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Selective Inhibitors of Human mPGES-1 from Structure-Based Computational Screening

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Abstract

Human mPGES-1 is recognized as a promising target for next generation of anti-inflammatory drugs. Although various mPGES-1 inhibitors have been reported in literature, few have entered clinical trials and none has been proven clinically useful so far. It is highly desired for developing the next generation of therapeutics for inflammation-related diseases to design and discover novel inhibitors of mPGES-1 with new scaffolds. Here, we report the identification of a series of new, potent and selective inhibitors of human mPGES-1 with diverse scaffolds through combined computational and experimental studies. The computationally modeled binding structures of these new inhibitors with mPGES-1 provide some interesting clues for rational design of modified structures of the inhibitors to more favorably bind with mPGES-1.

Graphical Abstract

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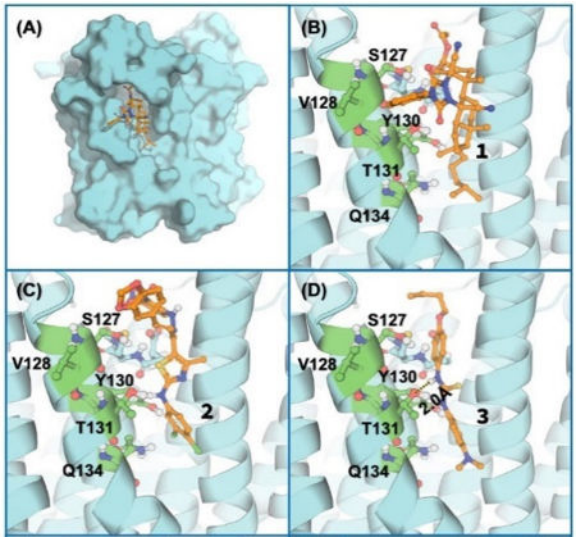
Supporting Information

Molecular structures of compounds **8** to **40** listed in Table 1.



A combined use of virtual screening and wet experimental activity assays has led to identification of new, potent and selective inhibitors of human mPGES-1 with diverse scaffolds.

Keywords

Prostaglandin; inflammation; selective inhibitor; inhibitor identification

Prostaglandin E2 (PGE_2) is known as the principal proinflammatory prostanoid and plays an important role in nociception.[1] The biosynthesis[2] of PGE_2 starts from arachidonic acid (AA) which is converted by cyclooxygenase COX-1 or COX-2 to prostaglandin H2 (PGH_2).[2] Then, PGH_2 is converted to PGE_2 by the prostaglandin E synthase (PGES) enzymes,[3] including microsomal PGES-1 (mPGES-1), an inducible enzyme.[4] It is known that mPGES-1 and COX-2 together[5, 6] play a key role in a number of inflammation-related diseases.[7–13] Hence, human mPGES-1 is recognized as a promising target for next generation of drugs to treat the inflammation-related diseases.[14]

There are a number of non-steroidal anti-inflammatory drugs (NSAIDs) available for current clinical practice. The available NSAIDs inhibit COX-1 and/or COX-2.[15] All of the available COX-1/2 inhibitors have significant adverse side effects.[16] The serious side effects led to withdrawal of rofecoxib (Vioxx), a selective COX-2 inhibitor. So, it is interesting to develop novel, improved anti-inflammatory drugs.[15] Through the action of the COX inhibitors, all prostaglandins downstream of PGH_2 cannot be produced, resulting in a variety of problems. For example, blocking the production of prostaglandin-I_2 (PGI_2) will cause significant cardiovascular problems.[17] Inducible enzyme mPGES-1 is a more promising target for anti-inflammatory drugs, because the mPGES-1 inhibition will only block the PGE_2 production without affecting PGI_2 and other prostaglandins for their production, as confirmed by reported knock-out studies.[18, 19] Thus, mPGES-1 inhibitors are expected to retain the anti-inflammatory effect of COX inhibitors, but without the side effects caused by the COX inhibition.

Although various mPGES-1 inhibitors have been reported in literature,[20–40] few have entered clinical trials[41] and none has been proven clinically useful so far due to various problems with the compounds themselves. It is highly desired for developing the next generation of therapeutics for inflammation-related diseases to design and discover new inhibitors of mPGES-1 with different scaffolds. Here, we report the identification of a set of new, potent and selective inhibitors of human mPGES-1 with various scaffolds through combined computational and experimental studies.

Our virtual screening was based on the X-ray crystal structure (PDB ID: 4BPM)[42] of human mPGES-1 and performed on the Development Therapeutics Program (DTP) Release 4 compound library including ~265,000 compounds available at the National Cancer Institute (https://cactus.nci.nih.gov/download/nci/). The virtual screening procedure used to screen the compounds in the library is similar to that we previously used to identify small-molecule inhibitors of various protein targets.[43, 44] First, the ~265,000 compounds were screened by performing receptor-rigid docking using AutoDock Vina,[45] leading to identification of top-100,000 compounds. Then, each of the top-100,000 compounds was further optimized using a four-step procedure (including 2,000 steps of energy-minimization, 20 ps of molecular dynamic simulation, 4,000 steps of energy-minimization, and then Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) binding energy calculation using AMBER 12 software package)[46, 47] similar to the known binding estimation after refinement (BEAR) protocol.[48, 49] The top-40 compounds were selected according to the ascending order of the MM/PBSA binding energies.

The computationally selected 40 compounds were tested for their inhibitory activity against human mPGES-1. Our protocol for the protein preparation and *in vitro* activity assays were the same as what we described previously.[50–52] All of the 40 compounds were assayed first for their inhibitory activity at a concentration of 10 μM. Then, the most active compounds were tested further for the dose-dependent inhibition in order to determine their IC_{50} values (Table 1) against mPGES-1. Finally, the most promising compounds were also assayed for their inhibitory activities against COX-1/2 (mixed COX-1 and COX-2) in order to know their selectivity for mPGES-1 over COX-1/2. The COX-1/2 assays were performed by using the COX (ovine/human) Inhibitor Screening Assay Kit (Item No. 560131) ordered from Cayman Chemical Company (Ann Arbor, MI). According to the kit, the COX activity assay utilizes the competition between prostaglandins (PGs) and a PG tracer, *i.e.* a PG-acetylcholinesterase (PG-AChE) conjugate, for a fixed amount of PG antiserum.[53, 54] Following the assay using the kit, we used a mixture of COX-1 and COX-2 (denoted as COX-1/2) with equal amount of each enzyme. The efficacies of tested compounds were determined as % inhibition against the COX enzymes at the concentration of 100 μM. All of the enzyme activity assays were carried out in triplicate.

According to the activity assays, all of the computationally selected 40 compounds showed significant inhibitory activity against human mPGES-1, with 10% to 100% inhibition at a concentration of 10 μM (see Table 1). Molecular structures of the most active compounds (top-7) are depicted in Figure 1, and those of the remaining compounds are provided in Supporting Information.

Based on the activity data summarized in Table 1, compounds 1 to 7 at a concentration of 10 μM inhibited the mPGES-1 activity by at least 75%. All of these compounds showed nanomolar IC_{50} values, 276 to 917 nM. Depicted in Figure 2 are their dose-response curves. The data in Table 1 also revealed that all of the top-7 compounds are highly selective for mPGES-1 over COX-1/2, as these compounds at a very high concentration (100 μM) showed no significant inhibition against COX-1 or COX-2, except for compound 6. Even for compound 6, the inhibition at 100 μM was only ~37%, suggesting that IC_{50} > 100 μM for compound 6 against COX-1/2.

Depicted in Figure 3 are the energy-minimized structures of human mPGES-1 binding with the top-7 compounds. In general, each of these compounds binds with the enzyme at the substrate-binding site and fit the binding site well. Figure 3(A) depicts the overall complex of the enzyme with **1**, and Figure 3(B) shows the structural detail of the binding site, showing that the main scaffold of **1** binds very well with the hydrophobic groove of the substrate-binding site of mPGES-1. The extended hydrocarbon side chain has hydrophobic interaction with the protein environment.

As shown in Figure 3(C), 2,4-dinitrobenzyl group of compound **2** stays in the bottom of the substrate-binding pocket of mPGES-1. The thiazole and dichlorobenzyl groups have the hydrophobic interaction with the protein. Compound **3** fits very well into the substrate-binding site of mPGES-1, as seen in Figure 3(D) showing a hydrogen bond (HB) between the NH group (including N9) and the hydroxyl oxygen on the side chain of residue T131. Compound **4** is huge in size, but it fits well in the substrate-binding site as seen in Figure 3(E). It is interesting to know that the binding site of the enzyme can accommodate a ligand as large as compound **4**.

As shown in Figure 3(F), there are two HBs between the protein and compound **5**. One HB is between N22 of **5** and the hydroxyl group of S127 side chain, and the other forms between and O12 of **5** and the hydroxyl group of T131 side chain. In addition, the benzyl rings of **5** have the hydrophobic interaction with the protein.

Figure 3(G) shows that, unlike the other compounds discussed above, compound **6** binds with the protein on the upper part of the substrate-binding groove of mPGES-1, with a HB between N7 of **6** and the hydroxyl group of S127 side chain. As seen in Figure 3(H), compound **7** occupies the substrate-binding pocket with both of the phenyltriazolothiadiazole rings. N30 of compound **7** forms a HB with the hydroxyl group of Y130 side chain.

In summary, through structure-based virtual screening followed by *in vitro* activity assays, we have identified a series of new, potent and selective inhibitors of human mPGES-1 with diverse scaffolds. In addition, the diverse binding structures of these highly selective inhibitors with mPGES-1 depicted in Figure 3 provide some interesting clues concerning how to design modified structures of the inhibitors to more favorably bind with mPGES-1. Based on the structures in Figure 3, each inhibitor has some unique interaction with the protein. A more potent inhibitor/ligand could be designed to have more of these favorable protein-ligand interactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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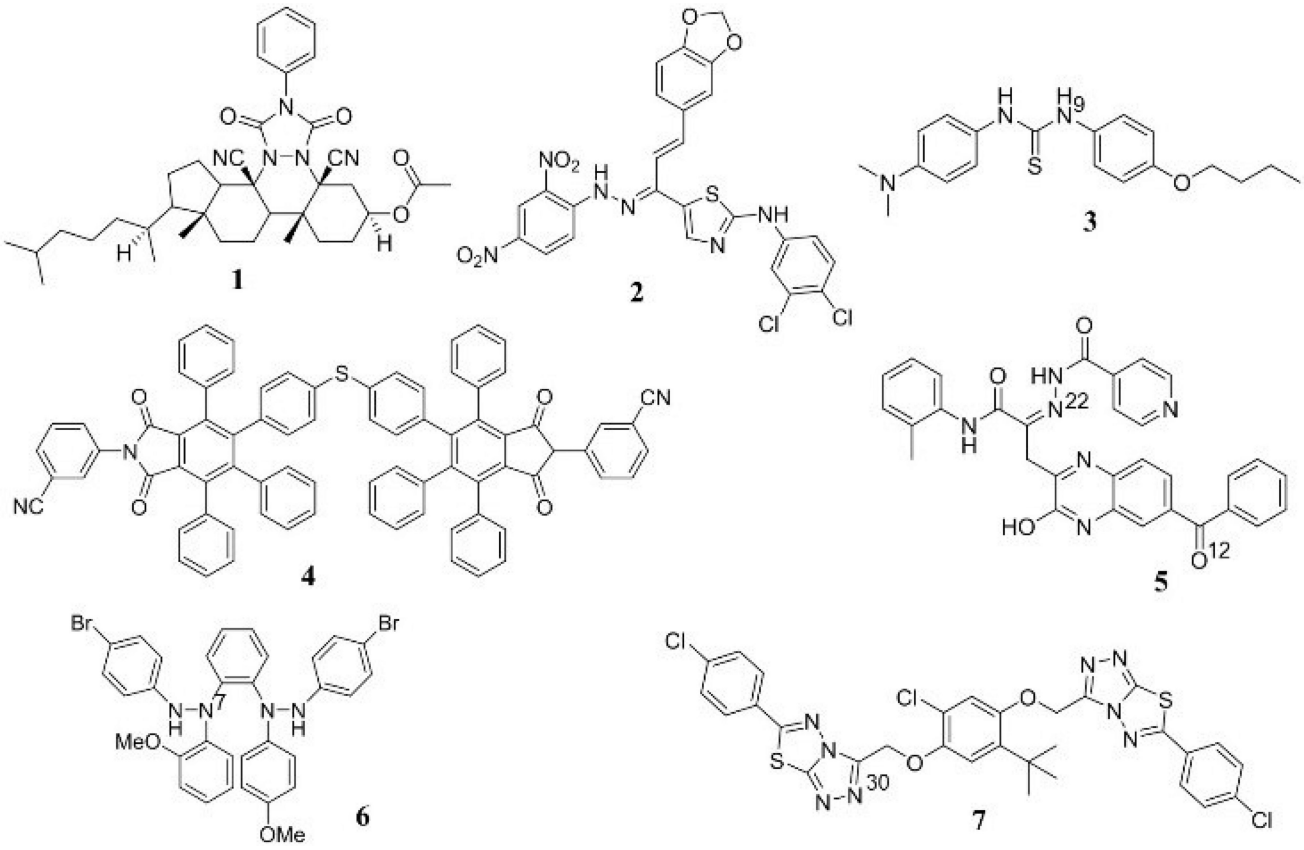

Figure 1.

Molecular structures of the top-7 inhibitors of human mPGES-1 identified. Some atoms with the numbering as superscripts are mentioned in the text for convenience of the discussion.

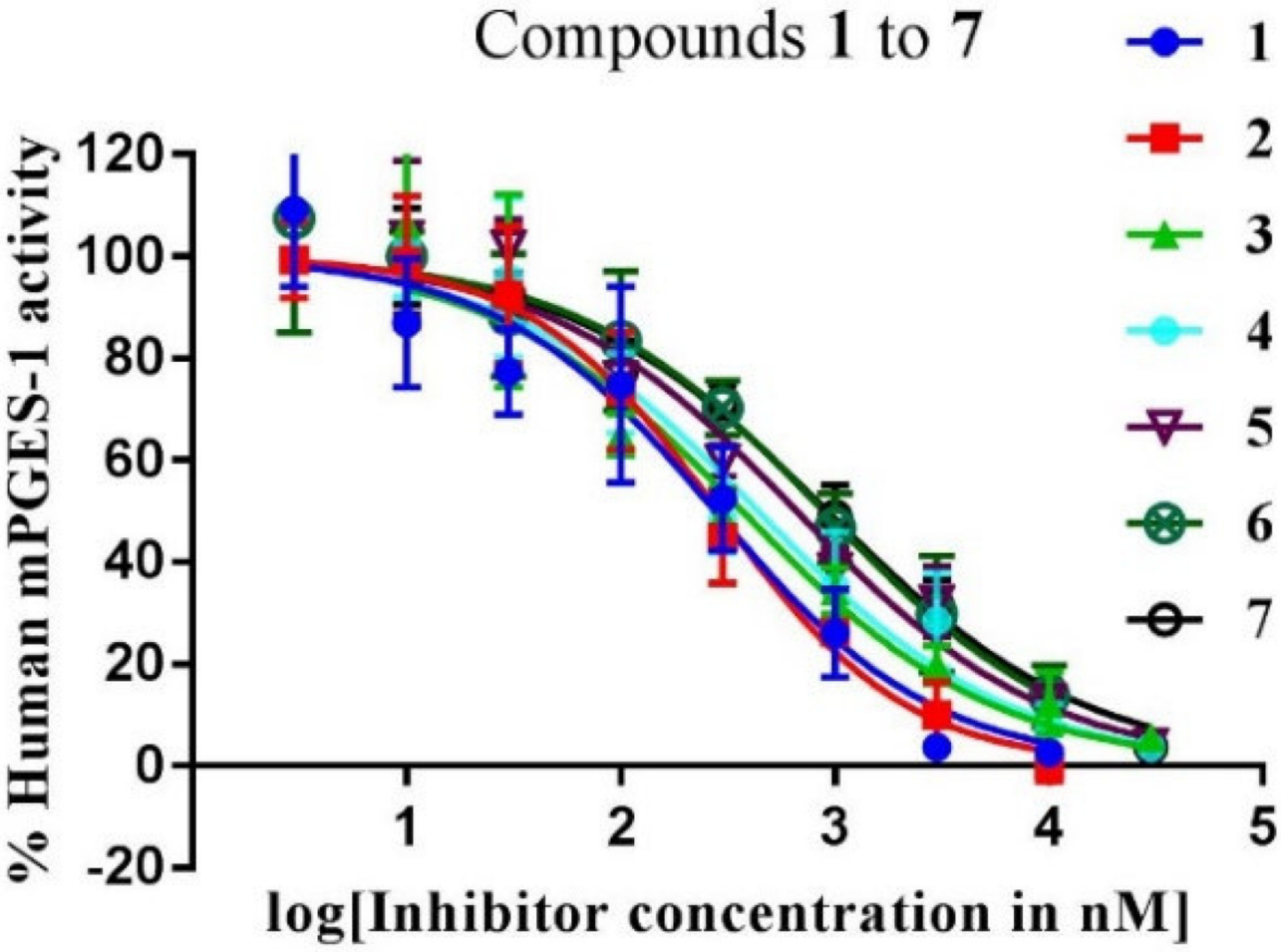

Figure 2.
Dose-dependent inhibition of human mPGES-1 by compounds **1** to **7**: plots of the remaining enzyme activity *vs* the inhibitor concentration.

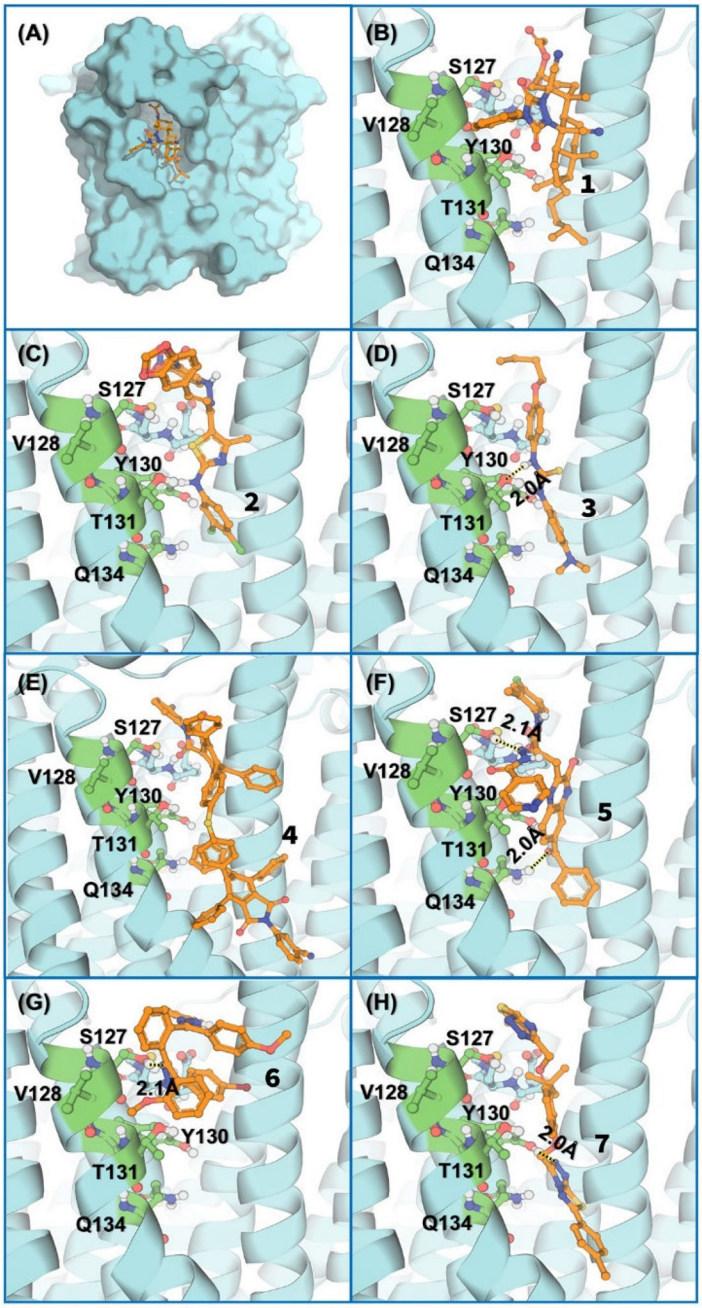

Figure 3.

Energy-minimized structures of human mPGES-1 binding with the identified inhibitors (1 to 7 depicted in Figure 1): (A) and (B) Compound 1; (C) 2; (D) 3; (E) 4; (F) 5; (G) 6; (H) 7. The protein is shown in cyan cartoon, and the key residues are shown in green ball-and-stick models. The ligand is shown in orange ball-and-stick models. Important polar interactions are shown in dashed lines.

Table 1

In vitro inhibitory activities of the newly identified mPGES-1 inhibitors.

Com-pound	%Inhibition of mPGES-1 at 10 μM[a]	IC_{50} (nM) for mPGES-1[b]	%Inhibition of COX-1/2 at 100 μM[c]
1	99	276 ± 60	14 ±13
2	98	284 ± 81	8 ± 20
3	99	370 ± 79	1 ± 3
4	100	439 ± 84	9 ± 22
5	94	664 ± 106	0 ± 3
6	100	889 ± 186	37 ± 4
7	75	917 ± 99	15 ± 2
8	71	N.D.	N.D.
9	70	N.D.	N.D.
10	70	N.D.	N.D.
11	69	N.D.	N.D.
12	65	N.D.	N.D.
13	65	N.D.	N.D.
14	64	N.D.	N.D.
15	59	N.D.	N.D.
16	59	N.D.	N.D.
17	59	N.D.	N.D.
18	57	N.D.	N.D.
19	53	N.D.	N.D.
20	50	N.D.	N.D.
21	49	N.D.	N.D.
22	49	N.D.	N.D.
23	48	N.D.	N.D.
24	47	N.D.	N.D.
25	46	N.D.	N.D.
26	46	N.D.	N.D.
27	46	N.D.	N.D.
28	44	N.D.	N.D.
29	43	N.D.	N.D.
30	40	N.D.	N.D.
31	37	N.D.	N.D.
32	36	N.D.	N.D.
33	32	N.D.	N.D.
34	30	N.D.	N.D.
35	29	N.D.	N.D.
36	28	N.D.	N.D.
37	26	N.D.	N.D.
38	25	N.D.	N.D.

Com-pound	%Inhibition of mPGES-1 at 10 μM[a]	IC_{50} (nM) for mPGES-1[b]	%Inhibition of COX-1/2 at 100 μM[c]
39	15	N.D.	N.D.
40	10	N.D.	N.D.

[a]The % inhibition of the compound at a concentration of 10 μM against human mPGSE-1.

[b]The determined IC_{50} against human mPGES-1 based on the data depicted in Figure 2.

[c]The % inhibition of the compound at a concentration of 100 μM against the COX-1/2 (mixed COX-1 and COX-2). The enzyme mixture contained equal amounts of COX-1 and COX-2 in terms of their enzyme activities. In this way, when a compound can significantly inhibit either COX-1 or COX-2, it will show the significant inhibitory effects against the mixed COX-1 and COX-2.