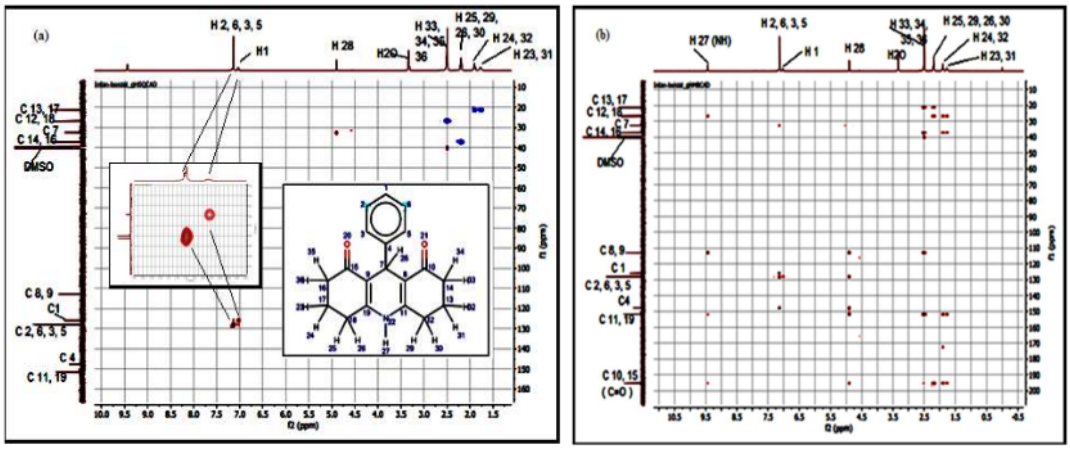


Fig.-2: The HSQC (a) and HMBC (b) spectra of compound 3a.

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	FRAP	% Inhibition ± SD
	% Scavenge ± SD	% Fe^{2+} equivalent	
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

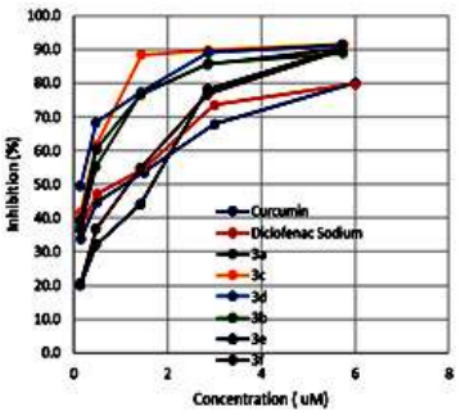


Fig.-3: Relation of Concentrations and Inhibition of Protein Denaturation Activity of Test Compounds

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

Compounds (Ligands)	IC_{50} (µM)	Binding Energy (Kcal/mol)	
		COX-1	COX-2
3a	0.316 ± 0.015	-8.83	-8.53
3b	0.258 ± 0.006	-10.32	-9.48
3c	0.203 ± 0.009	-7.55	-7.78
3d	0.125 ± 0.011	-8.32	-8.56
3e	0.942 ± 0.025	-8.46	-9.17
3f	0.811 ± 0.031	-9.79	-9.04
Diclofenac	0.706 ± 0.021	-7.70	-7.58
Curcumin	0.563 ± 0.017	-8.25	-7.70
FLP1650	-	-8.59	-
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	
	Hydrophobic	Hydrogen Bond
FLP1650	Val116, Leu359, Leu531, Ile523, Val349, Leu352, Phe381, Tyr385, Ala527	Arg120, Tyr355
Diclofenac	Leu531, Val349, Leu352, Phe381, Ala527, Leu384	Tyr385
Curcumin	Val116, Leu359, Leu531, Val349 Ala527, Met113, Leu357	-
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, Tyr348, 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	
	Hydrophobic	Hydrogen Bond
S58701	Leu359, Val116, Leu531, Ala527, Val349, Leu384, Tyr385, Phe381, Leu352, Val523, Tyr355	Phe518, Gln192, Ser353
Diclofenac	Leu531, Ala527,Val349, Phe518, Leu352, Val523	Tyr385, Ser530
Curcumin	Ala527, Val349, Phe518, Leu352, Val523, Tyr355, Arg120	-

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)

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.

ACKNOWLEDGMENT

We are full thanks to the Ministry of research and technology/NationallResearch &&Innovation Agency, Indonesia, which funded this study via PDUPT Grant on 2020; No.: NKB-91/UN2.RST/HKP.05.00/2020, the Molecular Structure Analysis Lab., ITB, Indonesia, and the Chemical Research Center, LIPI, Indonesia, measured NMR spectra and mass spectra.

REFERENCES

1. American College of Rheumatology, Enpowering Rheumatology Professionals, NSAIDs (nonsteroidal anti-inflammatory drugs) (2020), https://www.rheumatology.org/I-Am-A/Patient-Caregiver/Treatments/NSAIDs(accessed 21.08.21)
2. S. Wongrakpanich, A. Wongrakpanich, K. Melhado, J. Rangaswami, *Aging and disease*, **9(1)**, 143(2018), https://doi.org/10.14336/AD.2017.0306
3. K.G. Shilpa, K.H. Shivaprasad, and M.R. Archana, *Rasayan Journal of Chemistry*, **14(4)**, 2246(2021), http://doi.org/10.31788/RJC.2021.1446507
4. N. Ullah, F. Jamali, Z. Huang, F. Sanaee, A. Rodriguez-Dimitrescu, F. Aldawsari, F. Jamali, A. Bhardwaj, N.U. Islam, and C.A. Velázquez-Martínez, *Journal of Enzyme Inhibition Medicinal Chemisty*, **31(6)**, 1018(2016), https://doi.org/10.3109/14756366.2015.1088840
5. A.A. Abdelhamid, S.K. Mohamed, A. Maharramov, A. Khalilov and M. Allahverdiev, *Journal of Saudi Chemical Society*. **18**, 474(2014), https://doi.org/10.1016/j.jscs.2011.10.005
6. B. Zeynizadeh and M. Gilanizadeh, *Current Chemisty Letter,* **9**, 71(2020), https://doi.org/10.5267/j.ccl.2019.8.001
7. S. Sudha and M.A. Pasha, *The Scientific World Journal,* Article **ID 930787**, 6 pages(2013), https://doi.org/10.1155/2013/930787
8. M.M. Alam, A.T. Mubarak, M.A. Assiri, S.M. Ahmed, and A.M. Fouda. *BMC Chemistry,* **13**, 40(2019), https://doi.org/10.1186/s13065-019-0545-3

9. S.S. Mansoor, K. Aswin, K. Logaiya, and S.P.N. Sudhan, *Journal of Taibah University of Science,* **8**, 265(2014), https://doi.org/10.1016/j.jtusci.2014.03.003
10. J.-J. Xia and K.-H. Zhang, *Molecules*, **17**, 5339(2012), https://doi.org/10.3390/molecules17055339
11. G.M. Ziarani, S. Mousavi, N. Lashgari, A. Badiei, and M. Shakiba, *Iranian Journal of Chemistry and Chemical Engineering*, **32(4)**, 9(2013), https://doi.org/10.30492/IJCCE.2013.6739
12. G. Sujatha, V. Anusuya, and G.L. Balaji, *European Journal Molecule & Clinical Medicine*, **7(3),** 2211(2020)
13. M. Kidwai and D. Bhatnagar, *Tetrahedron Letter,* **51(20)**, 2700(2010), https://doi.org/10.1016/j.tetlet.2010.03.033
14. Y.-B. Shen and G.-W. Wang, *Arkivoc*, **xvi**, 1(2008)
15. H. Hayun, B.P. Maggadani, A. Kurnia, A. Hanifah, M. Yuliandi, I. Fitriyani and S.P. Hadrianti, *International Journal of Applied Pharmaceutics*, **11**, 246(2019), https://doi.org/10.22159/ijap.2019.v11s1.19448
16. T.N. Putri, A. Bachtiar and H. Hayun, *Journal of Applied Pharmaceutical Sciences,* **8**(**05**), 019(2018), https://doi.org/10.7324/JAPS.2018.8503
17. T. Okselni, A. Santoni, A. Dharma and M. Efdi, *Rasayan Journal of Chemistry*, **11(3)**, 1211(2018), http://dx.doi.org/10.31788/RJC.2018.1133058
18. H. Hayun, I. Gavrila, S. Silviana, A.E.K. Siahaan, R.F. Vonna and M.I. Latifah, *Rasayan Journal of Chemistry*, **13**(**1**), 131 (2020), https://doi.org/10.31788/RJC.2020.1315300
19. G. Wolber and T. Langer, *Journal of Chemical Information and Modeling,* **45(1)**, 160(2005), https://doi.org/10.1021/ci049885e
20. P. Tosco, N. Stiefl, and G. Landrum, *Journal of Cheminformatics*, **6**, 37(2014), https://doi.org/10.1186/s13321-014-0037-3
21. S. Megantara, M. Mutakin, E. Halimah, E. Febrina, and J. Levita, *Rasayan Journal of Chemisty*, **13(03)**,1321(2020), https://doi.org/10.31788/RJC.2020.1335766
22. D. Picot, P.J. Loll, and R.M. Garavito, *Nature*, **367**, 243(1997), https://doi.org/10.1038/367243a0
23. R.G. Kurumbail, A.M. Stevens, J.K. Gierse, J.J. Mcdonald, R.A. Stegeman, J.Y. Pak, D. Gildehaus, J.M. Miyashiro, T.D. Penning, K. Seibert, P.C. Isakson, and W.C. Stallings, *Nature,* **384,** 644(1996), https://doi.org/10.1038/367243a0
24. D. Ramírez and J. Caballero, *Molecules*, **23(5)**, 1038(2018), https://doi.org/10.3390/molecules23051038
25. H. Hariyanti, K. Kusmardi, A. Yanuar, and H. Hayun, *Indonesian Journal of Chem*isty, **21(1)**, 137(2021), https://doi.org/10.22146/ijc.54745
26. R.M. Silverstein, F.X. Webster, and D.J. Kiemle, Spectrometric Identification of Organic Compounds, 7th ed., John Wiley & Sons, Inc., New York, NY, USA, (2005)
27. MarvinSketch 20.8.0. ChemAxon Ltd., 1998–2020, http://www.chemaxon.com (accessed 14.11.20)
28. M. Marius, D. Amadou, A.A. Donatien, A. Gilbert, Y.N. William, K. Rauf, et al., *Gastroenterology Research and Practice,* Article **ID 4253174**, (2020), https://doi.org/10.1155/2020/4253174
29. N.I. Osman, N.J. Sidik, A. Awal, N.A.M. Adam, and N.I. Rezali, *Journal of Intercultural Ethnopharmacology*, **5(4)**, 343(2016), https://doi.org/10.5455/jice.20160731025522
30. E.L. Opie, *Journal of Experimental Medicine*, **115**, 597(1962), https://doi.org/10.1084/jem.115.3.597
31. L. Saso, G. Valentini, M.L. Casini, E. Grippa, M.T. Gatto, M.G. Leone, and B. Silvertini, *Archives of Pharmacal Research*, **24**, 150(2001), https://doi.org/10.1007/BF02976483
32. S. Yesmin, A. Paul, T. Naz, A.B.M.A. Rahman, S.F. Akhter, M.I.I. Wahed, T.B. Emran, and S.A. Siddiqui, *Clinical Phytoscience*, **6(59)**, 1(2020), https://doi.org/10.1186/s40816-020-00207-7
33. A.L. Blobaum and L.J. Marnett, *Journal of Medicinal Chemistry*, **50**, 1425(2006), https://doi.org/10.1021/jm0613166
34. H. Hayun, A. Yanuar, M. Hanafi, and P.W.S.S. Hudiyono, *Bioinformation*, **7(5)**, 246(2011), https://doi.org/10.6026/97320630007246

[RJC-6775/2021]

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

ORIGINALITY REPORT

12%	8%	10%	6%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to School of Business and Management ITB Student Paper	1%
2	patentscope.wipo.int Internet Source	1%
3	www.rasayanjournal.co.in Internet Source	1%
4	L. A. Latifah, N. H. Soekamto, A. Tahir. "NEW ANTIBACTERIAL ACTIVITIES OF BROMINATED C18 AND C20 FATTY ACIDS ISOLATED FROM MARINE SPONGE Xestospongia testudinaria AGAINST SHRIMP PATHOGENIC BACTERIA", Rasayan Journal of Chemistry, 2021 Publication	<1%
5	Sundberg, R.J.. "Mechanistic aspects of the formation of anhydrovinblastine by Potier-Polonovski oxidative coupling of catharanthine and vindoline. Spectroscopic	<1%

observation and chemical reactions of intermediates", Tetrahedron, 1992
Publication

6	link.springer.com Internet Source	<1%
7	Submitted to Academic Library Consortium Student Paper	<1%
8	bmcchem.biomedcentral.com Internet Source	<1%
9	file.scirp.org Internet Source	<1%
10	Anna L. Blobaum, Lawrence J. Marnett. "Structural and Functional Basis of Cyclooxygenase Inhibition", Journal of Medicinal Chemistry, 2007 Publication	<1%
11	bjbas.springeropen.com Internet Source	<1%
12	Submitted to Jawaharlal Nehru Technological University Student Paper	<1%
13	Submitted to Jawaharlal Nehru Technological University Anantapur Student Paper	<1%
14	Phannarath Phansavath, Robert Stammler, Corinne Aubert, Max Malacria. "Synthesis of	<1%

Variously Substituted Spirobenzocyclobutenes", Synthesis, 1998
Publication

15	Submitted to North West University Student Paper	<1%
16	Submitted to University of Sheffield Student Paper	<1%
17	Apriliana Cahya Khayrani, Rafidha Irdiani, Reza Aditama, Diah Kartika Pratami et al. "Evaluating the potency of Sulawesi propolis compounds as ACE-2 inhibitors through molecular docking for COVID-19 drug discovery preliminary study", Journal of King Saud University - Science, 2021 Publication	<1%
18	Sugey M. Martinez Gomez, Diego M. Alzate Sanchez, William Rodríguez-Córdoba, Cesar A. Sierra, Cristian Ochoa-Puentes. "Competitive One-Pot Reactions: Simultaneous Synthesis of Decahydroacridine-1,8-diones and 1,8-Dioxo-octahydroxanthenes and Photophysical Characterization", Synthetic Communications, 2013 Publication	<1%
19	repositorio.fciencias.unam.mx:8080 Internet Source	<1%

20	Abouzid, K.. "Novel anti-inflammatory agents based on pyridazinone scaffold; design, synthesis and in vivo activity", Bioorganic & Medicinal Chemistry, 20080515 Publication	<1 %
21	Hoda Z. Shams, Rafat M. Mohareb, Maher H. Helal, Amira E. Mahmoud. "Synthesis, Structure Elucidation, and Biological Evaluation of Some Fused and/or Pendant Thiophene, Pyrazole, Imidazole, Thiazole, Triazole, Triazine, and Coumarin Systems Based on Cyanoacetic 2-[(Benzoylamino)thioxomethyl] Hydrazide", Phosphorus, Sulfur, and Silicon and the Related Elements, 2007 Publication	<1 %
22	Manisha R. Bhosle, Moseen A. Shaikh, Dhananjay Nipate, Lalit D. Khillare, Giribala M. Bondle, Jaiprakash N. Sangshetti. "ChCl:2ZnCl2 Catalyzed Efficient Synthesis of New Sulfonyl Decahydroacridine-1,8-Diones via One-Pot Multicomponent Reactions to Discover Potent Antimicrobial Agents", Polycyclic Aromatic Compounds, 2019 Publication	<1 %
23	N. Srividya, P. Ramamurthy, P. Shanmugasundaram, V. T. Ramakrishnan. "Synthesis, Characterization, and	<1 %

	Electrochemistry of Some Acridine-1,8-dione Dyes", The Journal of Organic Chemistry, 1996 Publication	
24	S. Megantara, I. Musfiroh, D. Lestari. "In-silico STUDY OF DITERPENOID LACTONES FROM Andrographis paniculate TO INTERLEUKIN-6 (IL-6) PROTEIN TARGET", RASAYAN Journal of Chemistry, 2022 Publication	<1 %
25	Submitted to University of Colorado, Denver Student Paper	<1 %
26	asianpubs.org Internet Source	<1 %
27	epdf.pub Internet Source	<1 %
28	japsonline.com Internet Source	<1 %
29	jppipa.unram.ac.id Internet Source	<1 %
30	patents.justia.com Internet Source	<1 %
31	Grice, Cheryl A., Kevin L. Tays, Brad M. Savall, Jianmei Wei, Christopher R. Butler, Frank U. Axe, Scott D. Bembenek, Anne M. Fourie, Paul J. Dunford, Katherine Lundeen, Fawn Coles, Xiaohua Xue, Jason P. Riley, Kacy N. Williams,	<1 %

Lars Karlsson, and James P. Edwards. "Identification of a Potent, Selective, and Orally Active Leukotriene A_4 Hydrolase Inhibitor with Anti-Inflammatory Activity", Journal of Medicinal Chemistry, 2008.
Publication

---

32 S. Sheik Mansoor, K. Aswin, K. Logaiya, S.P.N. Sudhan. "Aqua-mediated synthesis of acridinediones with reusable silica-supported sulfuric acid as an efficient catalyst", Journal of Taibah University for Science, 2018 <1%
Publication

---

33 Jonathan B. Chaires, Jinsong Ren, Donald Hamelberg, Arvind Kumar, Vandna Pandya, David W. Boykin, W. David Wilson. "Structural Selectivity of Aromatic Diamidines", Journal of Medicinal Chemistry, 2004 <1%
Publication

---

34 Song Ja Kim, Alamgeer, Munaza Kanwal, Mubashir Hassan, Sahar Abdullah, Mamuna Waheed, Haseeb Ahsan, Zaman Ashraf. "Flurbiprofen–antioxidant mutual prodrugs as safer nonsteroidal anti-inflammatory drugs: synthesis, pharmacological investigation, and computational molecular modeling", Drug Design, Development and Therapy, 2016 <1%
Publication

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		