Supplemental Information for:

Tuaimenal A, a Meroterpene from the Irish Deep-Sea Soft Coral *Duva florida*, Displays Inhibition of the SARS-CoV-2 3CLpro Enzyme.

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Figure S1: Tuaimenal A (1) ESI-HRMS.  
Figure S2: Tuaimenal A (1) ¹H NMR spectrum (400 MHz, CDCl₃). 
Figure S3: Tuaimenal A (1) ¹³C NMR spectrum (100 MHz, CDCl₃). 
Figure S4: Tuaimenal A (1) gCOSY spectrum (400 MHz, CDCl₃). 
Figure S5: Tuaimenal A (1) HSQC spectrum (400 MHz, CDCl₃). 
Figure S6: Tuaimenal A (1) HMBC spectrum (400 MHz, CDCl₃). 
Figure S7: Tuaimenal A (1) UV spectrum in ACN (10 μg/mL). 
Figure S8: Tuaimenal A (1) FTIR spectrum, thin film. 
Figures S9: Final pose from the flexible docking of tuaimenal A (1) into PLpro. 
Figure S10: Final pose from the flexible docking of tuaimenal A (1) into TMPRSS2. 
Figure S11: Final pose from the flexible docking of tuaimenal (1) A into RdRp.
Figure S12: Glide scores for SARS-CoV-2 proteins targets and tuaimenal A (I).  
Figure S13: Final pose of tuaimenal A (I) Glide docking with 3CLpro.  
Figure S14: Structure of Mpro with the final ligand poses.  
Rigid Docking Methods and Results  
Table S1: Residue numbers selected to define the active site of each protein target.  
Table S2: Glide scores received by tuaimenal A (I) against protease target poses.  
Table S3: Absolute Configuration Determination Report.

Figure S1: Tuaimenal A (I) ESI-HRMS. The base peak of 371.2216 is consistent with the [M + H]^+ (calculated for C_{23}H_{31}O_{4} 371.2217, Δmmu 0.1).
Figure S2: Tuaimenal A (1) $^1$H NMR spectrum (400 MHz, CDCl$_3$).

Figure S3: Tuaimenal A (1) $^{13}$C NMR spectrum (100 MHz, CDCl$_3$).

Figure S4: Tuaimenal A (1) HSQC spectrum (400 MHz, CDCl$_3$).
Figure S5: Tuaimenal A (1) gCOSY spectrum (400 MHz, CDCl₃).

Figure S6: Tuaimenal A (1) HMBC spectrum (400 MHz, CDCl₃).
**Figure S7:** Tuaimenal A (1) UV spectrum in ACN (10 μg/mL); $\lambda_{\text{max}}$ (log $\varepsilon$) 198 nm (3.2), with additional peaks noted at $\lambda$ (log $\varepsilon$) 258 nm (1.4), 307 nm (1.5), and 369 nm (0.3).
Figure S8: Tuaimenal A (1) FTIR spectrum, thin film.

Figure S9: Final pose from the flexible docking of tuaimenal A (1) into PLpro. Dashed yellow lines indicate a hydrogen bond to Ser260.
**Figure S10:** Final pose from the flexible docking of tuaimenal A (1) into TMPRSS2. Dashed yellow lines indicate a hydrogen bond to Thr314.

**Figure S11:** Final pose from the flexible docking of tuaimenal (1) A into RdRp. Dashed yellow lines indicate a hydrogen bond to Ser679.
Figure S12. Glide scores for SARS-CoV-2 protein targets for the ligand conformations of tuaimenal A (1).

Figure S13. Final pose of tuaimenal A (1) Glide docking with 3CLpro, highlighting the two pi-pi stacking interactions (in dashed blue lines) with His41. Hydrogen bonds with Ser46, Met49, and Gln189 are to backbone atoms and not pictured.
Figure S14. Structure of Mpro with the final ligand poses from rigid and flexible docking superimposed. The flexible docking pose, in purple, can be found deeper in the active site than that of the rigid docking pose, in yellow.

Rigid Docking Methods and Results. Schrödinger’s Glide application was used to conduct the initial molecular docking of tuaimenal A (1). Ligand conformations were sampled flexibly while the protein receptor remained rigid. The van der Waals radii of the ligand atoms was set to a scaling factor of 0.80 and a partial atomic charge cutoff of 0.15 to soften the potential when considering nonpolar regions of the ligands. The prepared ligands were then computationally bound to the respective targets using extra precision (XP) virtual screening settings.

Validation of binding affinity is observed when multiple ligand conformations are bound to multiple target conformations of the same protein. Six favorable poses of tuaimenal A were observed across three of the 3CLpro structures (i.e., three different protein conformations of 3CLpro out of the five total initial receptors), of which the lowest Glide score was -8.925. Eleven favorable poses resulted for three of the PLpro conformations (lowest Glide score of -8.533, average -7.585). Seven favorable poses resulted for three human TMPRSS2 structures (lowest Glide score of -8.282, average -7.750). Only one conformation of tuaimenal A bound favorably to the viral RdRp structure (Glide score of -7.419).
Table S1: Information on the residue numbers selected in the definition of the active site of each protein target.

|     | 3CLpro | PLpro | RdRp | TMPRSS2 |
|-----|--------|-------|------|---------|
| 20  | 160    | 465   | 135  |
| 25  | 162    | 473   | 136  |
| 26  | 164    | 475   | 151  |
| 27  | 165    | 476   | 152  |
| 28  | 206    | 478   | 157  |
| 40  | 244    | 542   | 200  |
| 41  | 245    | 543   | 290  |
| 42  | 246    | 600   | 292  |
| 140 | 247    | 602   | 293  |
| 141 | 261    | 607   | 294  |
| 142 | 262    | 608   | 315  |
| 143 | 263    | 610   | 316  |
| 144 | 265    | 611   | 317  |
| 145 | 267    | 612   | 318  |
| 146 | 268    | 614   | 319  |
| 147 | 269    | 678   | 320  |
| 162 | 270    | 680   | 321  |
| 163 | 271    | 681   | 325  |
| 164 | 272    | 733   | 326  |
| 165 | 734    | 327   |
| 189 |        | 328   |
|     |         |       | 329  |
Table S2:
Favorable Glide scores received by 6 poses of tuaimenal A (1) docked into 5 conformations of each protein target.

| Protein target | Glide score |
|----------------|-------------|
| 3CLpro         | -8.925      |
| 3CLpro         | -8.925      |
| 3CLpro         | -8.760      |
| PLpro          | -8.533      |
| PLpro          | -8.533      |
| TMPR           | -8.282      |
| TMPR           | -8.067      |
| TMPR           | -7.896      |
| TMPR           | -7.896      |
| PLpro          | -7.747      |
| PLpro          | -7.747      |
| TMPR           | -7.721      |
| PLpro          | -7.600      |
| PLpro          | -7.600      |
| 3CLpro         | -7.577      |
| 3CLpro         | -7.577      |
| PLpro          | -7.465      |
| RdRp           | -7.419      |
| TMPR           | -7.295      |
| 3CLpro         | -7.250      |
| TMPR           | -7.090      |
| PLpro          | -7.066      |
| PLpro          | -7.066      |
| PLpro          | -7.039      |
| PLpro          | -7.039      |
# Absolute Configuration Determination Report

## GENERAL INFORMATION

| Customer          | USF / Nicole |
|-------------------|--------------|
| Sales Order Number| 2020-115 LSV |
| Sample code (Our ref.) | Tuaimenal A |
| Sample description (Your ref.) | Tuaimenal A |
| VCD-spectrometer | ChiralIR w/ DualPEM |
| Report prepared by | Jordan Nafie |
| Report validated and signed by | Rina K Dukor |
| Date              | February 12, 2021 |

## RESULTS

**Absolute Configuration of Tuaimenal A is (R)**

Confidence Level: **86%**

## MEASUREMENT PARAMETERS

| Parameter                          | Value               |
|------------------------------------|---------------------|
| Concentration                      | 5.7mg / 220uL       |
| Solvent                            | CDCl₃               |
| Resolution                         | 4 cm⁻¹              |
| PEM setting                        | 1400 cm⁻¹           |
| Number of scans/Measurement time   | 24 hours            |
| Sample cell                        | BaF₂                |
| Path length                        | 100 µm              |

## CALCULATION DETAILS

| Component                                      | Value                                      |
|-----------------------------------------------|--------------------------------------------|
| Gaussian version                              | Gaussian 09                                |
| Total low-energy conformers used for Boltzmann sum | 338                                        |
| Methodology and basis set for DFT calculations | B3LYP/6-31Gd w/CPCM (chloroform)           |
| Enantiomer used for calculation               | R                                          |
| Total calculated conformers                   | 818                                        |
| Number of low-energy conformations shown in report | 4                                          |

## COMMENTS

The confidence level is a measure of the degree of congruence between a calculated and measured spectrum. If identical spectra are being compared the confidence level is 100%. The confidence level (CL) is not the likelihood that the assignment is correct. Rather it's a measure of quality or degree of agreement between calculated and measured spectra. With a CL of 86% for this molecule, the visual agreement between measured and calculated spectra is good – this is a high confidence assignment. Due to the flexibility and high number of active conformers, the VCD intensity for this molecule was very low. Despite some noise in the measured spectrum, the results clearly point to an R configuration.
Structure of Tuaimenal A:

Tuaimenal A

CompareVOA Results.
Please note: In this plot the frequency scaling factor is not applied.
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Table 1. Numerical comparison describing the similarity in the range of 950 - 1800 cm\(^{-1}\) between the calculated IR and VCD spectra for the (R) enantiomer at the B3LYP / 6-31Gd w/ CPCM (Chloroform) level and the observed IR and VCD spectra for **Tuaimenal A**.

| Cal. (950-1800cm\(^{-1}\)) | Numerical comparison | Observed Tuaimenal A |
|-----------------------------|----------------------|----------------------|
| scaling factor              | 0.968                | 0.968                |
| IR similarity (%)           | 90.6                 | 90.6                 |
| \(\Sigma\) (%)              | 57.465               | 57.465               |
| \(\Delta\) (%)              | 35.587               | 35.587               |
| Confidence Level (%)        | 86                   | 86                   |

\(\Sigma\): single VCD similarity, gives the similarity between the calculated and observed VCD spectra.

\(\Delta\): enantiomeric similarity index, gives the difference between the values of \(\Sigma\) for both enantiomers of a given diastereoisomer.
IR (lower frame) and VCD (upper frame) spectra of *Tuaimenal A* in CDCl$_3$; 100um path-length cell with BaF$_2$ windows; 24 h collection for enantiomer and solvent; instrument optimized at 1400 cm$^{-1}$. Solvent subtracted IR and VCD spectra are shown. Uppermost trace is the VCD noise spectrum.
IR (lower frame) and VCD (upper frame) spectra observed for Tuaimenal A (left axes) compared with Boltzmann-averaged spectra of the calculated conformations for the (R) configuration, (right axes).
IR (lower frame) and VCD (upper frame) spectra observed for Tuaimenal A (left axes) compared with Boltzmann-averaged spectra of the calculated conformations for the (S) configuration, (right axes).
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Lowest energy conformers - (R) Configuration:
Plot of ESI (similarity of correct enantiomer minus incorrect enantiomer to calculated) vs SNS (overall similarity of correct enantiomer to calculated) for a library of correct assignments verified independently by X-Ray other method (Black X marks). Red X is Tuaimenal A.