Structural insights for the optimization of dihydropyrimidin-2(1H)-one based mPGES-1 inhibitors

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Supporting information

Computational details

The chemical structures of investigated compounds were built with Maestro (version 9.6)[1] Build Panel and then processed with LigPrep, version 2.8,[1] generating all the possible stereoisomers, tautomers, protonation states at a pH of 7.4 ± 1.0, and finally minimized using OPLS 2005 force field.

Protein 3D model was prepared using the Schrödinger Protein Preparation Wizard,[1] starting from the mPGES-1 X-ray structure in the active form co-crystallized with the inhibitor LVJ (PDB code: 4BPM). In particular, crystallized water molecules were removed, all hydrogens were added, and bond orders were assigned. Protein .pdb file obtained was then converted in .mae format.

Docking calculations were performed using Glide software (SP and XP mode, version 6.1, Schrödinger package).[2] Glide docking experiments were performed generating a receptor grid focused on the mPGES-1 binding site (considering co-crystallized ligand LVJ as reference

structure) and centered at -10.0557 (x), 16.6230 (y), 45.7128 (z), with inner box and outer box dimensions of 16×26×22 and 27×37×33, respectively.

A first docking step was performed using the SP (Standard Precision) Glide mode, sampling ligands as flexible and producing two outputs after setting their halogens as H-bonds acceptors or donors. The enhanced sampling mode (4 times) was chosen for the sampling step, keeping 10000 poses for ligand for the initial phase of docking, selecting 800 poses per ligand for energy minimization. 100 maximum output structures were saved for each ligand, choosing 0.8 as scaling factor related to van der Waals radii with a partial charge cutoff of 0.15; a post-docking optimization of the obtained docking outputs was performed, accounting 100 maximum number of poses, and choosing 0.5 kcal/mol as cutoff for rejecting obtained minimized poses. For each SP docking step, the top-ranked 5 poses were selected and re-docked into the mPGES-1 binding site, using the extra precision mode (XP) of Glide, setting all parameters as previously described for the SP mode.

Docking results were analyzed with Maestro (version 9.6). Illustrations of the 3D models were generated using VMD software[3] and Maestro.[1]

General synthetic methods

All commercially available starting materials were purchased from Sigma-Aldrich and were used as received. All solvents used for the synthesis were of HPLC grade, and were purchased from Sigma-Aldrich and Carlo Erba Reagenti. All NMR spectra were recorded on a Bruker Avance 600 MHz instrument. All compounds were dissolved in 0.5 mL of 99.95% $CDCl_3$ (Carlo Erba, 99.95 Atom % D).

Coupling constants (*J*) are reported in Hertz, and chemical shifts are expressed in parts per million (ppm) on the delta (δ) scale relative to $CHCl_3$ (7.26 ppm for ^{1}H and 77.2 ppm for ^{13}C) as internal reference. Electrospray mass spectrometry (ESI-MS) was performed on a LCQ DECA TermoQuest (San Josè, California, USA) mass spectrometer. High resolution mass spectra were acquired on a LTQ Orbitrap XL (Thermo Scientific).

Reactions were monitored on silica gel 60 F_{254} plates (Merck). Analytical and semi-preparative reversed-phase HPLC was performed on Agilent Technologies 1200 Series high performance liquid chromatography using a Jupiter Proteo C_{18} reversed-phase column (250 x 4.60mm, 4μ, 90 Å, flow rate = 1 mL/min; 250 x 10.00mm, 10μ, 90 Å, flow rate = 4 mL/min respectively, Phenomenex®). The binary solvent system (A/B) was as follows: 0.1% TFA in water (A) and 0.1% TFA in CH_3CN (B). The absorbance was detected at 280 nm. The purity of all tested compound (>98%) was determined by HPLC analysis.

Microwave Irradiation Experiments.

All microwave irradiation experiments were carried out in a dedicated CEM-Discover® Focused Microwave Synthesis apparatus, operating with continuous irradiation power from 0 to 300 W utilizing the standard absorbance level of 300 W maximum power. The reactions were carried out in 10 mL sealed microwave glass vials. The temperature was monitored using the CEM-Discover built-in-vertically-focused IR temperature sensor. After the irradiation period, the reaction vessel was cooled rapidly (60-120 s) to ambient temperature by air jet cooling.

General procedure for Microwave-assisted Biginelli reaction.

A mixture of 5-[3-(trifluoromethyl)phenyl]furfural (1.0 mmol), urea or 3-ureidopropionic acid (1.5 mmol), 1,3-dicarbonyl compound (1.0 mmol) in acetonitrile (1.5 mL) were placed in a 10 mL microwave glass vial equipped with a small magnetic stirring bar. TMSCl (1.0 mmol) was added and the mixture was then stirred under microwave irradiation at 120°C for 15-20 min. After irradiation, the reaction mixture was cooled to ambient temperature by air jet cooling, cold water was added and the vial was poured into crushed ice and then at 4°C overnight. The resulting precipitate was filtered and washed with a cold mixture of ethanol/water (1:1) (3x3 mL) to give the desired product. HPLC purification was performed by semi-preparative reversed-phase HPLC (on a Jupiter Proteo C_{18} column: 250 x 10.00mm, 10μ, 90 Å, flow rate = 4 mL/min) using the gradient

conditions reported below for each compound. The final products were obtained with high purity > 98% detected by HPLC analysis and were fully characterized by ESI-MS, and NMR spectra.

1.1. Compound **2** was obtained by following the general procedure as a red gelatinous solid (3.5 mg, 40% yield after HPLC purification). RP-HPLC t_R = 34.9 min, gradient condition: from 5% B to 35% B in 15 min, increased to 100 % B in 40 min, flow rate of 4 mL/min, λ= 280 nm. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.20 (s, 3H), 5.53 (s, 1H), 6.37 (brs, 1H), 6.64 (brs, 1H), 7.49 (br s, 2H), 7.62-7.69 (m, 3H), 7.79 (s, 1H), 8.06 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 19.5, 50.0, 108.1, 109.7, 121.5, 124.6, 126.3, 129.5, 130.7, 133.7. ESMS, calcd for $C_{23}H_{16}BrF_3N_2O_4$ 521.29; found m/z = 522.7 $[M + H]^+$. HRMS, calcd for $C_{23}H_{17}BrF_3N_2O_4$ $[M+H]^+$ 521,03183, found 522.03227.

1.2. Compound **3** was obtained by following the general procedure as a yellow gelatinous solid (15.6 mg, 50% yield after HPLC purification). RP-HPLC t_R = 32.7 min, gradient condition: from 5% B to 25% B in 5 min, increased to 100 % B in 40 min, flow rate of 4 mL/min, λ= 280 nm. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.40 (s, 3H), 5.13 (dd, J= 25.5, 12.5 Hz, 2H), 5.58 (s, 1H), 6.12 (brs, 1H), 6.18 (br s, 1H), 6.57 (br s, 1H), 7.21 (br s, 2H), 7.24 (br s, 1H) 7.44-7.48 (m, 2H), 7.71 (br s, 1H), 7.81 (br s, 1H), 8.05 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 19.7, 50.1, 66.4, 108.1, 109.7, 121.5, 124.6, 126.3, 128.1, 129.5, 130.7. ESMS, calcd for $C_{24}H_{19}F_3N_2O_4$ 456.42; found m/z = 457.1 $[M + H]^+$. HRMS, calcd for $C_{24}H_{20}F_3N_2O_4$ $[M+H]^+$ 457.13697, found 457.13765.

1.3. Compound **4** was obtained by following the general procedure as a red gelatinous solid (5.6 mg, 47% yield after HPLC purification). RP-HPLC t_R = 29.6 min, gradient condition: from 5% B to 30% B in 5 min, increased to 100 % B in 45 min, flow rate of 4 mL/min, λ= 280 nm. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.55 (s, 3H), 2.74-2.79 (m, 2H), 3.91-4.12 (m, 2H), 5.15 (s, 2H) 5.53 (s, 1H),

6.12 (brs, 1H), 6.33 (d, *J*=3.3Hz, 1H), 6.57 (d, J= 3.4Hz, 1H), 7.28 (br s, 4H), 7.44 (br s, 2H), 7.66-7.70 (m, 1H), 7.78 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 19.3, 36.6, 50.3, 66.2, 108.1, 109.6, 121.5, 124.4, 126.3, 128.1, 129.5, 130.8. ESMS, calcd for $C_{27}H_{23}F_3N_2O_6$ 528.48; found m/z = 529.0 $[M + H]^+$. HRMS, calcd for $C_{27}H_{24}F_3N_2O_6$ $[M+H]^+$ 529.15810, found 529.15826.

Induction of mPGES-1, isolation of microsomes, and determination of mPGES-1 activity in microsomes of A549 cells

Human A549 cells was treated and prepared as described.[4] In brief, cells (2×10^6/20 mL DMEM/High glucose (4.5 g/l) medium containing FCS (2%, v/v)) were incubated for 16 h at 37 °C and 5% CO_2. Then, the culture medium was replaced by fresh medium, interleukin-1β (1 ng/ml) was added, and cells were incubated for another 72 h. Cells were detached with trypsin/EDTA, washed with PBS and frozen in liquid nitrogen. Ice-cold homogenization buffer (0.1 M potassium phosphate buffer pH 7.4, 1 mM phenylmethylsulfonyl fluoride, 60 µg/ml soybean trypsin inhibitor, 1 µg/ml leupeptin, 2.5 mM glutathione, and 250 mM sucrose) was added and after 15 min, cells were resuspended and sonicated (3 × 20 sec) on ice. The homogenate was subjected to differential centrifugation (10,000×g for 10 min and at 174,000×g for 1 h at 4 °C). The pellet (microsomal fraction) was resuspended in 1 ml homogenization buffer, and protein concentration was determined by the Coomassie protein assay. The microsomal membranes were then diluted in potassium phosphate buffer (0.1 M, pH 7.4) containing 2.5 mM glutathione (100 µl total volume) and test compounds or vehicle (DMSO) were added. After 15 min, PGE_2 formation was initiated by addition of 20 µM PGH_2 (final concentration). After 1 min at 4 °C, the reaction was terminated with 100 µl of stop solution (40 mM $FeCl_2$, 80 mM citric acid and 10 µM of 11β-PGE_2), PGE_2 was separated by solid phase extraction on reversed phase (RP)-C18 material using acetonitrile (200 µl) as eluent, and analyzed by RP-HPLC (30% acetonitrile aqueous + 0.007% TFA (v/v), Nova-Pak® C18 column, 5 × 100 mm, 4 µm particle size, flow rate 1 ml/min) with UV detection at 195 nm.

11β-PGE_2 was used as internal standard to quantify PGE_2 formation by integration of the area under the peaks.

Molecular docking models of compounds 1-3.

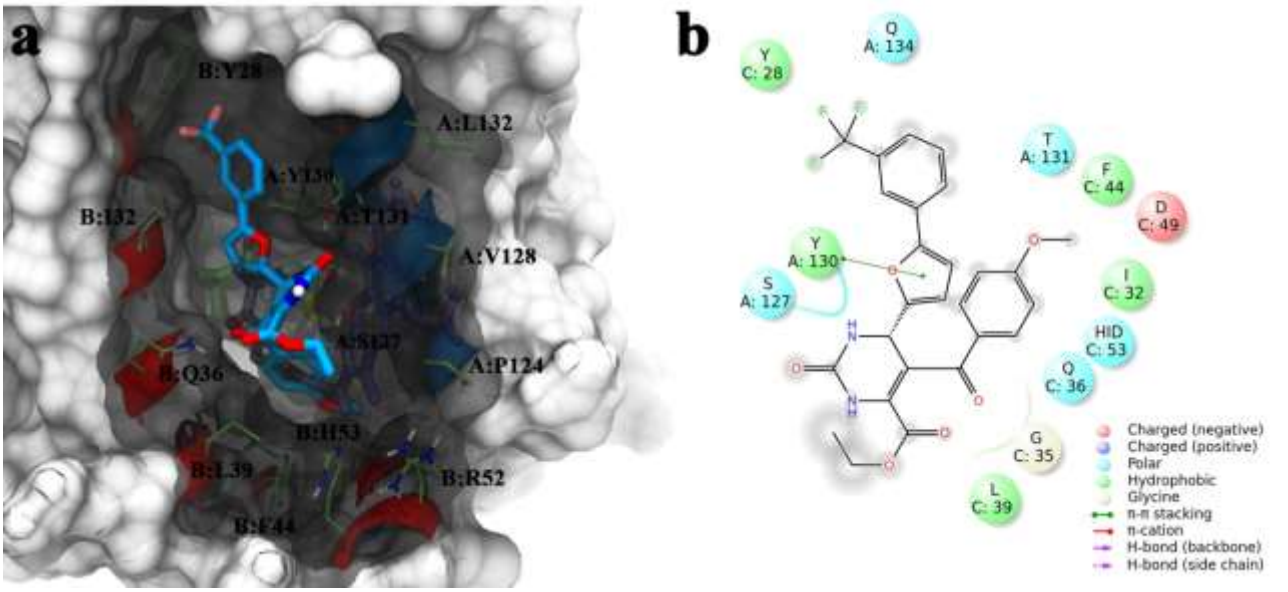

Fig. S1. a) 3D model of **1** (colored by atom types: C cyan, N blue, O red, H light gray, F pink) in docking with mPGES-1 (molecular surface represented in white); residues in the active site represented in licorice (colored by atom types: C green, N blue, O red, S yellow, H light gray) and related molecular surfaces depicted in transparent silver; superimposed structure of LVJ is depicted in transparent iceblue licorice. b) 2D panel representing interactions between **1** and residues in mPGES-1 binding site.

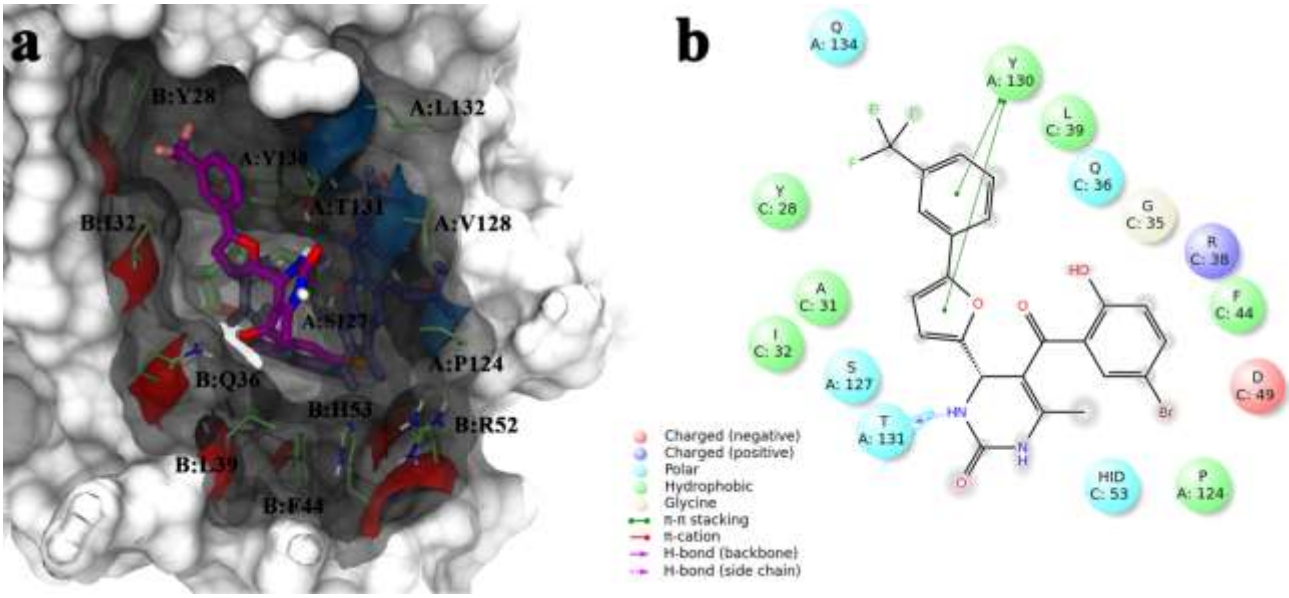

Fig. S2. a) 3D model of **2** (colored by atom types: C violet, N blue, O red, H light gray, F pink) in docking with mPGES-1 (molecular surface represented in white); residues in the active site represented in licorice (colored by atom types: C green, N blue, O red, S yellow, H light gray) and related molecular surfaces depicted in transparent silver; superimposed structure of LVJ is depicted in transparent iceblue licorice. b) 2D panel representing interactions between **2** and residues in mPGES-1 binding site.

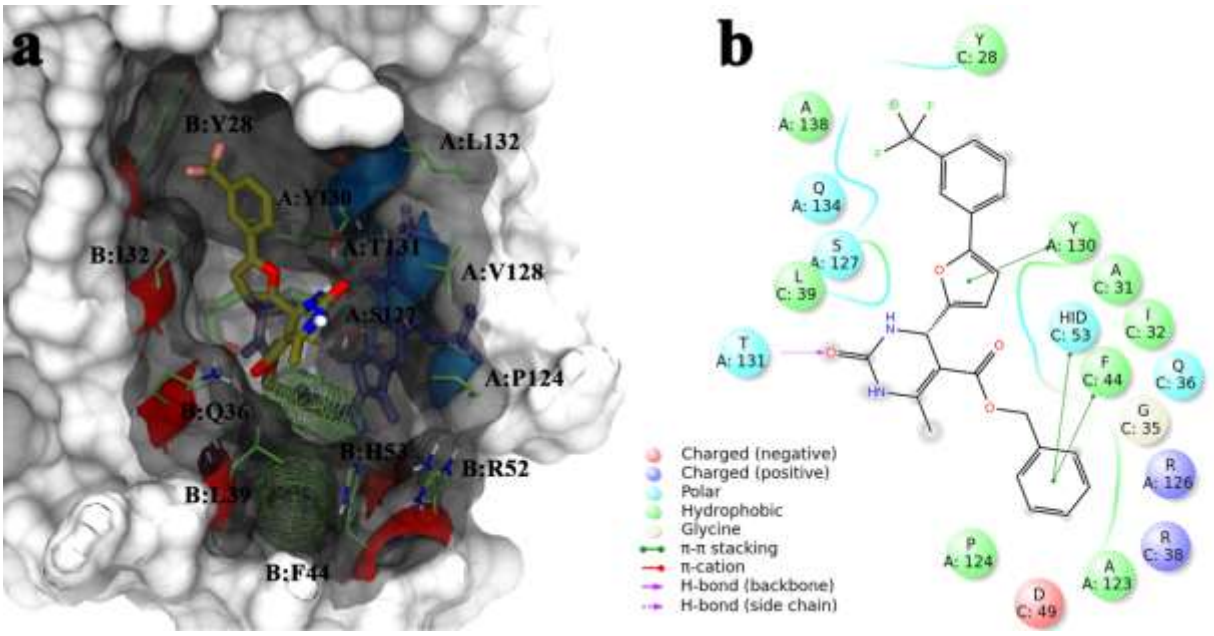

Fig. S3. a) 3D model of **3** (colored by atom types: C tan, N blue, O red, H light gray, F pink) in docking with mPGES-1 (molecular surface represented in white); residues in the active site represented in licorice (colored by atom types: C green, N blue, O red, S yellow, H light gray) and related molecular surfaces depicted in transparent silver; superimposed structure of LVJ is depicted in transparent iceblue licorice. b) 2D panel representing interactions between **3** and residues in mPGES-1 binding site.

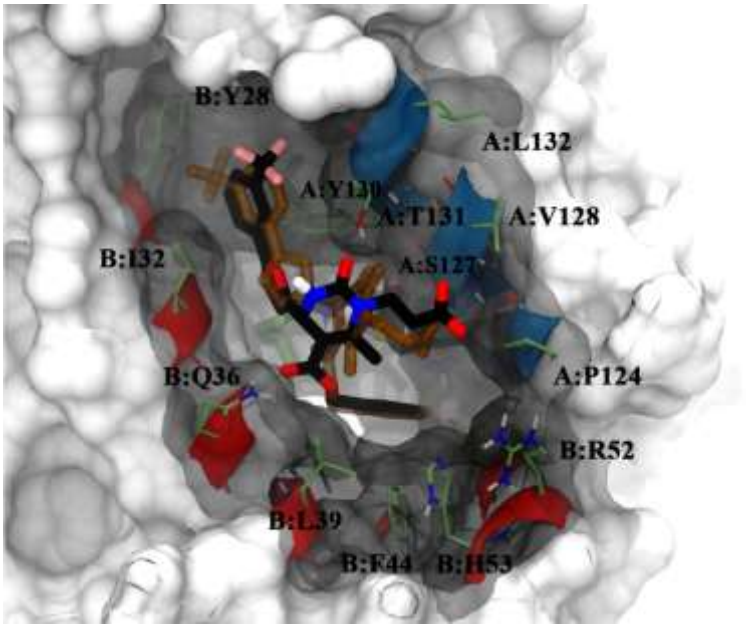

Fig. S4. Superposition between the two possible enantiomers at C4 of **4** (*R* enantiomer at colored by atom types: C black, N blue, O red, H light gray, F pink; *S* enantiomer colored in transparent orange) in docking with mPGES-1 (molecular surface represented in white); residues in the active site represented in licorice (colored by atom types: C green, N blue, O red, S yellow, H light gray).
Predicted binding affinities, as calculated with Glide software: -7.48 kcal/mol (*R* enantiomer); -8.83 kcal/mol (*S* enantiomer).

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