

## Supplementary data

### **IDO1 and TDO inhibitory evaluation of analogues of the marine pyrroloiminoquinone alkaloids:wakayin and tsitsikammamines**

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**Chemistry:** <sup>1</sup>H and <sup>13</sup>C NMR spectra were performed on Bruker Avance 300 or Avance 500 (cryoprobe TCI <sup>1</sup>H{<sup>13</sup>C, <sup>31</sup>P}) spectrometers with the chemical shifts of the remaining protons of the deuterated solvents serving as internal standards. IR spectra were obtained on a Thermo Nicolet 6700 spectrophotometer. Mass spectra were recorded on a GCT premier waters mass spectrometer for CI and High resolution mass spectra (HRMS) were recorded on a UPLC Xevo G2 Q TOF Waters spectrometer for ESI. Reagents were purchased from commercial sources and used as received. Flash-chromatography was performed on silica gel (15-40 μM) by means of the solvent systems indicated below.

*5-((2-(1H-indol-3-yl)ethyl)amino)-1-tosyl-1H-indole-4,7-dione (1a)* and *6-((2-(1H-indol-3-yl)ethyl)amino)-1-tosyl-1H-indole-4,7-dione (2a)* and *5-((2-(1H-indol-3-yl)ethyl)amino)-1H-indole-4,7-dione (1)* and *6-((2-(1H-indol-3-yl)ethyl)amino)-1-tosyl-1H-indole-4,7-dione (2)*

To a solution of indoledione **21** (480 mg, 1.59 mmol) in absolute ethanol (140 mL) was added portionwise tryptamine (1.3 g, 7.95 mmol) and the mixture was stirred at room temperature for 2h. After concentration over vacuum, the crude product was purified by flash-chromatography (CH<sub>2</sub>Cl<sub>2</sub>) to give the expected compounds: (CH<sub>2</sub>Cl<sub>2</sub>) to give the two expected compounds **1a** and **2a** and the elution solvent was changed (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 99: 1 to give the second ones **1** and **2**:

**1a:** pink fuschia solid (59 mg, 8%), 166 °C. HRMS (DCI-CH<sub>4</sub>) calcd for C<sub>25</sub>H<sub>22</sub>N<sub>3</sub>O<sub>4</sub>S 460.1331, found 460.1331. <sup>1</sup>H NMR (CDCl<sub>3</sub>): 2.45 (s, 3H); 3.12 (t, 2H, J = 6.9

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Hz); 3.43 (q, 2H, J = 6.9 Hz); 5.26 (s, 1H) 5.84 (br t, 1H, J = 5.6 Hz); 6.67 (d, 1H, J = 3.3 Hz); 7.07 (d, 1H, J = 2.4 Hz); 7.16 (td, 1H, J = 1 Hz and 8 Hz); 7.25 (td, 1H, J = 1 Hz and 8 Hz); 7.36 (d, 2H, J = 8.2 Hz); 7.41 (d, 1H, J = 8.2 Hz); 7.59 (d, 1H, J = 7.8 Hz); 7.70 (d, 1H, J = 3.3 Hz); 8.04 (d, 2H, J = 8.2 Hz); 8.10 (br s, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) 21.78, 24.05, 42.77, 97.49, 107.16, 111.40, 112.00, 118.43, 119.70, 122.16, 122.44, 126.83, 126.93, 127.71, 129.13 (2C), 129.46 (2C), 132.69, 134.27, 136.43, 145.85, 147.02, 175.08, 178.82. IR (ATR) 3357, 1678, 1593, 1371 cm<sup>-1</sup>.

**2a:** pink fuschia solid (80 mg, 11%), mp 111 °C. HRMS (DCI-CH<sub>4</sub>) calcd for C<sub>25</sub>H<sub>22</sub>N<sub>3</sub>O<sub>4</sub>S 460.1331, found 460.1343. <sup>1</sup>H NMR (CDCl<sub>3</sub>): 2.53 (s, 3H, CH<sub>3</sub>); 3.10 (t, 2H, J = 6.6 Hz); 3.43 (q, 2H, J = 6.7 Hz); 5.37 (s, 1H); 5.93 (t, 1H, J = 5.6 Hz); 6.74 (d, 1H, J = 3.1 Hz); 7.06 (d, 1H, J = 2.3 Hz); 7.15 (dq; 1 H, J = 1 Hz and 7 Hz); 7.24 (dq, 1H, J = 1 Hz and 7 Hz); 7.35 (d, 2H, J = 1 Hz and 8.7 Hz); 7.41 (td, 1H, J = 1 Hz and 8.2 Hz); 7.58 (dd, 1H, J = 1 Hz and 7.9 Hz); 7.81 (d, 1H, J = 3.2 Hz); 7.99 (d, 2H, J = 8.4 Hz); 8.16 (br s, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) 21.79, 24.13, 42.95, 96.64, 108.62, 111.41, 111.95, 118.46, 119.67, 122.20, 122.42, 126.91, 126.92, 128.87 (2C), 129.79 (2C), 131.27, 133.95, 134.53, 136.46, 146.17, 148.09, 170.12, 181.37. IR (ATR) 3308, 1653, 1632, 1375 cm<sup>-1</sup>.

**1:** pink fuschia solid (224 mg, 46%), mp 228 °C. HRMS (DCI-CH<sub>4</sub>) calcd for C<sub>18</sub>H<sub>16</sub>N<sub>3</sub>O<sub>2</sub> 306.1243, found 306.1247. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) 3.01 (t, 2H, J = 7.4 Hz); 3.40 (q, 2H, J = 6.9 Hz); 5.21 (s, 1H), 6.41 (d, 1H, J = 2.5 Hz); 7.00 (td, 1H, J = 1.1 Hz and 8 Hz); 7.09 (td, 1H, J = 1 Hz and 8 Hz); 7.15 (t, 1H, J = 5.9 Hz); 7.25 (t, 1H, J = 2.5 Hz); 7.26 (s, 1H, J = 1.8 Hz); 7.36 (d, 1H, J = 8 Hz); 7.56 (d, 1H, J = 8 Hz); 10.88 (br s, 1H, NH); 12.62 (br s, 1H, NH). <sup>13</sup>C NMR (DMSO-d<sub>6</sub>) 23.73, 43.38, 107.40, 111.65, 111.90, 118.67, 118.81 (2C), 121.22, 123.50, 124.11, 127.57, 134.80, 136.72, 150.03, 177.13, 178.37. IR (ATR) 3420, 3288, 1673, 1603, 1392 cm<sup>-1</sup>.

**2:** pink fuschia solid (107 mg, 22%), mp 209 °C. HRMS (DCI-CH<sub>4</sub>) calcd for C<sub>18</sub>H<sub>16</sub>N<sub>3</sub>O<sub>2</sub> 306.1243, found 306.1230. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): 3.01 (t, 2H, J = 7.4 Hz); 3.42 (q, 2H, J = 6.9 Hz); 5.20 (s, 1H) 6.48 (d, 1H, J = 2.5 Hz); 7.00 (m, 2H); 7.08 (td, 1H, J = 1 Hz and 8 Hz); 7.25 (d, 1H, J = 2.5 Hz); 7.32 (t, 1H, J = 5.9 Hz); 7.35 (d, 1H, J = 8 Hz); 7.57 (d, 1H, J = 8 Hz); 10.87 (br s, 1H, NH); 12.41 (br s, 1H, NH). <sup>13</sup>C NMR (DMSO-d<sub>6</sub>) 23.76, 43.34, 96.31, 107.92, 111.71, 111.89, 118.67, 118.80, 121.46, 123.46, 127.59, 128.42, 128.60, 129.20, 136.72, 148.89, 172.30, 182.20. IR (ATR) 3388, 1654, 1623, 1600, 1397 cm<sup>-1</sup>.

5-((2-(1*H*-indol-3-yl)ethyl)(methyl)amino)-1-tosyl-1*H*-indole-4,7-dione (**1b**) and 6-((2-(1*H*-indol-3-yl)ethyl)(methyl)amino)-1-tosyl-1*H*-indole-4,7-dione (**2b**)

A solution of methyl tryptamine (1.09 g, 6.3 mmol) in absolute ethanol (15 mL) was added dropwise to a solution of indole-dione **21** (0.38 g, 1.25 mmol) in absolute ethanol (16 mL). After 2h stirring at room temperature, the mixture was concentrated over vacuum and the crude product was purified by flash-chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 99.5: 0.5) to give the expected compounds:

**1b**: pink fuschia solid (180 mg, 12%), mp 175 °C. HRMS (DCI-CH<sub>4</sub>) calcd for C<sub>26</sub>H<sub>23</sub>N<sub>3</sub>O<sub>4</sub>S 474.1488, found 474.1483. <sup>1</sup>H NMR (CDCl<sub>3</sub>) 2.45 (s, 3H), 2.95 (s, 3H), 3.10 (t, 2H, J = 7.2 Hz), 3.97 (t, 2H, J = 7.2 Hz), 5.34 (s, 1H), 6.55 (d, 1H, J = 3.3 Hz), 7.00 (d, 1H, J = 2.2 Hz), 7.16 (m, 2H), 7.36 (d, 2H, J = 8.1 Hz), 7.63 (d, 1H, J = 3.3 Hz), 8.05 (d, 2H, J = 8.1 Hz). <sup>13</sup>C NMR (CDCl<sub>3</sub>) 21.78, 24.38, 41.11, 56.10, 104.49, 107.69, 111.18, 112.45, 118.79, 119.66, 122.28, 122.65, 127.02, 127.42, 128.80, 129.10 (2C), 129.44, 131.09 (2C), 134.54, 136.31, 145.75, 151.28, 174.22, 184.42. IR (ATR) 3366, 1674, 1624, 1369 cm<sup>-1</sup>.

**2b**: pink fuschia solid (710 mg, 48%), mp 175 °C. HRMS (DCI-CH<sub>4</sub>) calcd for C<sub>26</sub>H<sub>23</sub>N<sub>3</sub>O<sub>4</sub>S 474.1488, found 474.1491. <sup>1</sup>H NMR (CDCl<sub>3</sub>) 2.43 (s, 3H), 2.98 (s, 3H), 3.11 (t, 2H, J = 7.2 Hz), 3.92 (t, 2H, J = 7.2 Hz), 5.38 (s, 1H), 6.66 (d, 1H, J = 3.3 Hz), 7.08 (d, 1H, J = 2.2 Hz), 7.22 (m, 2H), 7.39 (d, 2H, J = 8.1 Hz), 7.64 (br.s, 1H), 7.75 (d, 1H, J = 3.3 Hz), 8.13 (d, 2H, J = 8.1 Hz). <sup>13</sup>C NMR (CDCl<sub>3</sub>) 21.76, 24.28, 41.01, 55.67, 103.82, 107.56, 111.18, 112.57, 118.98, 119.72, 122.27, 122.59, 127.19, 127.33, 128.98, (2C), 129.65 (2C), 130.22, 132.20, 134.49, 136.24, 145.90, 152.80, 175.08, 183.67. IR (ATR) 3365, 1670, 1618, 1370 cm<sup>-1</sup>.

3-(1*H*-indol-3-yl)-1-methyl-5-tosyl-2,3-dihydropyrrolo[2,3-*f*]indole-4,8(1*H*,5*H*)-dione (**3**)

To a solution of compound **2a** (140 mg, 0.3 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (9 mL), was added DDQ (140 mg, 0.6 mmol). The mixture was stirred for 2h at room temperature. After concentration over vacuum, the crude product was purified by flash-chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 99.5: 0.5) to give the expected product as a violin solid (40 mg, 28%), mp > 260 °C. HRMS (DCI-CH<sub>4</sub>) calcd for C<sub>26</sub>H<sub>20</sub>N<sub>3</sub>O<sub>4</sub>S 470.1175, found 470.1173. <sup>1</sup>H NMR (CDCl<sub>3</sub>) 2.43 (s, 3H), 4.10 (s, 3H), 6.78 (d, 1H, J = 3.3 Hz), 7.17-7.27 (m, 3H), 7.33 (d, 2H, J = 8.4 Hz), 7.48 (d, 1H, J = 8.2 Hz), 7.72 (d, 1H, J = 7.8 Hz), 7.76 (d, 1H, J = 3.3 Hz), 8.04 (d, 2H, J = 8.4 Hz), 8.25 (d, 1H, J = 3.0 Hz), 8.35 (br. s, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) 21.74, 36.94, 107.43, 107.68, 111.52, 119.64, 120.21, 120.31, 122.12, 126.22, 126.78, 128.95, 128.98 (2C), 129.16, 129.49 (2C),

129.87, 131.49, 132.54, 134.60, 136.01, 145.58, 171.84, 174.06. IR (ATR) 3369, 1646, 1629, 1376  $\text{cm}^{-1}$

*3-(1H-indol-3-yl)-1-methyl-7-tosylpyrrolo[3,2-f]indole-4,8(1H,7H)-dione (4)*

Same procedure as for compound **3** involving compound **2b** (200 mg, 0.4 mmol),  $\text{CH}_2\text{Cl}_2$  (12 mL) and DDQ (200 mg, 0.9 mmol). Purification by flash-chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  99.5: 0.5) led to the expected product as a violin solid (150 mg, 75%), mp > 260 °C. HRMS (DCI- $\text{CH}_4$ ) calcd for  $\text{C}_{26}\text{H}_{20}\text{N}_3\text{O}_4\text{S}$  470.1175, found 470.1157.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 2.47 (s, 3H), 4.07 (s, 3H), 6.81 (d, 1H, J = 3.2 Hz), 7.24 (m, 2H), 7.21-7.27 (m, 3H), 7.38 (d, 2H, J = 8.5 Hz), 7.46 (d, 1H, J = 7.3 Hz), 7.77 (d, 1H, J = 3.2 Hz), 8.07 (d, 2H, J = 8.5 Hz), 8.35 (br. s, 1H), 8.39 (d, 1H, J = 3.2 Hz).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 21.78, 37.28, 107.74, 108.34, 111.59, 119.57, 119.80, 120.30, 122.17, 122.21, 126.13, 126.70, 128.82, 128.92 (2C), 129.29, 129.59 (2C), 130.49, 130.61, 133.90, 134.53, 136.09, 145.79, 167.13, 178.84. IR (ATR) 3306, 1652, 1631, 1374  $\text{cm}^{-1}$ .

**Determination of IDO1 and TDO activities:** the assay was performed in 96-well flat bottom plates seeded with  $2 \times 10^5$  cells in a final volume of 200  $\mu\text{L}$ . To determine TDO or IDO activity, the cells were incubated overnight at 37 °C in IMDM supplemented with 2% FBS (Invitrogen). The plates were then centrifuged 10 min at 300 g, and 150  $\mu\text{L}$  of the supernatant were collected. The supernatant was analyzed by HPLC to measure the concentration of residual tryptophan and produced kynurenine, based on the retention time and the UV absorption (280 nm for tryptophan, 360 nm for kynurenine). For the HPLC analysis, 55  $\mu\text{L}$  of supernatant were mixed with 55  $\mu\text{L}$  of 6% (wt/vol) trichloroacetic acid to precipitate the proteins. After centrifugation, the supernatant (100  $\mu\text{L}$ ) was collected and injected onto an Onyx Monolithic C18 column (Phenomenex). The inhibition of tryptophan degradation and kynurenine production was expressed as a percentage of the values obtained in the absence of inhibitor. A dose/response assay was then performed at four concentrations. For each compound, the cell viability, expressed in percentage, was evaluated at the end of the incubation period.

**Structural classification, molecular visualization and molecular docking**

Molecular graphics were performed with the UCSF Chimera package. Chimera is developed by the Ressource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by the NIGMS P41-GM103311).

The protein structures used in this paper were downloaded from the RCSB Protein Database and were structurally aligned with structure 2DT0 (chain A, formerly cited as 2DT0a) set as reference and using UCSF Chimera/Matchmaker program. The protein structures, were prepared (structure checks, rotamers, hydrogenation, splitting of chains) using Biovia ([www.3dsbiovia.com](http://www.3dsbiovia.com)) Discovery Studio Visualizer 2016 (DSV) and UCSF Chimera.

The new compounds were sketched using ChemAxon Marvin 16, ([www.chemaxon.com](http://www.chemaxon.com)).

All ligands were checked (hybridization, hydrogenation, some geometry optimizations, 3D sketching) and merged in SDF libraries using DSV.

The structural classification is based on combination of descriptors (at ligand and protein levels) such as position, orthogonal projection, decomposition for ligands, presence for cofactor, aperture level for protein, interaction networks. All the chains of 48 IDO1 liganded (heme or ligands) structures corresponding to Uniprot domain P14902 were spitted, individually aligned and the similar chains were reduced to one representative structure. Finally the 52 chains studied were classified in five clusters, and a representative structure was chose for each cluster. The first cluster [A] is characteristic of an opened binding cavity and was mainly subdivided in [A] opened) and [A1] (more opened). The [A] cluster was represented by the reference chain 2DT0a and included 2D0Ua, 4U72a, 4U74a, 6E40a, 6E42c, 6E41a, E41b, 5XE1a, 5W8Na chains. The [A1] cluster was represented by 5EK2a used for docking studies and included 4PK5a, 4PK5b, 4PK6a, 4PK6b, 5EK4a, 5EK3a, 5EK2a, 5ETWa, 6PU7b, 6KW7a, 6KOFa, 6KPSa, 6O3Ia, 6V52a, 6WJY chains.

Molecular modeling studies were carried with Molegro Virtual Docker 6 software ([www.clcbio.com](http://www.clcbio.com)) using the A chain of structure 5EK2a as target.

A search space volume of 15 Å radius centered in the binding pocket around ligand 5PJ (from 5EK2a) was used. The ligands were set flexible during the docking, and two different docking protocols (P1-GPU and P2-OPT) were used.

The protocol P1-GPU is based on rigid docking at protein level and a GPU (Nvidia Tesla CUDA hardware, [www.nvidia.com](http://www.nvidia.com)) screening algorithm. Docking process used the MolDock function (Moldock [grid] with a resolution of 0.3 Å) for scoring and CUDA optimizer (MVD, 6000 iteration steps, other parameters let as default, no water molecule was taken in account during calculations. The protocol returns 100 independent runs. Post-docking, all the poses were re-ranked using MolDock and Rerank scoring systems.

The protocol P2-OPT is based on flexible docking at protein level (softened potentials during docking phase) and Moldock optimizer was used as searching algorithm. According to structural study, 20 residues were defined as flexible during the docking: ALA260, ALA264, ARG231, CYS129, GLN266, ILE354, LEU154, LYS238, MET295, PHE163, PHE164, PHE226, SER167, SER235, SER263, TYR126, TYR233, VAL125 and VAL130. Docking process used 3000 iteration steps, (convergence was reached for all ligands) other parameters let as default. The protocol returns 20 independent runs. A final minimization (per run) was parameterized using 2000 steps for lateral chains and protein backbone; other parameters were let with default values. A displaceable water molecule was taken in account in the calculations. MolDock and Rerank scores were calculated post-docking and post-minimization.

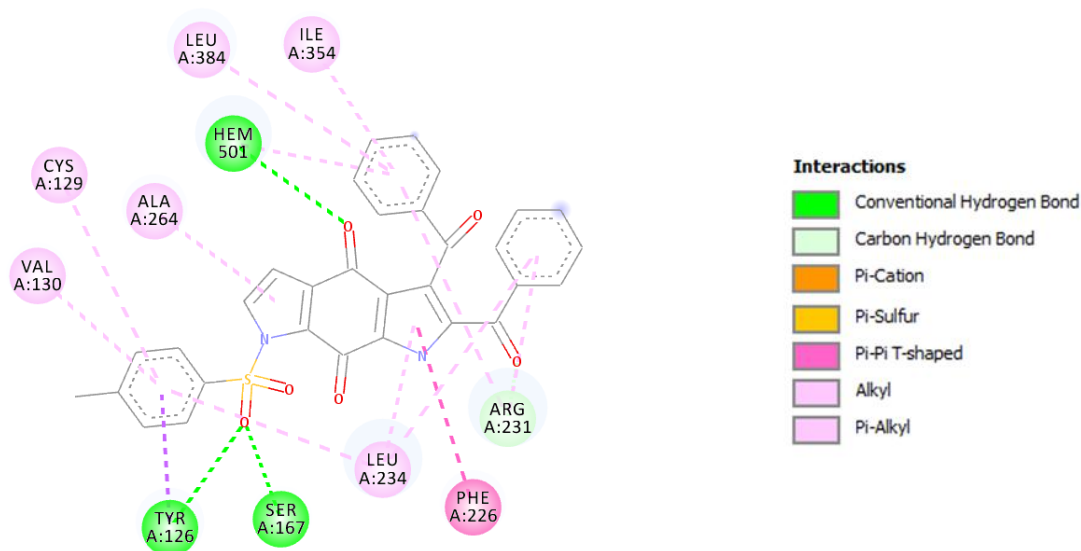
The two protocols used a pharmacophoric model (template) with a grid resolution of 0.3 Å and strength of 500. The definition of template was based on aligned protein structures in the same reference space. The conformations of co-crystallized IDO1 complexes making coordinate bonding involving the nitrogen of ligand and Fe of heme, showed clearly that it was possible to set a minimal template based on the mean position of nitrogen. Four similarity groups (using spheres of 1.8 Å radiuses) and one atom per group were defined as ring, negative charge, hydrogen acceptor, steric hindrance.

The clustering parameters (RMSD threshold 1.7 Å, other parameters let as default) were the same for P1-GPU and P2-OPT protocols.

The protocol P1-GPU was used on all ligands and worked well with fragment-like molecules; the crystallographic reproduction of 5PJ conformation of 5EK2a was reached with a RMSD of 0.62 Å. As protocol P2 gave similar orientation of Tosyl groups in the binding site, the best compounds were investigated using P2-OPT protocol and full site flexibility. As controls, the PKJ (from 4PK5a and ROX ligands were docked in 5EK2a in order to check the reproduction of crystallography (RMSD of 1.47 Å) and docking results (same conformation and orientation). We noticed also that 5EK2a and 4PK5a are included in the same protein structural cluster (open++ class).

DSV generated 2D interaction diagram of **5** best pose (best Rerank) using protocol OPT.

Two groups of hydrogen bonds are found, the first for oxygen of Ts group (two interactions), the other implying one of the carbonyl group and Heme (one interaction and potent metal donor/acceptor).



DSV generated 2D interaction diagram of **6** best pose (best Rerank) using protocol OPT.

Three hydrogen bonds are found for this compound with additional Pi-sulfur (CYS129) and Pi-Cation (ARG231) interactions. The corresponding pose of **6** is close to the Heme center

than in the case of the counterpart

