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FULL PAPER

A Combinatorial Virtual Screening Approach Driving the Synthesis of 2,4-Thiazolidinedione-Based Molecules as New Dual mPGES-1/5-LO Inhibitors

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Abstract: Dual inhibition of microsomal prostaglandin E_2 synthase-1 (mPGES-1) and 5-lipoxygenase (5-LO), two key enzymes involved in pro-inflammatory eicosanoid biosynthesis, represents a new strategy for treating inflammatory disorders. Herein we report the discovery of 2,4-thiazolidinedione-based mPGES-1/5-LO dual inhibitors following a multidisciplinary protocol, involving virtual combinatorial screening, chemical synthesis, and validation of the biological activities for the selected compounds. Following the multicomponent-based chemical route for the decoration of the 2,4-thiazolidinedione core, a large library of virtual compounds was built (~2.0×10^4 items) and submitted to virtual screening. Nine selected molecules were synthesized and biologically evaluated, disclosing among them four compounds able to reduce the activity of both enzymes in the mid- and low- micromolar range of activities. These results are of interest for further expanding the chemical diversity around the 2,4-thiazolidinedione central core, facilitating the identification of novel anti-inflammatory agents endowed with a promising and safer pharmacological profile.

Introduction

Prostaglandin E_2 synthases (PGES, namely mPGES-1, mPGES-2 and cPGES) are terminal enzymes involved in the conversion of prostaglandin H_2 (PGH_2), enzymatically produced by cyclooxygenases (COXs), to prostaglandin E_2 (PGE_2)[1-3]. Different from the constitutively expressed mPGES-2 and cPGES isoforms, mPGES-1 is an inducible membrane-bound form and it is involved in PGE_2-related acute and chronic disorders[4], such as pain[5], fever[6], rheumatoid arthritis[7], arthritis[8], inflammation[9], and cancer[10, 11]. Recently, mPGES-1 inhibitors emerged as new valuable and safer drugs with potentially reduced side effects as compared with COX inhibitors, thus representing a promising therapeutic option especially in the treatment of chronic inflammation related disorders. On the other hand, the use of classical nonsteroidal anti-inflammatory drugs (NSAIDs) is limited by several side effects, such as cardiovascular, gastrointestinal and renal side[12]. In the last few years, the identification of novel dual inhibitors of mPGES-1 and 5-lipoxygenase (5-LO) received a strong interest due to the involvement of these enzymes in the biosynthesis of pro-inflammatory PGE_2 and leukotrienes[13]. The combined modulation of both enzymes represents a valuable strategy to intervene with inflammatory pathologies in view of a higher efficacy and safety. In the frame of our ongoing efforts to develop new attractive anti-inflammatory agents targeting mPGES-1[14-19], we took advantage from the high-resolution X-ray structures of human mPGES-1 in complex with new and potent inhibitors recently published (e.g. PDB code: 4BPM, 4YL1, 5BQH, and 5BQI)[20-22].

From a structural point of view, mPGES-1 is a glutathione-dependent membrane protein located on the endoplasmic reticulum and structurally organized as homotrimer, with three equivalent active site cavities within the membrane-spanning region at each monomer interface[20]. Each asymmetric monomer is characterized by a four-helix bundle motif, while each active site is toward the cytoplasmic part of the protein between the N-terminal parts of helix II and IV of one monomer and the C-terminal part of helix I and the cytoplasmic domain of the adjacent monomer (Figure 1)[20]. The inhibitor-binding site of mPGES-1 features several regions (Figure 1); firstly, a binding groove is located between the GSH binding site and a molecular surface nearby the cytoplasmic part of the protein, mainly composed by aromatic (C:Phe44, C:His53) and polar (C:Arg52) residues (Figure 1). The cofactor GSH is in a profound cavity mainly characterized by polar residues, and it adopts a U-shape due to the strong interactions between its two terminal carboxylic functions and the positively charged residues in the deeper part of the binding site (C:Arg38, A:Arg73) (Figure 1). Importantly, the phenol group in the side chain of A:Tyr130 is involved in a π-stacking with the gamma peptide linkage between the cysteine and the glutamate of GSH.

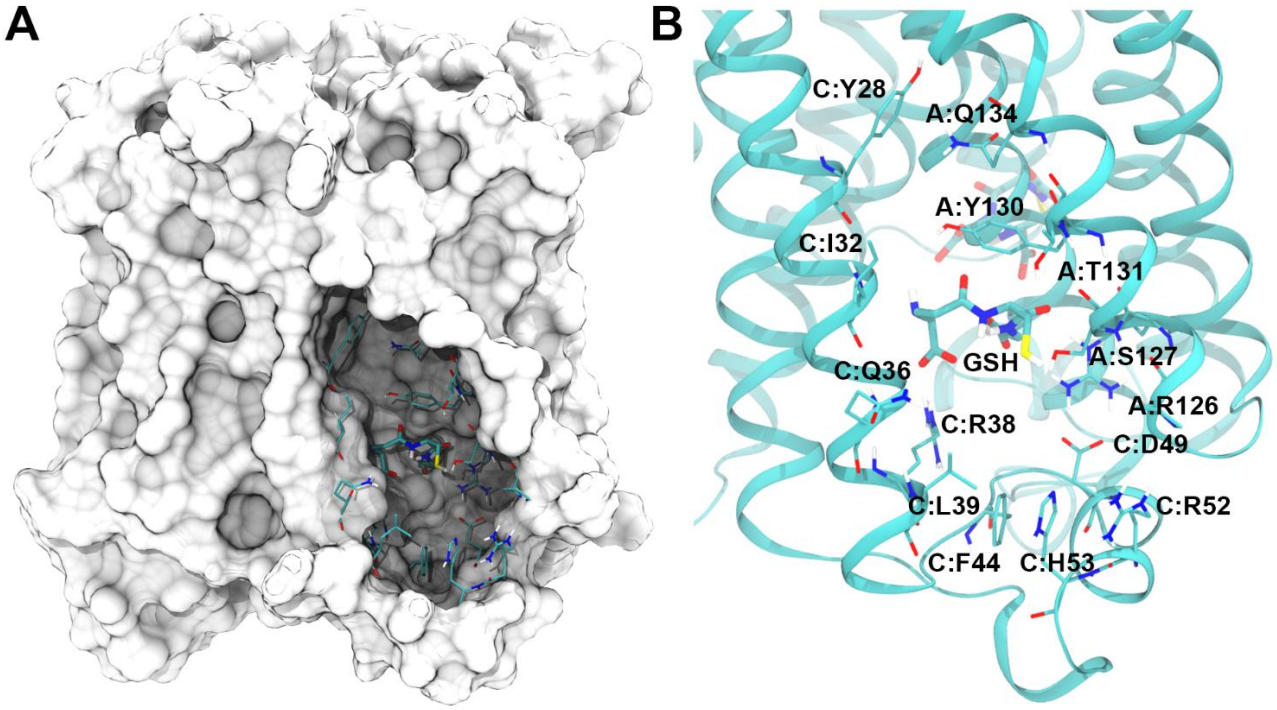


Figure 1. mPGES-1 structure: a) molecular surface representation of the mPGES-1 trimer ; b) secondary structure focused to the mPGES-1 binding site (colored in cyan); glutathione (GSH) cofactor and key-residues in the mPGES-1 binding site are represented in sticks (C: cyan, O red, N blue, S yellow, H light gray).

Finally, moving from the external part of the endoplasmic reticulum membrane to the cytoplasmic part of the protein, a binding groove is evident at the intersection between helix 1 of chain B and helix 4 of chain A, with polar (A:Gln134), aliphatic (C:Val24) and aromatic (C:Tyr28) residues, and could be bound by long molecular functions (Figure 1).

In this context, the catalysis mechanism for the conversion of PGH_2 to PGE_2 has been recently elucidated, revealing novel structural insights useful for the design of new mPGES-1 inhibitors[23]. In detail, Arg126 and Asp49 on the adjacent chain (see Figure 1), interacting within the crystal structure, were shown to be essential during catalysis. Also, the interruption of this arginine–aspartate interaction can facilitate their participation in the chemical mechanism, and then ligands able to interact with these two residues can represent potential mPGES-1 inhibitors[23].

In our research group, the careful analysis of these structural information has been extensively employed for the identification of several new chemotypes featuring mPGES-1 inhibitory activity[14-16, 18, 19, 24, 25].

Here, we have investigated the interesting 2,4-thiazolidinedione chemical core[26] as a new possible template for developing anti-inflammatory/anticancer agents. The “privileged scaffold” feature of the 2,4-thiazolidinedione chemical template has been widely analyzed and discussed, being it endowed with several relevant pharmacological effects (e.g. antihyperglycemic, hypolipidemic, antimicrobial, anticancer, anti-inflammatory, anti-oxidant).[27, 28] On the other hand, the absence of any “promiscuous binding” by this scaffold has been also demonstrated in a wok by Klein et al. by testing a high number of 2,4-thiazolidinedione-based compounds featuring different substituents against various targets, confirming it as a template for developing pharmacologically interesting compounds.[29]

Starting from these premises, the interference of 2-4-thiazolidinedione-based compounds on mPGES-1 activity was here investigated, while further evaluating their inhibition on 5-lipooxygenase (5-LO) as another key enzyme involved in the arachidonic acid cascade. Indeed, 5-LO catalyzes the initial transformation of arachidonic acid (AA) to 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and, afterwards, its subsequent conversion to LTA4. Subsequently, starting from LTA4, further enzymatic reactions cause the synthesis of LTB4 and the cysteinyl-LTs C4, D4, and E4, playing fundamental roles in inflammatory and allergic reactions. For all these reasons, mPGES-1/5-LO dual inhibition is considered particularly advantageous if compared with single interference in terms of both efficacy and adverse effects, representing the main aim of this work.

Results and Discussion

The chemical decoration of the 2,4-thiazolidinedione chemical core can be easily accomplished through a Knoevenagel condensation between the commercially available 2,4-thiazolidinedione with different aromatic aldehydes[30, 31], expanding the core in C-5 direction (Scheme 1) while N-3 position can be further modified through a reaction with different aliphatic halides (Scheme 1). Concerning the molecular modeling tasks, the 2,4-thiazolidinedione core (with minimal substitutions at positions 3 and 5) was firstly docked onto the mPGES-1 crystal structure (See Experimental Section) in order to evaluate its binding mode and the possibility of decorating it according to modifiable positions while gaining further interactions with the receptor counterpart. The analysis of the docking poses highlighted the accommodation of the 2,4-thiazolidinedione core in the central part of the mPGES-1 binding site close to the glutathione (GSH) cofactor (vide infra), thus making the

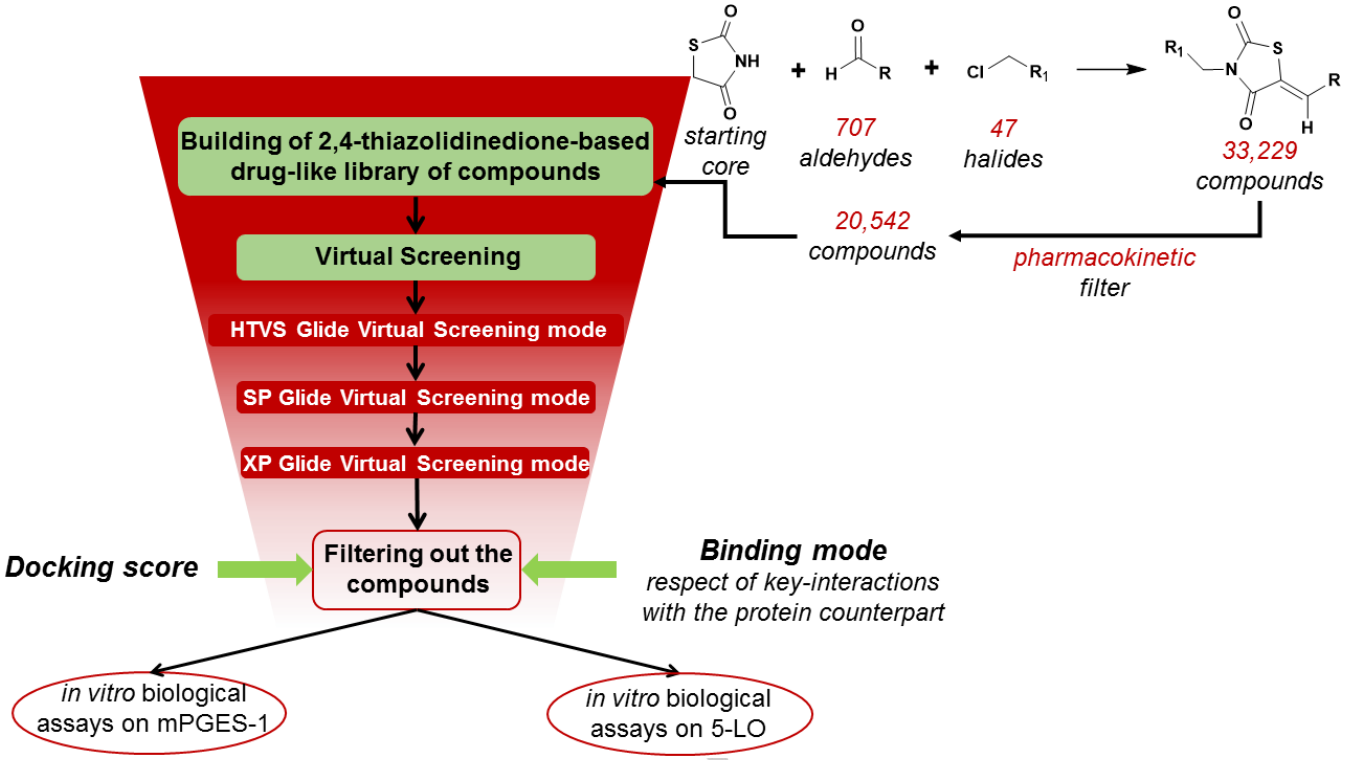


Figure 2. The workflow for the identification of the 2,4-thiazolidinedione-based mPGES-1/5-LO inhibitors.

modifications in the 3- and 5- positions desirable for the chemical optimization (See Figure S1, Supporting Information). Moreover, preliminary substitutions of the C-5 position with the aldehydes oriented the new introduced group towards the cytoplasmic part of the protein, thus close to C:His53 and C:Phe44 residues. For these reasons, we chose aromatic aldehydes in order to establish the edge-to-face π-π interactions with these residues. Also, we considered this position as the most important one for the substitution on the original core, since the establishment of these interactions was recognized as essential for the enzyme inhibition, as reported in different papers by us and other groups.[14, 16] Then, the other available position (N-3) was that related to the exploration of the external part of the endoplasmic reticulum membrane, with the aim of establishing further edge-to-face π-π interactions (with A:Phe130 and/or C:Tyr28) and polar interactions (with A:Gln134 and peptidic bond of the close residues). For these reasons, we selected a small set of halides featuring aromatic and polar groups; also, the choice of these reactants was limited by both the respect of molecular weight filter on the final molecules, in order to obtain compounds with drug-like properties, while assuring a certain variability for the final library preparation.

Starting from these preliminary data, we built *in silico* a large library of compounds featuring the 2,4-thiazolidinedione chemical core and substituted in 3- and 5- positions, considering the commercial availability of chemical synthons (33,229 compounds, considering commercially available aldehydes and commercially available/in-house synthesized halides) finally reduced to 20,542 items after filtering out "non-drug like" compounds (See Experimental Section) (Figure 2). The obtained library was then screened by means of molecular docking calculations on mPGES-1. Specifically, the semi-flexible docking procedure, namely considering the protein as rigid, and the ligands as flexible, was used. Indeed, after carefully analyzing and superimposing

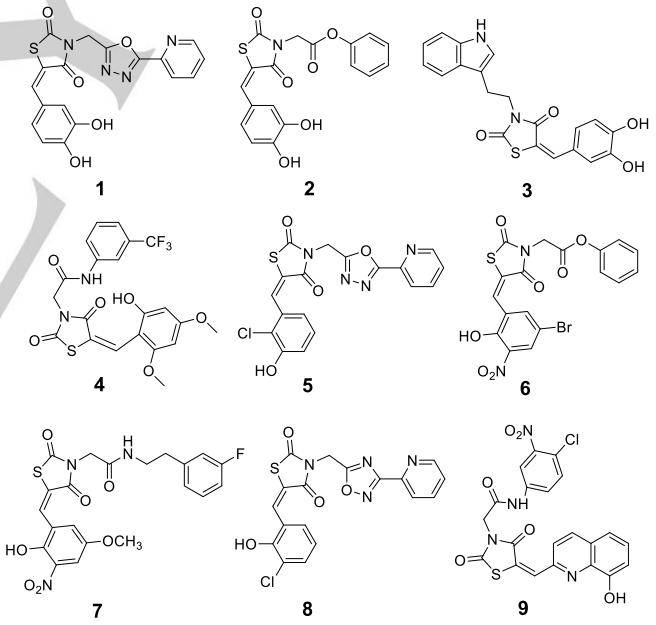
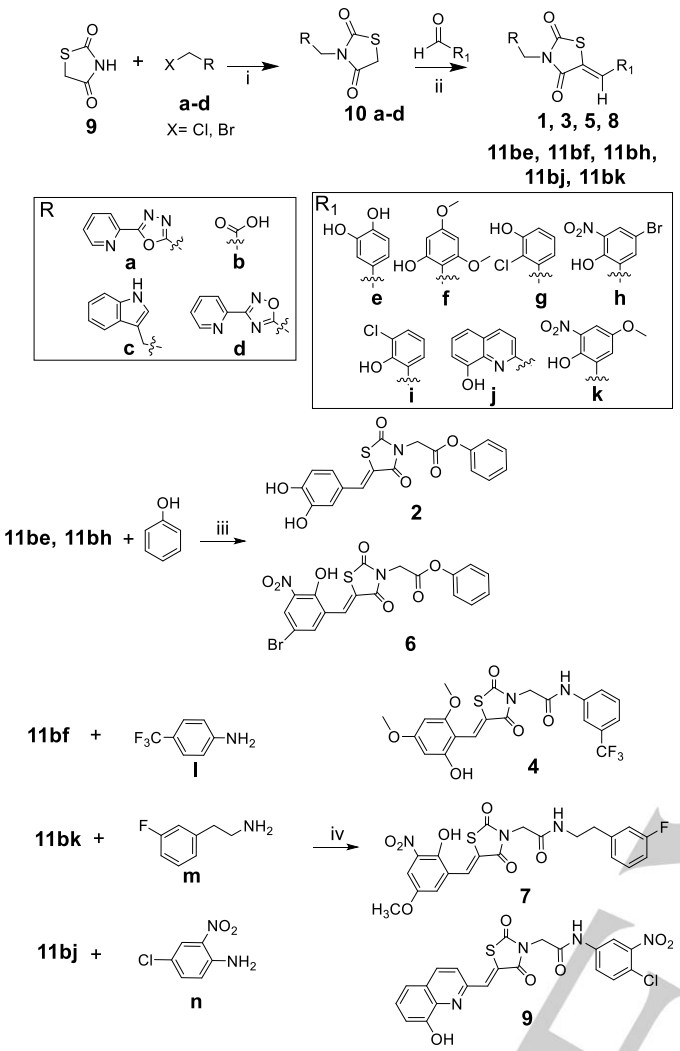


Figure 3. Chemical structure of compounds **1-9**

twelve mPGES-1 structures co-complexed with potent inhibitors (See Figures S2 and S3, Supporting Information), we noticed a high degree of similarity on the side chain positions of the key residues in the ligand binding site. Also, we excluded ligand-based procedures, since a high number of mPGES-1 inhibitors have been discovered so far, and each of them can be used in principle as a putative template against the large built 2-4-thiazolidinedione-based virtual library, thus complicating the calculation procedures. On the other hand, the analysis of the only one human 5-LO protein structure (wild-type) available in the



Scheme 1. Chemical route for the synthesis of compounds **1**-**9**. *Reagents and conditions:* (i) NaH, THF dry, reflux, 3h; (ii) piperidine, EtOH, reflux, overnight; (iii) DMAP, DIC, DCM, r.t., overnight; (iv) HOBt, DIC, DMF, r.t., overnight.

Protein Data Bank (PDB code: 3O8Y), not co-complexed with an inhibitor, disclosed a partially accessible ligand binding site, thus not compatible for the semi-rigid molecular docking procedure. For these reasons, in this specific case, Induced Fit experiments should be performed[32] and, considering the high number of compounds to be tested, this should require high computational times.

The 3D- protein structure used for the molecular modeling experiments was related to the crystal structure of the enzyme co-complexed with the potent inhibitor 4UL (PDB code: 5BQI)[21].

The "Virtual Screening Workflow", as implemented in Schrödinger Suite,[33] was employed (Figure 2) following these steps:

- High-Throughput Virtual Screening (HTVS) phase; saved first 60% of compounds ranked by docking score for the subsequent step;
- Standard Precision phase (SP); saved first 60% of compounds ranked by docking score for the subsequent step;
- Extra Precision phase (XP), saved first 70% of compounds ranked by docking score.

The binding mode of the filtered compounds was investigated by analyzing the related docking poses and checking the establishment of specific sets of interactions while showing a favorable accommodation in the binding sites. The application of several filters (selection of the most affine poses by docking score, analysis of the ligand-protein interactions, visual inspection) then led to the selection of nine compounds (**1**-**9**) for subsequent chemical synthesis (Figures 2-3 and Scheme 1) and biological evaluation (Figure 4 and Table 1). Specifically, the most promising compounds were selected ascertaining the respect of the following key interactions:

- edge-to-face π-π interaction with C:Phe44 and/or C:His53;
- contacts with A:Arg126, A:Thr131, C:Gln36, C:Asp49;
- contacts with GSH; also, the interaction with A:Tyr130. A key residue interacting with the GSH cofactor was considered specifically evaluating the possible edge to face π-π contacts with ligand counterpart.

For the synthesis of selected compounds (**1**-**9**, Figure 3) we started with the *N*-alkylation of commercially available 2,4-thiazolidiendione (**9**) to obtain *N*-substituted derivatives **10a**-**d** which were subjected to a Knoevenagel condensation using different substituted aromatic aldehydes (**e-k**)[26, 30, 31]. According to this procedure we synthesized compounds **1**, **3**, **5**, and **8** and the intermediates **11be**, **11bf**, **11bh**, **11bj**, and **11bk** (Scheme 1). The synthesis of **2** and **6** were performed by esterification of **11be** and **11bh** with phenol, respectively; while compounds **4**, **7** and **9** were obtained by amide coupling of **11bf**, **11bk** and **11bj** with different decorated anilines (**l**-**n**) (Scheme 1).

The ability of compounds **1**-**9** to interfere with the activity of mPGES-1 was determined by a cell-free assay, using the microsomal fractions of interleukin-1β (IL-1β)-stimulated A549 cells as a source for human mPGES-1.

Firstly, the compounds (solubilized in DMSO as vehicle) were tested at a final concentration of 10 μM. Among the tested compounds, **7** showed modest inhibition (~25% of inhibition), **3** and **6** partially inhibited the enzyme (~50% of inhibition), whereas **8** revealed the most promising inhibitory activity (~85% of inhibition) (Figure 4).

The biological activities of compounds **3**, **6**-**8** were further investigated by determining the IC_{50} for mPGES-1, confirming their interesting biological profile (see Table 1), and disclosing four compounds, among nine tested, with interesting biological activities spanning from the mid- to the low-micromolar range. Among these, compound **8** (IC_{50}= 3.5 ± 0.4 μM) turned out to be the most potent inhibitor against mPGES-1.

Docking results highlighted the favorable accommodation of **8** in the mPGES-1 binding site and the establishment of a large set of interactions with key residues, as above reported. In particular, edge-to-face π-π interactions between the terminal 3-chloro-2-hydroxyphenyl group of **8** with C:Phe44 and C:His53 were detected; also, a series of polar interactions were established with A:Arg126, A:Thr131, C:Gln36, and C:Asp49 as well as edge-to-face π-π interaction and hydrogen bond with A:Tyr130 in a region close to the GSH cofactor (Figure 5). Similar sets of interactions were found for compounds **3**, **6**, and **7** (See Figures S5-S7, Supporting Information)

Several mPGES-1 inhibitors were reported to suppress also 5-LO activity, the pivotal enzyme responsible for leukotriene (LT) biosynthesis[34]. Thus, to better investigate the biological profile of the disclosed molecules, we decided to evaluate their effects also

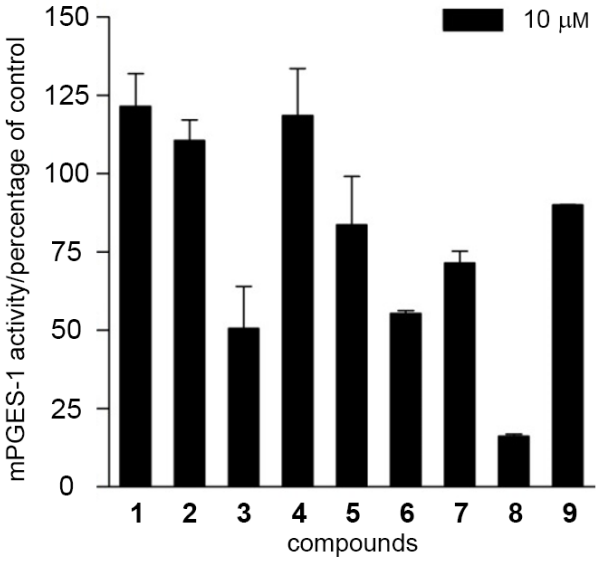


Figure 4. mPGES-1 remaining activity in the presence of compounds **1**-**9** at 10 μM final concentration. Data are given as mean ± S.E.M., number of replicates (n) = 3.

Table 1. Cell-free mPGES-1 remaining activity at [ligand]=10 μM, cell-free mPGES-1 and 5-LO IC_{50} values for tested compounds **1**-**9**.

Compound	Cell-free mPGES-1 remaining activity at 10 μM compound	Cell-free mPGES-1 IC_{50} (μM)[a]	Cell-free 5-LO IC_{50} (μM)[a]
1	100%	ND	ND.
2	100%	ND	ND
3	50%	10.2 ± 3.6	0.2 ± 0.1
4	100%	ND.	ND
5	80%	ND	ND.
6	60%	14.0 ± 1.7	3.9 ± 0.8
7	70%	22.5 ± 4.9	11.8 ± 2.4
8	15%	3.5 ± 0.4	9.4 ± 4.0
9	90%	ND	ND

[a] Data are given as mean ± S.E.M., n = 3.

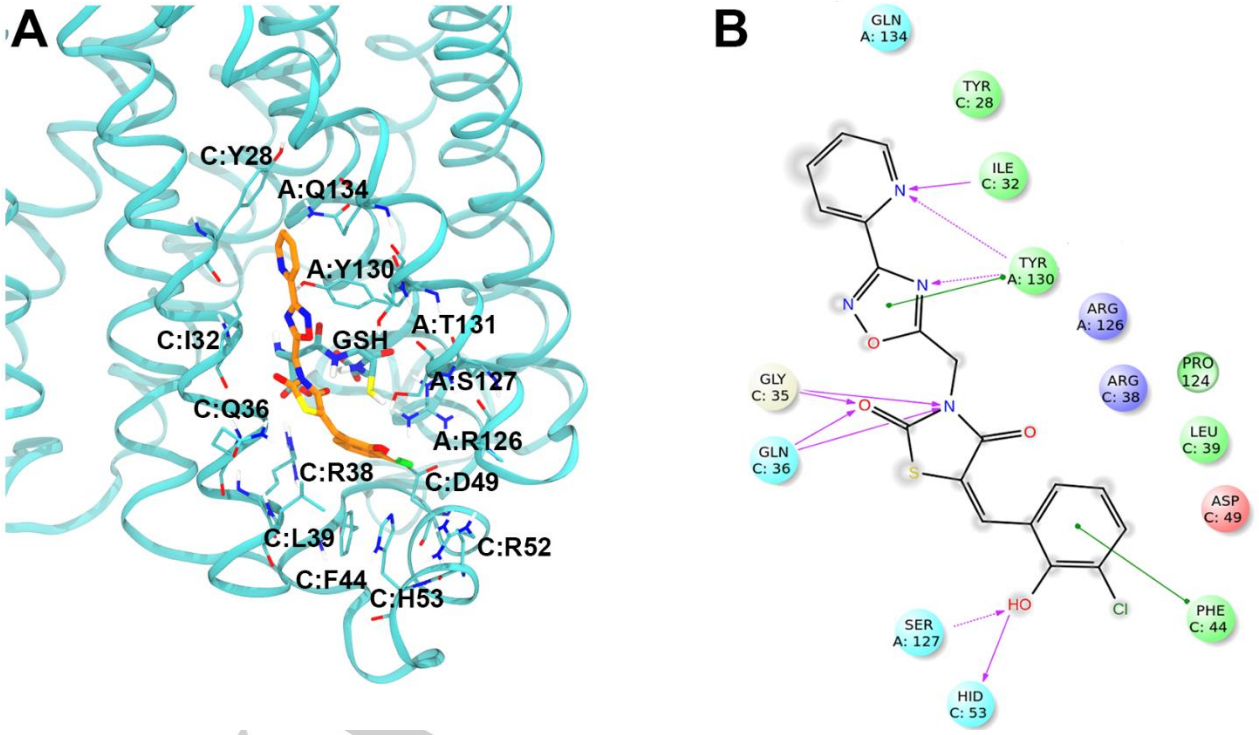


Figure 5. a) Selected 3D pose of **8** (colored by atom types: C, orange; N, blue; O, red; S, yellow; polar H, light grey; Cl, light green) in docking with human mPGES-1 (secondary structure focused to the mPGES-1 binding site colored in cyan; glutathione (GSH) cofactor and key-residues in the mPGES-1 binding site are represented in sticks colored by atom types: C, cyan; O, red; N, blue; S, yellow; H light gray); b) related two-dimensional panels representing interactions (violet arrows representing H-bonds, and green lines representing π-π stacking interactions).

against 5-LO, the pivotal enzyme responsible for leukotriene (LT) biosynthesis.

In detail, compounds **3**, **6-8** were tested against 5-LO activity in cell-free assays using partially purified human recombinant 5-LO[35]. The obtained results highlighted inhibitory activities for all the four tested compounds with IC_{50} values in the low micromolar range (Table 1). Compound **3** displayed the highest inhibitory activity against 5-LO among the tested compounds with IC_{50}= 0.2 ± 0.1 μM. Note that compared to the reference inhibitor zileuton (IC_{50} = 0.6 μM, data not shown) used as drug against asthma[36], compound **3** was even three-fold more potent.

Conclusions

In conclusion, based on the promising *in vitro* pharmacological activity, 2-4 thiazolidinedione-based compounds emerged as a new class of dual mPGES-1/5-LO inhibitors (See Table 1). The

reported results confirmed the applicability of the virtual screening workflow for accelerating the discovery of novel agents able to interact with pharmacologically relevant targets. Indeed, among nine compounds selected from the virtual screening procedure, three items showed mPGES-1 inhibition in the mid- micromolar range of activities and, interestingly, one in low-micromolar range. The selected four compounds were also able to inhibit 5-LO, another key enzyme involved in the arachidonic acid cascade for the leukotriene biosynthesis. The combined application of virtual combinatorial screening, easily accessible chemical synthesis and biological investigation of the predicted activities determined the selection of 2,4-thiazolidinedione-based small molecules able to inhibit mPGES-1 and 5-LO as attractive candidates for anti-inflammatory drug development. Furthermore, the computational indications obtained by the careful analysis of the docking poses related to the most active compounds (e.g. compound **8**) will be exploited for future optimization steps, in order to obtain derivatives with improved biological activity. Computationally demanding methods (e.g. molecular dynamics-based approaches) could be useful for an accurate prediction of the binding affinities. On the other hand, ligand-based methods seem to be not particularly reliable for this biological system, since the similarity analysis of the nine selected compound with twelve co-crystallized mPGES-1 inhibitors (See Figure S3, Supporting Information) computing Tanimoto coefficients showed overall poor values (See Table S1, Supporting Information).
On the other hand, this procedure could be reiterated for investigating novel possible chemical cores, especially considering the reasonable computational times for the building of the virtual library and the subsequent virtual screening.

Experimental Section

Computational studies.

Building of a combinatorial library and Virtual Screening. According to the chemical route for the decoration of 2,4-thiazolidinedione chemical core, we built a virtual library of compounds as input files for the subsequent molecular docking calculations, using CombiGlide software[37]. Briefly, this computational tool allows the decoration of the central core with a large set of substituents related to specific chemical synthons. Specifically, the starting reagents were considered, checking the commercial availability from Sigma-Aldrich:
- 707 commercially available aldehydes
- 47 halides, of which 13 commercially available, and 34 subsequently functionalized and showing ester and amide groups.
The starting core and all the reagents were then combined for producing the final library of compounds (33,229 molecules). On the produced starting virtual library, pharmacokinetic properties were computed using QikProp software[37], and finally "non-drug like" compounds were discarded using Reactive filter tool, then providing a final library of 20,542 2,4-thiazolidinedione–based derivatives.

Virtual Screening on mPGES-1. Protein 3D model was prepared using the Schrödinger Protein Preparation Wizard[37], starting from the mPGES-1 X-ray structure in the active form co-complexed with the inhibitor 4UL (2-(difluoromethyl)-5-{[(2-methylpropanoyl)amino]methyl}-N-{5-methyl-4-[4-(trifluoromethyl)phenyl]-1H-imidazol-2-yl}pyridine-3-carboxamide) (PDB code: 5BQI). The visual inspection of the protein crystal structure employed (PDB code: 5BQI) revealed that the binding of the reference co-crystallized inhibitor (4UL) was not assisted by water molecules and, for these reasons, we removed them for the subsequent molecular docking experiments (Figure S4, Supporting Information). All hydrogen atoms were added, and bond orders were assigned. Docking calculations were performed on the protein structure in the presence of the cofactor GSH, whereas 4UL was removed. The Virtual Screening was performed following the Virtual Screening Workflow using Glide software[37], following the scheme:
- High-Throughput Virtual Screening scoring and sampling (HTVS), saved 1 pose for each compound, saved first 60% of ranked compounds;
- Standard Precision scoring and sampling phase (SP), saved 1 pose for each compound, saved first 60% of ranked compounds
- Extra Precision scoring and sampling phase (XP), saved 10 maximum number of poses for each compound, saved first 70% of ranked compound poses as final output (~14000 poses).
The selected compounds were then ranked considering docking score (XP Glide Score), and visually inspected and filtered evaluating the respect of the key interactions as reported in the Results and Discussion section, leading to a small set of molecules as candidate items for the subsequent chemical synthesis.

Chemical synthesis.

General methods
All commercially available starting materials were purchased from Sigma-Aldrich and were used as received. The solvents used for the synthesis were of HPLC grade (Sigma-Aldrich). NMR spectra were recorded on a Bruker Avance 400 MHz instrument at *T*=298 K Compounds **1-9** were each dissolved in 0.5 mL of $CDCl_3$, or CD_3OD (Sigma-Aldrich, 99.98 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 solvent peak as internal reference. Electrospray mass spectrometry (ESI-MS) was performed on a LCQ DECA TermoQuest (San Josè, California, USA) mass spectrometer. Reactions were monitored on silica gel 60 F_{254} plates (Merck) and visualized under UV light (λ = 254nm, 365nm). Analytical and semi-preparative reversed-phase HPLC was performed on Agilent Technologies 1200 Series high performance liquid chromatography using a Fusion-RP, C_{18} reversed-phase column (100 x 2mm, 4μM, 80 Å, flow rate = 1 mL/min; 250 x 10.00 mm, 4 μM, 80 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 240 nm. The purity of all tested compound (>96%) were determined by HPLC analysis and NMR data. The compound **4** was selected as representative for fully NMR characterization of the series.

General Method for synthesis of N-substituted thiazolidine-2,4-diones 10a-d
To a solution of thiazolidine-2,4-dione (**9**) (1.0 equiv.) in dry THF (3.0 mL) NaH (1.2 equiv.) was added dropwise and the mixture was stirred for 30 min at 80°C; then the mixture was cooled to room temperature and halides **a**-**d** (1.5 equiv.) were added. The reaction was stirred at 80°C for 3h, and poured into ice-cold water.

The solvent was removed in vacuo, the mixture was extracted with AcOEt (3 x 20 mL) and the combined organic phases were washed with water (20.0 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo.

The desired compounds **10a-d** were confirmed by analytical HPLC and ESI-MS and used without any further purification for the next step.

General Method (A) for the Synthesis of 5-arylidene-thiazolidine-2,4-diones (1,3,5,8, 11be, 11bf, 11bh, 11bj, 11bk)

A mixture **10a-d** (1.0 equiv.), aromatic aldehydes **e-k** (1.0 equiv.), piperidine (0.8 equiv.), and ethanol (1.5 mL) were placed in a 25 ml bottom flask. The reaction mixture was continuously stirred and refluxed overnight. The course of the reaction was monitored by TLC. The reaction mixture was poured into water and acidified with AcOH, extracted with AcOEt (3 x 20 mL) and the combined organic phases were washed with water (20.0 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo.

HPLC purification was performed by semi-preparative reversed-phase HPLC (Fusion-RP, C_{18} reversed-phase column: 250 x 10.00mm, 4μM, 80 Å, flow rate = 4 mL/min) using the gradient conditions reported below and the final products **1**,**3**,**5**,**8** were characterized by ESI-MS and NMR spectra.

5-(3,4-Dihydroxy-benzylidene)-3-(5-pyridin-2-yl-[1,3,4]oxadiazol-2-ylmethyl)-thiazolidine-2,4-dione) (1).

Compound **1** was obtained by following the general procedure (A) as a pale yellow powdery solid (79.5 mg, 22% yield after HPLC purification); RP-HPLC t_R = 20.5 min, gradient condition: from 5% B to 100 % B over 50 min, flow rate of 4 mL/min, λ = 240 nm. ^{1}H NMR (400 MHz, CD_3OD): δ_H = 7.81 (s, 1H), 7.76 (s, 1H), 6.97-6.94 (m, 2H), 6.93-6.89 (m, 2H), 6.81 (d, *J*= 3.1 Hz, 1H), 6.79 (d, *J*= 3.1 Hz, 1H), 5.19 (s, 2H). ESMS, calcd for $C_{18}H_{12}N_4O_5S$ 396.38; found m/z = 395.5 $[M-H]^-$.

5-(3,4-Dihydroxy-benzylidene)-3-[2-(1H-indol-3-yl)-ethyl]-thiazolidine-2,4-dione (3).

Compound **3** was obtained by following the general procedure (A) as a pale yellow solid (91,8 mg, 20% yield after HPLC purification); RP-HPLC t_R = 30.2 min, gradient condition: from 5% B ending to 100% B over 50 min, flow rate of 4 mL/min, λ = 240 nm. ^{1}H NMR (400 MHz, CD_3OD) δ_H= 7.63 (s, 1H), 7.53 (d, *J* = 7.9 Hz, 1H), 7.22 (d, *J* = 8.0 Hz, 1H), 7.01-6.95 (m, 2H), 6.93-6.85 (m, 3H), 6.77 (d, *J*= 8.2 Hz, 1H), 3.92-3.87 (m, 2H), 3.02-2.98 (m, 2H). ESMS, calcd for $C_{20}H_{16}N_2O_4S$ 380.42; found m/z = 381,4 $[M + H]^+$.

5-(2-Chloro-3-hydroxy-benzylidene)-3-(5-pyridin-2-yl-[1,3,4]oxadiazol-2-ylmethyl)-thiazolidine-2,4-dione (5).

Compound **5** was obtained by following the general procedure (A) as pale yellow solid (60,6 mg, 15% yield after HPLC purification); RP-HPLC t_R = 23.9 min, gradient condition: from 5% B to 100 % B over 50 min, flow rate of 4 mL/min, λ = 240 nm. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 8.94-8.86 (m, 2H), 8.74 (s, 1H), 8.20 (s, 1H), 8.03-7.96 (m, 1H), 7.19 (t, *J*= 8.0 Hz, 1H), 7.12-7.06 (m, 1H), 6.99 (d, *J*= 7.6 Hz, 1H), 4.27 (s, 2H). ESMS, calcd for $C_{18}H_{11}ClN_4O_4S$ 414.82; found m/z = 415.3 $[M + H]^+$.

5-(3-Chloro-2-hydroxy-benzylidene)-3-(3-pyridin-2-yl-[1,2,4]oxadiazol-5-ylmethyl)-thiazolidine-2,4-dione (8).

Compound **8** was obtained by following the general procedure (A) as a yellow solid (63.7 mg, 41% yield after HPLC purification); RP-HPLC t_R = 25.3 min, gradient condition: from 5% B to 100 % B over 50 min, flow rate of 4 mL/min, λ = 240 nm. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 8.94-8.86 (m, 2H), 8.74 (s, 1H), 8.20 (s, 1H), 8.03-7.96 (m, 1H), 7.19 (t, *J*= 8.0 Hz, 1H), 7.12-7.06 (m, 1H), 7.01 (t, *J*= 8.0 Hz, 1H), 4.40 (s, 2H). ESMS, calcd for $C_{18}H_{11}ClN_4O_4S$ 414.82; found m/z = 415.4 $[M + H]^+$.

[5-(3,4-Dihydroxy-benzylidene)-2,4-dioxo-thiazolidin-3-yl]-acetic acid phenyl ester (2).

1.0 equiv. of **11be** was dissolved in DCM (4 mL), and DMAP (1.0 equiv.), phenol (1.0 equiv.) and DIC (1.0 equiv.) were added. The mixture was stirred overnight at room temperature and the reaction was monitored by TLC, analytical RP-HPLC and ESI-MS. The mixture was diluted with 10 mL of water, extracted with EtOAc (3 x 20 mL), and the combined organic layers were dried over sodium sulfate.

A portion of the crude product was purified by semi-preparative reversed-phase HPLC (Fusion-RP, C_{18} reversed-phase column, using the gradient conditions reported below), affording pure product **2** as white powdery solid (153.7 mg, 33% yield after HPLC purification);

RP-HPLC t_R = 20.1 min, gradient condition: from 5% B ending to 100% B over 50 min, flow rate of 4 mL/min, λ = 240 nm. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H= 8.30-8.24 (m, 1H), 8.03 (s, 1H), 7.83-7.77 (m, 1H), 7.50-7.43 (m, 2H), 7.37-7.35 (m, 3H), 3.59 (s, 2H). ESMS, calcd for $C_{18}H_{13}NO_6S$ 371.36; found m/z = 395.0 $[M + Na]^+$.

[5-(5-Bromo-2-hydroxy-3-nitro-benzylidene)-2,4-dioxo-thiazolidin-3-yl]-acetic acid phenyl ester (6).

1.0 equiv. of **11bh** was dissolved in DCM (4 mL), and DMAP (1.0 equiv.), phenol (1.0 equiv.) and DIC (1.0 equiv.) were added. The mixture was stirred overnight at room temperature and the reaction was monitored by TLC, analytical RP-HPLC and ESI-MS. The mixture was diluted with 10 mL of water, extracted with EtOAc (3 x 20 mL), and the combined organic layers were dried over sodium sulfate.

A portion of the crude product was purified by semi-preparative reversed-phase HPLC (Fusion-RP, C_{18} reversed-phase column, using the gradient conditions reported below), affording pure product **6** as white solid (48.5 mg, 25% yield after HPLC purification);

RP-HPLC t_R = 32.4 min, gradient condition: from 5% B to 100 % B over 50 min, flow rate of 4 mL/min, λ = 240 nm. ^{1}H NMR (400 MHz, CD_3OD): δ_H = 8.18 (d, *J*= 8.8 Hz, 1H), 7.91 (s, 1H), 7.80-7.74 (m, 1H), 7.51-7.44 (m, 2H), 7.34 (d, *J*= 7.3 HZ, 1H), 7.28-7.23 (m, 2H), 5.19 (s, 2H). ESMS, calcd for $C_{18}H_{11}BrN_2O_7S$ 479.26; found m/z = 518.2 $[M + K]^+$.

General method (B) for synthesis of compounds 4,7,9:

1.0 equiv. of **11bf**, **11bk**, **11bj** were dissolved in DCM (4 mL), amines **l-n** (2.0 equiv.), HOBt (1.0 equiv) and DIC (1.5 equiv) were added. The mixture was stirred overnight at room temperature to and the reaction was monitored by TLC. After completation, the reaction mixture was extracted with EtOAc (3x20mL) and the organic phase was anhydrified with Na_2SO_4 and then evaporated under vacuum to give the desired products. HPLC purification was performed by semi-preparative reversed-phase HPLC (Fusion-RP, C_{18} reversed-phase column, using the gradient conditions reported below) and the final products were characterized by ESI-MS and NMR spectra.

2-[5-(2-Hydroxy-4,6-dimethoxy-benzylidene)-2,4-dioxo-thiazolidin-3-yl]-N-(3-trifluoromethyl-phenyl)-acetamide (4)
Compound **4** was obtained by following the general procedure (B) as a pale yellow solid (48.6 mg, 24% yield after HPLC purification); RP-HPLC t_R = 24.1 min, gradient condition: from 5% B to 100 % B over 50 min, flow rate of 4 mL/min, λ = 240 nm. ^{1}H NMR (400 MHz, CD_3OD): δ_H = 8.66 (s, 1H), 8.00-7.87 (m, 2H), 7.63 (*t*, *J*=7.7Hz, 1H), 7.53-7.45 (m, 1H), 6.63 (s, 1H), 6.58 (s, 1H), 5.47 (s, 2H), 3.99 (s, 3H), 3.95 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ = 174.9, 172.7, 172.1, 168.5, 167.3, 152.1, 137.5, 136.8, 134.3, 132.5, 131.7, 129.2, 128.6, 120.0, 116.8, 112.2, 103.0, 99.4, 54.1, 51.3, 33.0. ESMS, calcd for $C_{21}H_{17}F_3N_2O_6S$ 482.43; found m/z = 505.4 $[M + Na]^+$.

N-[2-(3-Fluoro-phenyl)-ethyl]-2-[5-(2-hydroxy-5-methoxy-3-nitro-benzylidene)-2,4-dioxo-thiazolidin-3-yl]-acetamide (7)

Compound **7** was obtained by following the general procedure (B) as a pale yellow solid (122.0 mg, 60% yield after HPLC purification); RP-HPLC t_R = 35.9 min, gradient condition: from 5% B to 100 % B over 50 min, flow rate of 4 mL/min, λ = 240 nm. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H= 7.87 (s, 1H), 7.77 (s, 1H), 7.32 (s, 1H), 6.98-6.84 (m, 4H), 3.94 (s, 3H), 3.70-3.64 (m, 2H), 3.43 (s, 2H) 2.89 (q, *J*= 6.8 Hz, 2H) , ESMS, calcd for $C_{21}H_{18}FN_3O_7S$ 475.45; found m/z = 476.5 $[M + H]^+$.

N-(4-Chloro-3-nitro-phenyl)-2-[5-(8-hydroxy-quinolin-2-ylmethylene)-2,4-dioxo-thiazolidin-3-yl]-acetamide (9)
Compound **9** was obtained by following the general procedure (B) as a yellow solid (116.1 mg, 72% yield after HPLC purification); RP-HPLC t_R = 23.6 min, gradient condition: from 5% B to 100 % B over 50 min, flow rate of 4 mL/min, λ = 240 nm. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 8.23 (s, 1H), 8.19-8.06 (m, 1H), 7.81 (d, *J*= 8.6 Hz, 1H), 7.62 (d, *J*= 8.6 Hz, 1H), 7.22-7.14 (m, 4H), 6.81 (d, *J*= 8.6 Hz, 1H), 3.84 (s, 2H). ESMS, calcd for $C_{21}H_{13}ClN_4O_6S$ 484.87; found m/z = 507.3 $[M + Na]^+$.

Biological assays.

Cell-free mPGES-1 activity assay. Microsomal preparations of interleukin-1β-treated A549 (human lung carcinoma) cells were used as source for mPGES-1. Expression of mPGES-1, preparation of microsomes and determination of mPGES-1 activity was performed as described previously[38]. This protocol showed a high robustness and accuracy, since it was applied in previous studies considering mPGES-1 inhibitors as standard drugs, like FLAP/mPGES-1 inhibitor MK-886 for which the related mPGES-1 IC_{50} value was correctly reproduced[38-40]. In brief, A549 cells were treated with Il-1β (1 ng/mL) for 48 h, cells were harvested, sonicated, and the homogenate was subjected to differential centrifugation at a) 10,000 × g for 10 min and b) 174,000 × g for 1 h at 4°C. The microsomal fraction (pellet) was resuspended in 1 mL homogenization buffer (0.1 M potassium phosphate buffer, pH 7.4, 1 mM phenylmethanesulfonyl fluoride, 60 mg/mL soybean trypsin inhibitor, 1 mg/mL leupeptin, 2.5 mM glutathione, and 250 mM sucrose), the total protein concentration was determined, and microsomes (2.5–5 µg total protein) were diluted in 50 µL potassium phosphate buffer (0.1 M, pH 7.4) containing 2.5 mM glutathione. The test compounds were solubilized in DMSO and were pre-incubated with microsomes (final DMSO concentration: 0.3%) for 15 min at 4°C. The reaction was started by addition of PGH_2 (50 µL in potassium phosphate buffer (0.1 M, pH 7.4) containing 2.5 mM glutathione; final PGH_2 concentration: 20 µM) and terminated after 1 min by addition of stop solution (100 µL; 40 mM $FeCl_2$, 80 mM citric acid and 10 µM of 11β-PGE_2 as internal standard). PGE_2 was separated by solid-phase extraction and analyzed by RP-HPLC as described previously[38]. Data were normalized to the vehicle control to avoid variations independent of test compounds. MK886 (at 1, 3, 10, and 30 µM) was used as reference drug.

Cell-free 5-LO activity assay.
E. coli (BL21) were transformed with pT3-5-LO plasmid, and human recombinant 5-LO protein was expressed and partially purified as described[41]. Briefly, cells were lysed in 50 mM triethanolamine-HCl pH 8.0, 5 mM EDTA, soybean trypsin inhibitor (60 µg/mL), 1 mM phenylmethanesulphonyl fluoride, and lysozyme (1 mg/mL), homogenized by sonication (for 3 × 15 s), and centrifuged at 40,000 × g (20 min at 4 °C). The 40,000 × g supernatant was applied to an ATP-agarose column to partially purify 5-LO as described[41]. Aliquots of purified 5-LO were immediately diluted with ice-cold PBS containing 1 mM EDTA. Samples were pre-incubated with the test compounds or vehicle (0.1% DMSO) as indicated. After 10 min at 4 °C, samples were pre-warmed for 30 s at 37 °C, and 2 mM $CaCl_2$ plus 20 µM arachidonic acid was added to start 5-LO product formation. The reaction was stopped after 10 min at 37 °C by addition of 1 mL ice-cold methanol, and the formed metabolites were analyzed by RP-HPLC as described[41]. 5-LO products include the all-trans isomers of LTB_4 as well as 5-HPETE and its corresponding alcohol 5-HETE. Zileuton (at 0.1, 0.3, 1 and 3 µM) was used as reference drug.

Statistical analysis. Data obtained are expressed as mean ± S.E. of single determinations performed in three or four independent experiments at different days. IC_{50} values were graphically calculated from averaged measurements at 4 different concentrations of the compounds using GRAPHPAD PRISM 4.0 software (San Diego, CA, USA).

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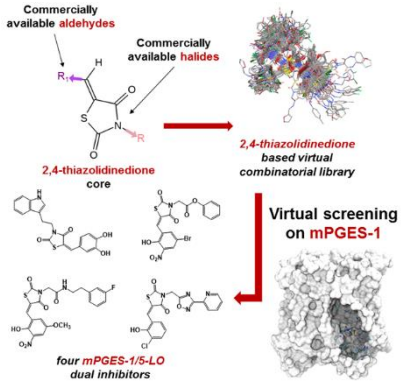
Conflict of interest

The authors declare no conflict of interest.

References:

[1] A. Koeberle, S. A. Laufer, O. Werz, *J. Med. Chem.* **2016**, *59,* 5970-5986.
[2] I. Kudo, M. Murakami, *J. Biochem. Mol. Biol.* **2005**, *38,* 633-638.
[3] A. Koeberle, O. Werz, *Biochem. Pharmacol.* **2015**, *98,* 1-15.
[4] B. Samuelsson, R. Morgenstern, P. J. Jakobsson, *Pharmacol. Rev.* **2007**, *59,* 207-224.
[5] D. Kamei, K. Yamakawa, Y. Takegoshi, M. Mikami-Nakanishi, Y. Nakatani, S. Oh-Ishi, H. Yasui, Y. Azuma, N. Hirasawa, K. Ohuchi, H. Kawaguchi, Y. Ishikawa, T. Ishii, S. Uematsu, S. Akira, M. Murakami, I. Kudo, *J. Biol. Chem.* **2004**, *279,* 33684-33695.
[6] D. Engblom, S. Saha, L. Engstrom, M. Westman, L. P. Audoly, P. J. Jakobsson, A. Blomqvist, *Nat. Neurosci.* **2003**, *6,* 1137-1138.
[7] M. Westman, M. Korotkova, E. af Klint, A. Stark, L. P. Audoly, L. Klareskog, A. K. Ulfgren, P. J. Jakobsson, *Arthritis Rheum.* **2004**, *50,* 1774-1780.
[8] H. Fahmi, *Curr. Opin. Rheumatol.* **2004**, *16,* 623-627.
[9] C. E. Trebino, J. L. Stock, C. P. Gibbons, B. M. Naiman, T. S. Wachtmann, J. P. Umland, K. Pandher, J. M. Lapointe, S. Saha, M. L. Roach, D. Carter, N. A. Thomas, B. A. Durtschi, J. D. McNeish, J. E. Hambor, P. J. Jakobsson, T. J. Carty, J. R. Perez, L. P. Audoly, *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100,* 9044-9049.
[10] D. Kamei, M. Murakami, Y. Nakatani, Y. Ishikawa, T. Ishii, I. Kudo, *J. Biol. Chem.* **2003**, *278,* 19396-19405.
[11] M. Nakanishi, V. Gokhale, E. J. Meuillet, D. W. Rosenberg, *Biochimie* **2010**, *92,* 660-664.
[12] K. D. Rainsford, *Am. J. Med.* **1999**, *107,* 27S-35S.
[13] O. Radmark, O. Werz, D. Steinhilber, B. Samuelsson, *Biochim. Biophys. Acta* **2015**, *1851,* 331-339.
[14] G. Lauro, P. Tortorella, A. Bertamino, C. Ostacolo, A. Koeberle, K. Fischer, I. Bruno, S. Terracciano, I. M. Gomez-Monterrey, M. Tauro, F. Loiodice, E. Novellino, R. Riccio, O. Werz, P. Campiglia, G. Bifulco, *ChemMedChem* **2016**, *11,* 612-619.
[15] G. Lauro, M. Manfra, S. Pedatella, K. Fischer, V. Cantone, S. Terracciano, A. Bertamino, C. Ostacolo, I. Gomez-Monterrey, M. De Nisco, R. Riccio, E. Novellino, O. Werz, P. Campiglia, G. Bifulco, *Eur. J. Med. Chem.* **2017**, *125,* 278-287.
[16] G. Lauro, M. Strocchia, S. Terracciano, I. Bruno, K. Fischer, C. Pergola, O. Werz, R. Riccio, G. Bifulco, *Eur. J. Med. Chem.* **2014**, *80,* 407-415.
[17] S. Terracciano, G. Lauro, M. Strocchia, K. Fischer, O. Werz, R. Riccio, I. Bruno, G. Bifulco, *ACS Med. Chem. Lett.* **2015**, *6,* 187-191.
[18] G. Lauro, V. Cantone, M. Potenza, K. Fischer, A. Koeberle, O. Werz, R. Riccio, G. Bifulco, *Medchemcomm* **2018**, *9,* 2028-2036.
[19] M. Iranshahi, M. G. Chini, M. Masullo, A. Sahebkar, A. Javidnia, M. Chitsazian Yazdi, C. Pergola, A. Koeberle, O. Werz, C. Pizza, S. Terracciano, S. Piacente, G. Bifulco, *J. Nat. Prod.* **2015**, *78,* 2867-2879.
[20] T. Sjogren, J. Nord, M. Ek, P. Johansson, G. Liu, S. Geschwindner, *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110,* 3806-3811.
[21] M. A. Schiffler, S. Antonysamy, S. N. Bhattachar, K. M. Campanale, S. Chandrasekhar, B. Condon, P. V. Desai, M. J. Fisher, C. Groshong, A. Harvey, M. J. Hickey, N. E. Hughes, S. A. Jones, E. J. Kim, S. L. Kuklish, J. G. Luz, B. H. Norman, R. E. Rathmell, J. R. Rizzo, T. W. Seng, S. J. Thibodeaux, T. A. Woods, J. S. York, X. P. Yu, *J. Med. Chem.* **2015**, *59,* 194-205.
[22] D. Li, N. Howe, A. Dukkipati, S. T. A. Shah, B. D. Bax, C. Edge, A. Bridges, P. Hardwicke, O. M. P. Singh, G. Giblin, A. Pautsch, R. Pfau, G. Schnapp, M. Wang, V. Olieric, M. Caffrey, *Cryst. Growth Des.* **2014**, *14,* 2034-2047.
[23] J. S. Brock, M. Hamberg, N. Balagunaseelan, M. Goodman, R. Morgenstern, E. Strandback, B. Samuelsson, A. Rinaldo-Matthis, J. Z. Haeggstrom, *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113,* 972-977.
[24] R. De Simone, M. G. Chini, I. Bruno, R. Riccio, D. Mueller, O. Werz, G. Bifulco, *J. Med. Chem.* **2011**, *54,* 1565-1575.
[25] S. Di Micco, S. Terracciano, V. Cantone, K. Fischer, A. Koeberle, A. Foglia, R. Riccio, O. Werz, I. Bruno, G. Bifulco, *Eur. J. Med. Chem.* **2018**, *143,* 1419-1427.
[26] S. Terracciano, G. Lauro, A. Russo, M. C. Vaccaro, A. Vassallo, M. De Marco, B. Ranieri, A. Rosati, M. C. Turco, R. Riccio, G. Bifulco, I. Bruno, *Chem. Commun.* **2018**, *54,* 7613-7616.
[27] T. Tomasic, L. P. Masic, *Curr. Med. Chem.* **2009**, *16,* 1596-1629.
[28] M. J. Naim, M. J. Alam, S. Ahmad, F. Nawaz, N. Shrivastava, M. Sahu, O. Alam, *Eur. J. Med. Chem.* **2017**, *129,* 218-250.
[29] T. Mendgen, C. Steuer, C. D. Klein, *J. Med. Chem.* **2012**, *55,* 743-753.
[30] G. Bruno, L. Costantino, C. Curinga, R. Maccari, F. Monforte, F. Nicolo, R. Ottana, M. G. Vigorita, *Bioorg. Med. Chem.* **2002**, *10,* 1077-1084.
[31] D. Rakowitz, R. Maccari, R. Ottana, M. G. Vigorita, *Bioorg. Med. Chem.* **2006**, *14,* 567-574.
[32] S. N. A. Bukhari, G. Lauro, I. Jantan, C. F. Chee, M. W. Amjad, G. Bifulco, H. Sher, I. Abdullah, N. A. Rahman, *Future Med. Chem.* **2016**, *8,* 1953-1967.
[33] LigPrep (www.schrodinger.com/LigPrep), Maestro (www.schrodinger.com/Maestro), Protein Preparation Wizard (www.schrodinger.com/Protein-Preparation-Wizard) and Glide (www.schrodinger.com/Glide) are all products of Schrödinger, LLC (New York, USA).
[34] A. Koeberle, O. Werz, *Biotechnol. Adv.* **2018**, *36,* 1709-1723.
[35] O. Radmark, B. Samuelsson, *J. Intern. Med.* **2010**, *268,* 5-14.
[36] W. Berger, M. T. De Chandt, C. B. Cairns, *Int. J. Clin. Pract.* **2007**, *61,* 663-676.
[37] QikProp (www.schrodinger.com/QikProp) is product of Schrödinger, LLC (New York, USA).
[38] A. Koeberle, U. Siemoneit, U. Buhring, H. Northoff, S. Laufer, W. Albrecht, O. Werz, *J. Pharmacol. Exp. Ther.* **2008**, *326,* 975-982.
[39] A. Koeberle, H. Northoff, O. Werz, *Biochem. Pharmacol.* **2009**, *77,* 1513-1521.
[40] U. Siemoneit, A. Koeberle, A. Rossi, F. Dehm, M. Verhoff, S. Reckel, T. J. Maier, J. Jauch, H. Northoff, F. Bernhard, V. Doetsch, L. Sautebin, O. Werz, *Br. J. Pharmacol.* **2011**, *162,* 147-162.
[41] L. Fischer, D. Szellas, O. Radmark, D. Steinhilber, O. Werz, *FASEB J.* **2003**, *17,* 949-951.

Entry for the Table of Contents



2,4- thiazolidinedione-based mPGES-1 inhibitors were identified applying a multidisciplinary protocol involving virtual combinatorial screening, chemical synthesis, and biological evaluation of the selected compounds. These compounds were also able to inhibit 5-LO, another key enzyme involved in the arachidonic acid cascade for the leukotriene biosynthesis. The reported results highlighted the applicability of this workflow for the discovery of pharmacologically relevant compounds.