Supplementary Information File1

Evaluation of the antiviral potential of halogenated dihydrorugosaflavonoids and molecular modeling with nsP3 protein of Chikungunya virus (CHIKV)

Ninad V. Puranik1,3#, Ruchi Rani2#, Vedita Anand Singh2, Shailly Tomar2*, Hemalata M. Puntambekar1,3, and Pratibha Srivastava1,3*

1Bioprospecting Group, Agharkar Research Institute, G. G. Agarkar Road, Pune-411004, Maharashtra, India
2Department of Biotechnology, Indian Institute of Technology Roorkee, Roorkee, Uttarakhand- 247667, India
3Savitribai Phule Pune University, Ganeshkhind, Pune-411007

# Both Ninad V. Puranik and Ruchi Rani have equal contribution in this piece of work.

*Corresponding author: Both Pratibha Srivastava Email: psrivastava@aripune.org

and Shailly Tomar Email: shailfbt@iitr.ac.in
**Figure S1**- Assessment of antiviral effect by CPE. Viral inhibition assessed by CPE images of Vero cells infected with CHIKV (MOI of 1) by treatment with 5c and 5d compounds. Cells were observed at 48hpi. Infected Vero cells with compound treated (5c - a, b and c while 5d - d, e, and f) and cell control (containing no virus - g) are shown. Virus control image is represented as h. Cells were observed by light microscope (Carl Zeiss, Germany) with a 5X objective lens.
Figure S2: LC-MS detail of 5c
Figure S3: $^1$H NMR of 5a
Figure S4: $^1$H NMR of 5a with splitting pattern
Figure S5: $^{13}$C NMR of 5a
Figure S6: LC-MS of 5b
Figure S7: $^1$H NMR of 5b
Figure S8: $^1$H NMR of 5b with splitting pattern
Figure S9: $^{13}$CNMR of 5b
Figure S10: HRMS of 5c
Figure S11: $^1$H NMR of 5c and $^1$H NMR of 5c with splitting pattern
Figure S12: $^{13}$C NMR of 5c
Figure S13: LC-MS of 5d
Figure S14: $^1$H NMR of 5d and $^2$H NMR of 5d with splitting pattern
Figure S16: LC-MS pf 5e
Figure S17: $^1$H NMR of 5e and $^1$H NMR of 5e with splitting pattern
Figure S18: $^{13}$C NMR of 5e
Figure S19: LC-MS of 5f
Figure S20: $^1$H NMR of 5f and $^1$H NMR of 5f with splitting pattern
Figure S21: $^{13}$C NMR of 5f
Methodology for Molecular Docking

Protein preparation

The selected PDB files of nsP3 (3GPO) for docking studies were taken from RCSB site (www.rcsb.org) and pre-treated before the docking calculation with the assistance of the Protein Preparation Wizard icon of Maestro 11.2 program by Schrodinger. The rules to use the protein preparation wizard was followed as per Maestro online tutorial. These steps were i) Addition of hydrogen atoms to protein structures, ii) assignment of bond orders, iii) deletion of crystallographic waters, iv) regeneration of states, v) optimization of hydrogen bonds using PROPKA program in Schrodinger before restrained minimization using the OPLS force field, and vi) setting the convergence for the heavy atoms were at RMSD 0.3 Å.

Ligand Preparation

The dihydrorugosaflavonoid derivatives (5a-f) were designated as ligands. They were prepared using the 2D program (ligand preparation wizard) of Maestro 11.2. They were transformed into a 3D model by using the preset option. Ligprep added several perfections on the ligands and generated low energy structure with ring conformation. Energy minimization, along with optimization, were performed using 'optimized potential for liquid simulations' (OPLS) force field. Subsequently, one confirmation for every ligand was created using Maestro 11.2 software from Schrodinger.

Receptor Grid Generation

GLIDE molecular docking enables one ligand to intermingle with the X-ray crystal structure of the specific protein for identification of the active site receptor grid. The benefits of ligands to interact in many conceivable conformations as a result of receptor grid-dependent molecular docking. Docking grid for protein structures 3GPO was generated using the receptor grid generation criteria of Maestro. The grid box was situated in the center at the cognate ligands of the protein structures complexed with natural cofactor while the maximum length of the dock molecules was fixed to 20 Å. The scaling factor and partial charge cut off of van der Waals radius scaling were 0.25 and 1 Å, respectively. Other features such as sites, constraints, rotatable groups, and excluded volume, which were the default setting of the Maestro 11.2 were used.

GLIDE molecular docking
After fabricating the ligand and target protein and specifying the grid on the active location of the protein, molecular docking measures were obtained. GLIDE molecular docking paraphernalia used efficient computational simulation technique for evaluation of particular poses and ligand flexibility. GLIDE systematic technique, an application for swift, accurate molecular docking, and its consequential GScore, which is an empirical scoring function and the combination of several parameters. The GScore was determined in kcal/mol, and it included ligand-protein interaction energies, hydrophobic interactions, hydrogen bonds, internal energy, pi-pi stacking interactions, and root mean square deviation (RMSD) and desolvation. GLIDE module of the XP visualizer identified the specific ligand-protein interactions. The dihydrorugosaflavonoids were docked with the 3D structure of 3GPO with the help of GLIDE. The precise fit compounds were defined for each target by thermodynamic optimal energy value, types of interactions, the potential of bonding, and conformations.

**ADME properties calculations**

ADME properties of ligand molecules were obtained by QikProp tool of Schrodinger 2017, which delivered information about Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADME/T) properties of the ligands. It provided data such as QP log Po/w, QP log BB, overall CNS activity, Caco-2, MDCK cell permeability, logK_{hsa} for human serum albumin binding, percentage of human oral absorption, etc.

**Chemistry**

All the compounds were synthesized as per the procedure reported in our previous published paper. The brief methodology has been given below.

**Synthesis of methyl 3,5-dimethoxybenzoate (2).**

3, 5-Dihydroxy benzoic acid (6 mmol) was taken in dry acetone. In the stirred mixture at 40°C for 15 min, K₂CO₃ (2.5 mmol) was added, and stirring was continued further at 60°C for 10 min. Then dimethyl sulfate (2.2 mmol) was added dropwise till a period of 30 min, and the temperature was increased slowly to 80°C. The reaction mixture was allowed to reflux for 6h. TLC checked the progress of the reaction. After completion, the reaction mixture was allowed to cool at room temperature and filtered through the celite bed. The filtered mixture was concentrated to get the crude product. The crude product was slowly
poured on crushed ice with constant stirring to obtain solid. The solid obtained was filtered and dried to get methyl 3,5-dimethoxybenzoate (2) in 91% yield.

**Synthesis of methyl 2-acetyl-3, 5-dimethoxybenzoate (3)**
Methyl 3,5-dimethoxybenzoate (2) (5 mmol) was mixed with acetyl chloride (25 mmol) and carbon disulfide (2 ml) under dry N$_2$ in an ice bath. In the reaction mixture AlCl$_3$ (15 mmol) was added under vigorous stirring. The reaction was allowed to stir for 15 min. TLC monitored the progress of the reaction. After completion, the reaction was quenched with ice and extracted with ethyl acetate. The organic layer was separated, dried over sodium sulfate and concentrated to obtain the crude product, which was purified by column chromatography (hexane-ethyl acetate, 70:30) to obtain methyl 2-acetyl-3, 5-dimethoxybenzoate (3) in 52 % yield.

**Synthesis of methyl 2-acetyl-3, 5-dihydroxybenzoate (4)**
Methyl 2-acetyl-3, 5-dimethoxybenzoate (3) (4 mmol) appended in chlorobenzene and AlCl$_3$ (10 mmol) was added slowly at room temperature. The reaction mixture was heated to reflux for 1h. TLC scrutinized the progress of the reaction. After completion, the reaction was cooled to room temperature and hydrolyzed using 1N HCl. The reaction mixture was extracted with ethyl acetate. The organic layer was separated, dried over sodium sulfate, and concentrated to obtain a crude product. The crude product was purified by column chromatography (hexane-ethyl acetate, 80:20) to acquire clean methyl 2-acetyl-3, 5-dihydroxybenzoate (4) with a 68% yield.

**General procedure for the synthesis of Methyl 7-hydroxy-2-(substituted phenyl)-4-oxo-3,4-dihydro-2H-chromene-5-carboxylate (5a-f)**
Methyl 2-acetyl-3,5-dihydroxybenzoate (4) (4.7 mmol) in DMSO was mixed with different substituted aromatic aldehydes (4.7 mmol), I$_2$ (0.23 mmol) and pyrrolidine (2.3 mmol) as per the reported procedure and the reaction was allowed to reflux for 8 hr. The TLC monitored the progress of the reaction. After completion, the mixture was cooled to room temperature and quenched with water. The aqueous layer was extracted with ethyl acetate. The organic layer was separated and washed with brine solution. The organic layer was dried concentrated on getting the crude product which was purified by column chromatography using hexane: ethyl acetate as a solvent system to obtain the products 5a-f.
