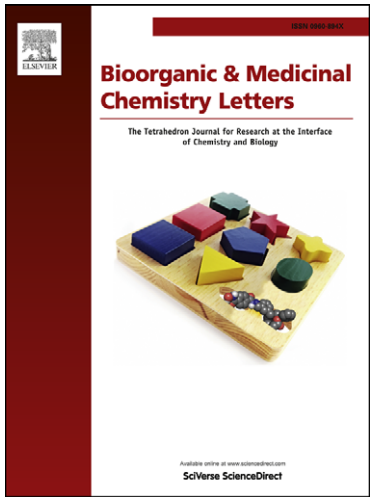


# Fragment-Based Discovery of Novel and Selective mPGES-1 Inhibitors Part 1: Identification of Sulfonamido-1,2,3-Triazole-4,5-Dicarboxlic acid

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# Fragment-Based Discovery of Novel and Selective mPGES-1 Inhibitors Part 1: Identification of Sulfonamido-1,2,3-Triazole-4,5-Dicarboxlic acid

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**Keywords:** Fragment-based discovery; mPGES-1; Partial Nuisance inhibitor; Inflammation; Triton X-100; Sulfonamido triazole-4,5-dicarboxylic acid

**Abstract:** Microsomal prostaglandin E synthase-1 (mPGES-1) is an inducible prostaglandin E synthase that catalyzes the conversion of prostaglandin $PGH_2$ to $PGE_2$ and represents a novel target for therapeutic treatment of inflammatory disorders. It is essential to identify mPGES-1 inhibitor with novel scaffold as new hit or lead compound for the purpose of the next-generation anti-inflammatory drugs. Herein we report the discovery of sulfonamido-1,2,3-triazole-4,5-dicarboxylic derivatives as a novel class of mPGES-1 inhibitors identified through fragment-based virtual screening and in vitro assays on the inhibitory activity of the actual compounds. 1-[2-(*N*-Phenylbenzenesulfonamido)ethyl]-1*H*-1,2,3-triazole-4,5-dicarboxylic acid (**6f**) inhibits human mPGES-1 ($IC_{50}$ of 1.1 μM) with high selectivity (*ca.*1000-fold) over both COX-1 and COX-2 in a cell-free assay. In addition, the activity of compound **6f** was again tested at 10 μM concentration in presence of 0.1% Triton X-100 and found to be reduced to 1/4 of its original activity without this detergent. Compared to the complete loss of activity of nuisance inhibitor with the detergent, therefore, compound **6f** would be regarded as a partial nuisance inhibitor of mPGES-1 with a novel scaffold for the optimal design of more potent mPGES-1 inhibitors.

Prostaglandin $E_2$ ($PGE_2$) is a key mediator in inflammation, pain, fever, atherosclerosis and tumorigenesis.[1] The biosynthetic pathway of $PGE_2$ involves two sequential enzymatic actions from arachidonic acid (AA). AA is released from the membrane and then converted to $PGH_2$ by cyclooxygenases (COX-1/COX-2),[2] followed by the subsequent isomerization of $PGH_2$ into $PGE_2$

by the terminal enzyme, microsomal prostaglandin E2 synthase (mPGES-1).[3] The inhibition of mPGES-1 would be expected to preclude only $PGE_2$ production without any potential side effects including ulcers, bleeding within the gastrointestinal tract, or increased risk of cardiovascular events resulting from the inhibition of cyclooxygenases.[4] Thus, mPGES-1 has been recognized as a promising target of next-generation therapeutics for the above $PGE_2$-related diseases.[5] Although several selective mPGES-1 inhibitors have been reported up date and a few inhibitors are currently in clinical trials,[6] no mPGES-1 inhibitor is available on the market. It is highly desirable to design and discover novel mPGES-1 inhibitors with different scaffolds for $PGE_2$-related diseases. Our ultimate goal of this study is to identify new mPGES-1 inhibitors with novel scaffold using fragment-based design and virtual docking with subsequent bioassay experiments.

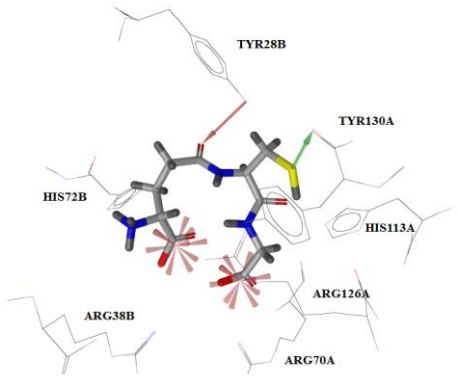



**Figure 1.** Co-crystal structure of glutathione bound to mPGES-1, showing four pharmacophores (two negative ionizable; one H-bond donor; one H-bond acceptor). Figure was made using LigandScout (PDB code: 3DWW)

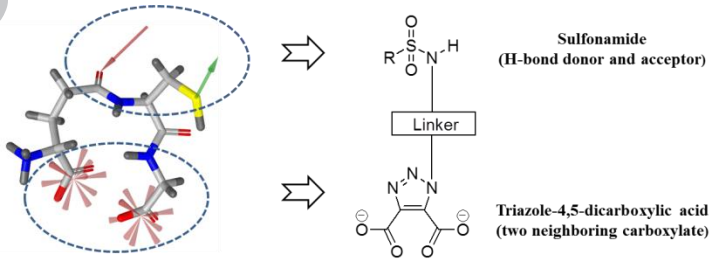



**Figure 2.** A glutathione bound to mPGES-1 can be divided into two parts. H-bonding acceptor/donor part can be substituted with privileged sulfonamido group and two-neighboring carboxylate part can be substituted with privileged triazole-4,5-dicarboxylate group. Then, two groups can be connected with short alkyl chain linker each other.

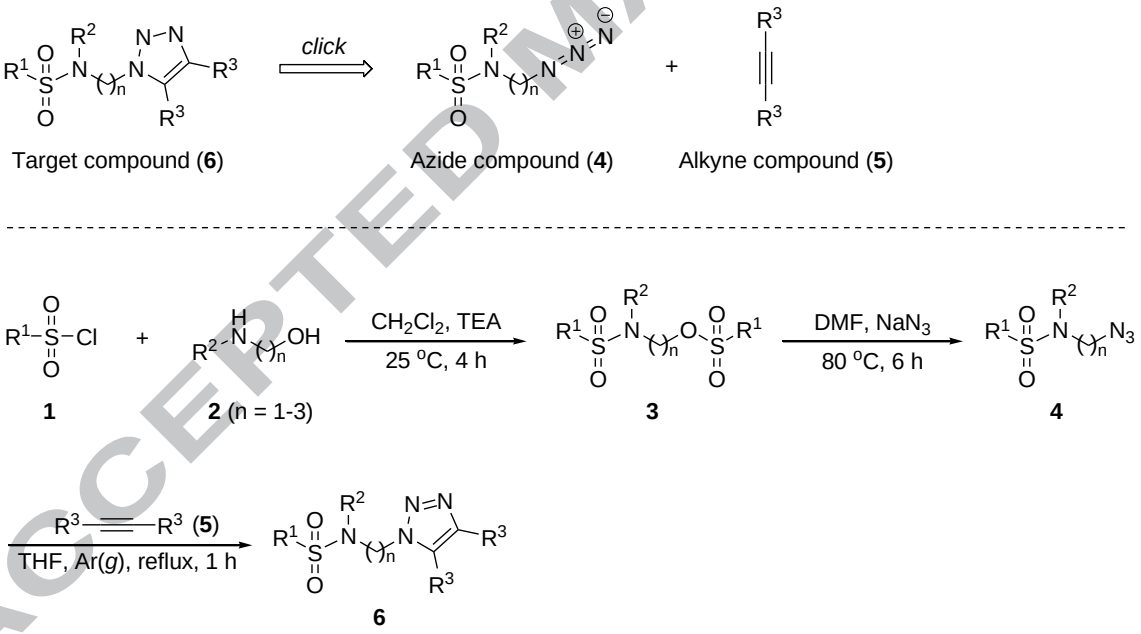
To date, there is no real three-dimensional X-ray crystal structure of mPGES-1 in the apo form or with an inhibitor bound with exception of electron crystallographic structure complexed with glutathione in its closed state (PDB code: 3DWW).[7] Therefore, our strategy for novel mPGES-

1 inhibitor is the replacement of glutathione with non-peptido mimetics via fragment-based design. The U-shaped conformation of glutathione bound to mPGES-1 showed four pharmacophores (two negative ionizable, one H-bond donor and one H-bond acceptor) built with LigandScout in its binding site of mPGES-1 as shown in Figure 1.[8] Based on this conformation, it is proposed that the H-bond acceptor/donor part of glutathione could be replaced with sulfonamide substructure because it would serve as a suitable mimic and also has been considered as small privileged molecular fragment in several groups of drugs.[9] Next, two proximally close carboxylate part of glutathione could be replaced with triazole-4,5-dicarboxylate substructure because it would serve as a suitable mimic and also could be readily installed using the powerful azide-alkyne click reaction.[10] Finally, two groups could be connected with short alkyl chain linker each other as shown in Figure 2. Synthetically, the proposed target compound would be accessed through retrosynthetic strategy as shown in Scheme 1.

Prior to the actual synthesis of target compounds, we decided to perform molecular docking studies to predict the binding interaction of virtual compound with mPGES-1. All the calculations were performed using Molegro Virtual Docker (MVD) 2010.4.2 for Windows.[11] The docking studies were carried out using the crystal structures of mPGES-1 complexed with glutathione (PDB code: 3DWW).[7] The active site of the enzyme was defined to include residues within a 10.0 Å radius to glutathione. The docking wizard of MVD2010.4.2 was used to dock all virtual compounds on the active sites of mPGES-1 enzyme. Among virtual compound library, only 13 compounds were selected according to the total interaction energy (cutoff point: -140.000 kcal/mol) between virtual compound and mPGES-1 predicted by the MVD scoring function and listed in Table 1. Among them, six compounds (**6a**, **6d**, **6e**, **6f, 6h, 6m**) have fortunately higher binding interaction than MK886 (-157.831 kcal/mol) as a positive control, of which compound **6h** showed the highest binging interaction with a value of -190.855 kcal/mol. In general, the top-ranking **3** (**6e**, **6f, 6h**)

compounds have a phenyl group at $R^1$ position, a carboxylic acid at $R^3$ position of triazole ring, and an ethylene (n =2) group as alkyl chain linker.

Based on this computational output, synthesis of the actual sulfonamido triazole-4,5-dicarboxylic acid derivative **6** was simply accomplished via the azide–alkyne click reaction as shown in Scheme 1. First, sulfonated-sulfonamide **3** was formed by the addition of sulfonyl chloride (**1**, 2 eq.) on (ω-hydroxyalkyl)amine **2** with stirring at room temperature. The intermediate *N*-(ω-azidoalkyl)sulfonamide **4** was prepared in high yield by the nucleophilic substitution ($S_N2$) of sulfonate group of **3** with sodium azide in DMSO at 80 °C. Subsequent click chemistry of azide **4** with alkyne **5** afforded the desired sulfonamido triazole-4,5-dicarboxylic acid (**6**) in 51- 90% yields under THF-reflux condition within 1 h.[10]



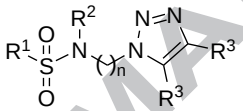

**Scheme 1**. Retrosynthesis and synthetic scheme for target compound **6a-m**

All 13 compounds were in vitro assayed with varying concentrations to determine their $IC_{50}$ against mPGES-1 using our two-enzyme coupled assay method as described previously.[12] Briefly,

110 nM of mPGES-1 and 220 nM of 15-PGDH were mixed in 200 μL of the reaction buffer (50 mM Tris-HCl, pH 7.5, 50 μM of PMA, 2 mM reduced form of glutathione, 1 mM $NAD^+$, 0.1 mM DTT) with tested compound 1 μL of dimethylsolfoxide (DMSO) and incubated in the plate for 30min. The reaction was initiated by adding cold $PGH_2$ to a final concentration of 14 μM. The amount of NADH in the reaction product was measured by fluorescence plate reader (DYNEX, USA) using an emission and excitation wavelength of 468 nm and 340 nm. As a result, each of the 13 compounds showed broad-range $IC_{50}$ values of 1.1 into 23.3 μM depending on the length of alkyl linker and the substituents of two key moieties, in particular, with $IC_{50}$ of 1.1 μM for compound **6f**. The same in vitro activity protocol was also performed for MK886, and we obtained $IC_{50}$ of 3.9 μM for MK886. Our determined $IC_{50}$ value of 3.9 μM for MK886 is slightly larger than the previously reported $IC_{50}$ value of 3.2 μM.[13] Thus, this in vitro data show that our designed assay protocol can be useful for screening another compound to identify potential mPGES-1 inhibitor. With respect to the preliminary structure–activity relationship (SAR) of this series, firstly, incorporation of ethylene linker in compound **6** yielded increased potency in the enzyme assay compared to methyl and propyl linker (**6g** vs. **6a** and **6m**). When fixed the ethylene linker, secondly, a larger phenyl group in both $R^1$ and $R^2$ positions led to increase the activity against mPGES-1 enzyme (**6b** vs. **6d; 6d** vs. **6f**) and in detail, introduction of an electron-donating or an electron-withdrawing substituent in phenyl ring of $R^1$ position mostly preserved or slightly increased enzyme potency (**6d** vs. **6g, 6i** and **6j**). Finally, replacement of carboxylic acid in $R^3$ position with ester or amide group suffered a severe loss of potency in the enzyme assay (**6g** vs. **6k** and **6l**), which is consistent with the above computational result. A brief SAR result reveals that a bulky group in both $R^1$ and $R^2$ positions should be necessary for improving the inhibitory activity against mPGES-1 enzyme together with both ethylene linker and carboxylic acid in $R^3$ positions. As a consequence, the most potent compound against isolated mPGES-1 in this series was compound **6f**, which was

about 3.5-fold more active ($IC_{50}$ of 1.1 μM) than MK886 ($IC_{50}$ of 3.9 μM) as shown in Table 1. Interestingly, compound **6h** ($IC_{50}$ of 3.8) showing top binding interesting was found to be less active then compound **6f** and **6i** ($IC_{50}$ of 3.3) in this actual inhibitory assay. Using purified ovine COX-1 and purified human recombinant COX-2,[14] the most potent compound **6f** in this series was next evaluated for its mPGES-1 selectivity over COX-1 and COX-2, and exhibited 50% COX-1 inhibition and little COX-2 inhibition at each 1 mM. These findings indicate that compound **6f** has *ca.*1000-fold mPGES-1 selectivity over COX-1 without COX-2 inhibitory activity.

**Table 1.** The estimated binding energies (kcal/mol) and experimentally determined actual inhibitory activity of compound **6a-m** against m-PGES-1



| Entry | n | $R^1$ | $R^2$ | $R^3$ | Yield[a] | MW | ESI-MS [M-H][b] | Binding energy[c] (PDB: 3DWW) | mPGES-1 ($IC_{50}$ : μM)[d] |
|---|---|---|---|---|---|---|---|---|---|
| **6a** | 1 | *p*-MePh | H | $CO_2H$ | 65 | 340.31 | 339.00 | -164.045 | 6.9 |
| **6b** | 2 | Me | H | $CO_2H$ | 72 | 278.24 | 277.00 | -145.623 | 10.3 |
| **6c** | 2 | Et | H | $CO_2H$ | 70 | 292.27 | 291.00 | -142.063 | 9.7 |
| **6d** | 2 | Ph | H | $CO_2H$ | 60 | 340.31 | 339.00 | -159.342 | 4.1 |
| **6e** | 2 | Ph | Me | $CO_2H$ | 81 | 354.34 | 353.10 | -166.356 | 4.0 |
| **6f** | 2 | Ph | Ph | $CO_2H$ | 79 | 416.41 | 415.10 | -167.249 | 1.1 |
| **6g** | 2 | *p*-MePh | H | $CO_2H$ | 90 | 354.34 | 353.10 | -154.603 | 4.2 |
| **6h** | 2 | *p*-MePh | *n*-Pr | $CO_2H$ | 88 | 410.44 | 409.10 | -190.855 | 3.8 |
| **6i** | 2 | *o*-$CF_3$Ph | H | $CO_2H$ | 67 | 408.31 | 407.00 | -156.255 | 3.3 |
| **6j** | 2 | *m*-$CF_3$Ph | H | $CO_2H$ | 71 | 408.31 | 407.00 | -154.634 | 4.4 |
| **6k** | 2 | Ph | H | $CO_2Me$ | 51 | 368.37 | 367.10 | -140.330 | 12.9 |
| **6l** | 2 | Ph | H | $CONH_2$ | 68 | 338.34 | 337.10 | -146.606 | 23.3 |
| **6m** | 3 | *p*-MePh | H | $CO_2H$ | 81 | 368.37 | 367.10 | -161.028 | 6.3 |
| **MK886**[e] | | | | | | | | -157.831 | 3.9 (3.2)[f] |

[a] Isolated yield of azide-alkyne click chemistry; [b] ESI-MS (negative mode) m/z; [c] Total interaction energy between virtual compound and mPGES-1; [d] $IC_{50}$ value is the compound concentration required to produce 50% inhibition of mPGES-1; [e] Positive control for mPGES-1 used; [f] Reported value.[13]

For understanding the easy relationship between the virtual and real results, the binding interaction energies and $IC_{50}$ values of **13** compounds were expressed as a diagram (Figure 3). The diagram shows that the predictions from the virtual screening were not perfect. The main reason about a low reliability of the *in silico*-docking studies is supposed to stem from using a closed inactive conformation of mPGES-1 (PDB Code: 3DWW) determined by Jegerschöld's group[7], that is not accessible by the real substrate $PGH_2$ (prostaglandin $H_2$). However, it did lead to the identification of hit compound **6f** with novel scaffold despite the early preliminary result. Based on the identification of hit compound **6f**, we further analyzed the detailed binding mode for mPGES-1 binding with compounds **6f**, as understanding the binding modes of hit **6f** will be of great help in further lead identification and optimization studies in the future. The binding modes of hit **6f** are quite different from those of glutathione in the active site of mPGES-1 (Figure 1 and Figure 4). The binding structure of compound **6f** revealed that the one carboxyl moiety of **6f** forms electrostatic interactions with HIS113A, ARG110A, and ARG126A (a key amino acid in mPGES-1 enzyme)[15] side chain. The other carboxyl moiety forms electrostatic interactions with ARG70A, ARG126A, ARG38B and LYS42B. The sulfonamide moiety of **6f** forms two hydrogen bonds with ARG126A side chain. Each phenyl ring of **6f** forms hydrophobic interactions with ALA31B, MET27B and TYR28B, ALA133A and TYR30A, respectively. These extra hydrophobic interactions afford **6f** the strong binding interaction against mPGES-1 compared to glutathione and.[16]

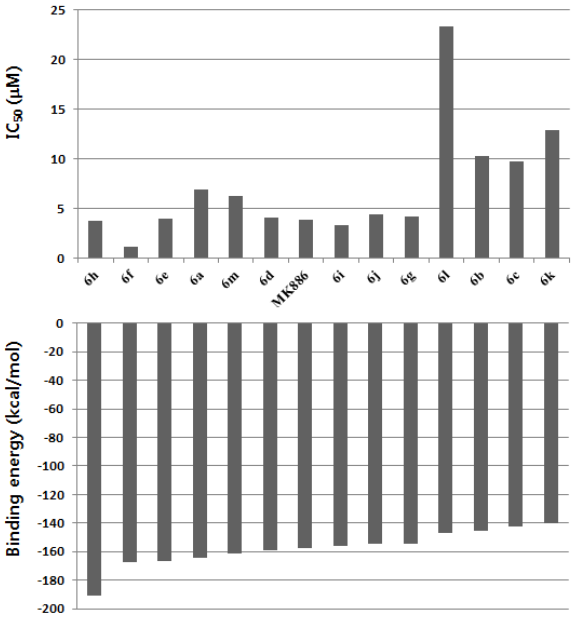



**Figure 3**. Diagram view of the binding energies and the inhibitory activities of compounds (13 compounds and positive control) according to the calculated binding energies for the easy comparison of virtual and actual results.

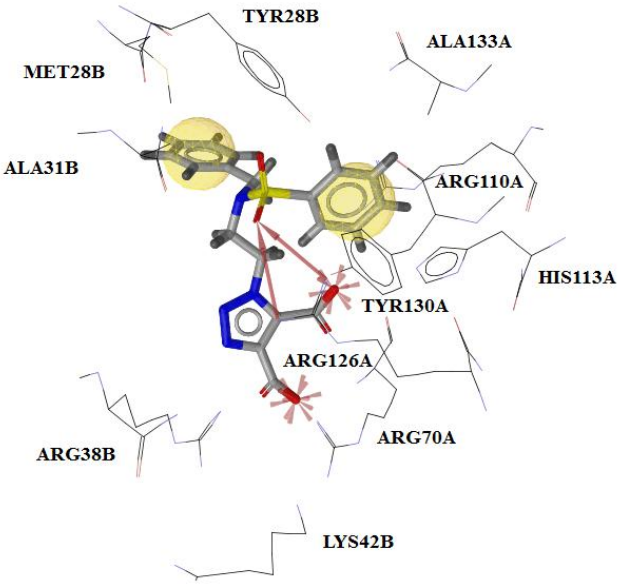



**Figure 4**. View of the binding for the structure of hit compound **6f** in the active site of mPGES-1. (Red arrow-hydrogen bonding acceptor; Yellow circle-hydrophobic interactions; Red flash-negative ionizable area Figure was made using LigandScout (PDB code: 3DWW).

To investigate the exact characteristics of compound **6f** as mPGES-1 inhibitor, the % inhibitory activity of **6f** was tested at 10 μM concentration with and without 0.1% Triton X-100 by using the Lehr group's protocol.[6a] As a result, the inhibitory activity of **6f** was reduced to 1/4 of its original activity without the detergent (69.1±1.5% into 18.1±9.1 %) as shown in Figure 5. This result would consist with the flat structure-activity relationship which was suggested by the publications of Brian K. Shoichet[17] and Matthias Lehr's group.[6a] Compound **6f** has the characteristics of flat structure and thus would make a less dense colloid-like aggregate than those of the reported compounds containing both flat structure and lipophilic long-alkyl side chain, which exhibited the complete loss of activity in the presence of 0.1% Triton X-100. Therefore, compound **6f** may be judged as a partial nuisance inhibitor of mPGES-1 instead of true mPGES-1 inhibitor.

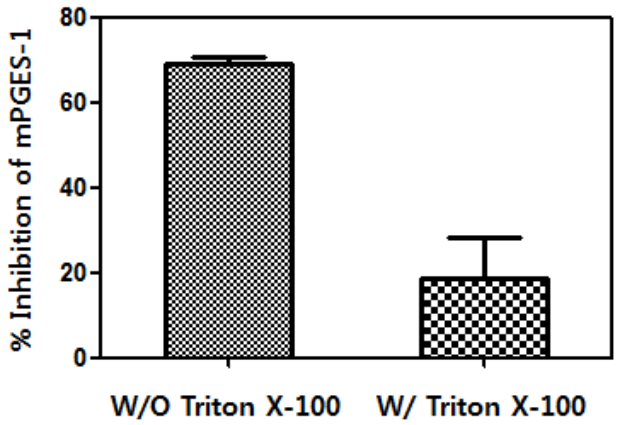



**Figure 5**. Inhibition of human mPGES-1 activity of compound **6f** at 10 μM concentration in absence and in presence of 0.1% Triton X-100. The inhibitory activity of **6f** was reduced to 1/4 of its original activity without the detergent (69% into 18%). The data are the mean ± SE of four independent determinations.

In conclusion, a fragment-based drug design and virtual screening coupled with biochemical assays was applied to identify new mPGES-1 inhibitors. This combined computational and experimental studies have led to identification of novel mPGES-1 inhibitor with novel scaffold, 1-[2-(*N*-phenylbenzenesulfonamido)ethyl]-1*H*-1,2,3-triazole-4,5-dicarboxylic acid (**6f**), which was active than MK886 with high mPGES-1 selectivity over COX-1 and no COX-2 inhibition. In addition, the activity of compound **6f** was again tested in presence of Triton X-100 and found to be reduced to 1/4 of its original activity without this detergent. Thus, compound **6f** would be regarded as a partial nuisance inhibitor of mPGES-1 with a novel scaffold for the optimal design of more potent mPGES-1 inhibitors.



## Acknowledgment

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**14.** **In vitro cyclooxygenase (COX) inhibition assay**: The ovine COX-1 and human recombinant COX-2 activity directly measures $PGF_{2\alpha}$ produced by $SnCl_2$ reduction of COX-derived $PGH_2$. The prostanoid product is quantified via enzyme immunoassay (EIA) using a broadly specific antibody that binds to all the major prostaglandin compounds using COX Inhibitor Screening Assay (Cayman Chemical, Ann Arbor, MI, USA). Briefly, recombinant COX-1 or COX-2 protein was pre-incubated with compound **6f** for 10 min in 37 °C. The reaction was started by the addition of 100 μM arachidonic acid and allowed to proceed for 2 min. The reaction was terminated by addition of HCl solution containing $SnCl_2$. The COX activity assay directly

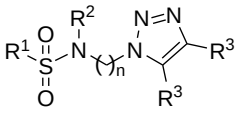
measures $PGF_{2\alpha}$ produced by $SnCl_2$ reduction of COX-derived $PGH_2$. The prostanoid product is quantified via EIA. As control inhibitors for COX-1 and COX-2, SC-560 (1 μM) or Dup-697 (10 μM) were used, respectively.

**15.** Hammarberg, T.; Hamberg, M.; Wetterholm, A.; Hansson, H.; Samuelsson, B.; Haeggström, J. Z. *J. Biol. Chem*. **2009**, *284*, 301.

**16.** Spectra data of **6f**: mp 151-152℃; MS ($ES^-$) m/z: 415.10$[M-H]^-$; $^1H$ NMR (400 MHz, acetone-$d_6$) δ 4.27 (2H, t, *J* = 5.2 Hz), 4.94 (2H, t, *J* = 5.2 Hz), 7.15-7.17 (2H, m), 7.34-7.40 (3H, m), 7.54-7.59 (4H, m), 7.69 (1H, m); $^{13}C$ NMR (100 MHz, acetone-$d_6$) δ 50.91, 51.11, 128.27, 129.11, 129.95, 130.04, 130.16, 132.77, 133.93, 139.22, 139.38, 139.84, 157.68, 167.47; HRMS (FAB, M+H) Calcd for $C_{18}H_{17}N_4O_6S$ 417.0869, found 417.0870.

**17.** Shoichet, B. K. *Drug Discov. Today* **2006**, *11*, 607.

**Table 1.** The estimated binding energies (kcal/mol) and experimentally determined actual inhibitory activity of compound **6a-m** against m-PGES-1

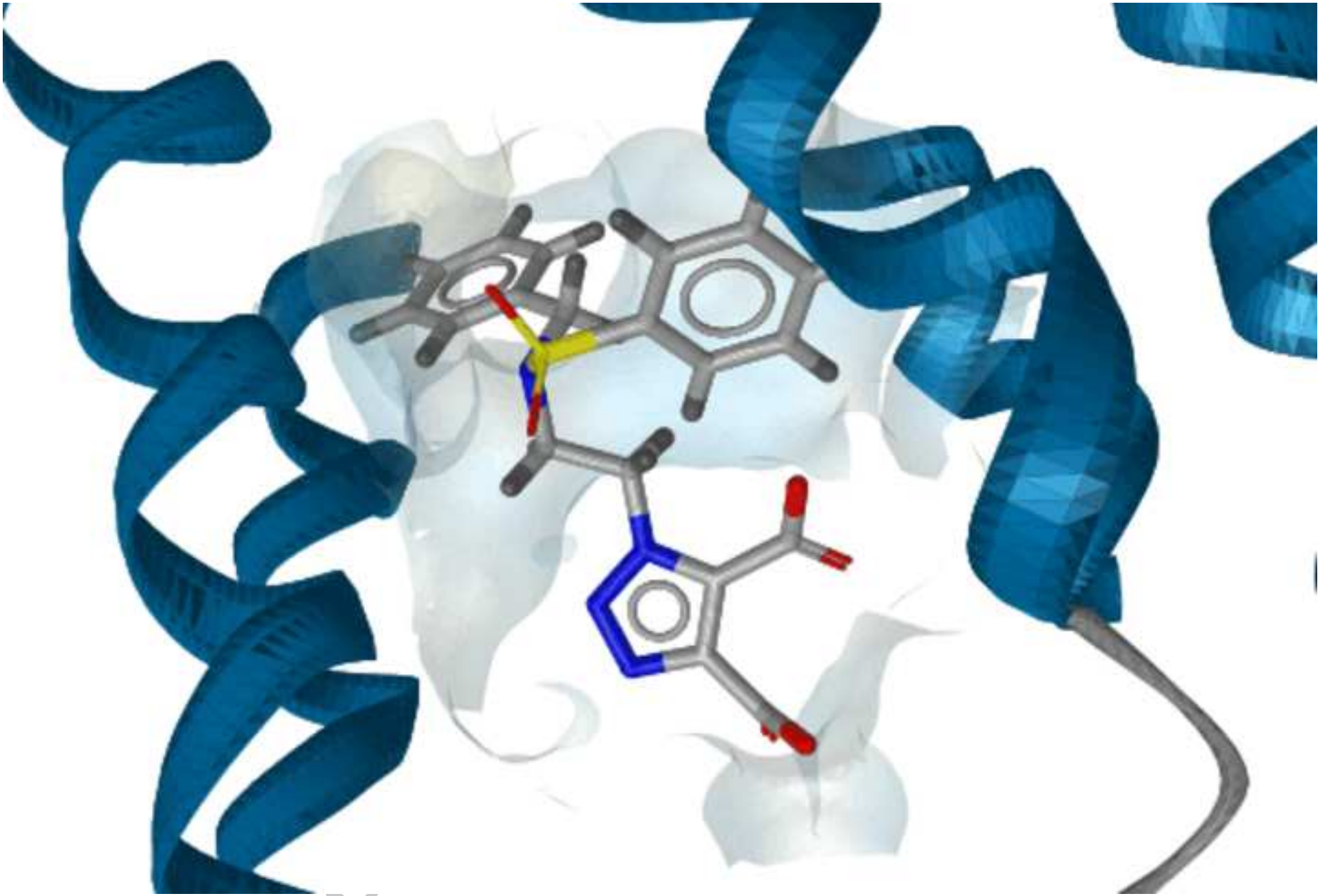


| Entry | n | $R^1$ | $R^2$ | $R^3$ | Yield[a] | MW | ESI-MS [M-H][b] | Binding energy[c] (PDB: 3DWW) | mPGES-1 ($IC_{50}$ : μM)[d] |
|---|---|---|---|---|---|---|---|---|---|
| **6a** | 1 | *p*-MePh | H | $CO_2H$ | 65 | 340.31 | 339.00 | -164.045 | 6.9 |
| **6b** | 2 | Me | H | $CO_2H$ | 72 | 278.24 | 277.00 | -145.623 | 10.3 |
| **6c** | 2 | Et | H | $CO_2H$ | 70 | 292.27 | 291.00 | -142.063 | 9.7 |
| **6d** | 2 | Ph | H | $CO_2H$ | 60 | 340.31 | 339.00 | -159.342 | 4.1 |
| **6e** | 2 | Ph | Me | $CO_2H$ | 81 | 354.34 | 353.10 | -166.356 | 4.0 |
| **6f** | 2 | Ph | Ph | $CO_2H$ | 79 | 416.41 | 415.10 | -167.249 | 1.1 |
| **6g** | 2 | *p*-MePh | H | $CO_2H$ | 90 | 354.34 | 353.10 | -154.603 | 4.2 |
| **6h** | 2 | *p*-MePh | *n*-Pr | $CO_2H$ | 88 | 410.44 | 409.10 | -190.855 | 3.8 |
| **6i** | 2 | *o*-$CF_3$Ph | H | $CO_2H$ | 67 | 408.31 | 407.00 | -156.255 | 3.3 |
| **6j** | 2 | *m*-$CF_3$Ph | H | $CO_2H$ | 71 | 408.31 | 407.00 | -154.634 | 4.4 |
| **6k** | 2 | Ph | H | $CO_2Me$ | 51 | 368.37 | 367.10 | -140.330 | 12.9 |
| **6l** | 2 | Ph | H | $CONH_2$ | 68 | 338.34 | 337.10 | -146.606 | 23.3 |
| **6m** | 3 | *p*-MePh | H | $CO_2H$ | 81 | 368.37 | 367.10 | -161.028 | 6.3 |
| **MK886**[e] | | | | | | | | -157.831 | 3.9 (3.2)[f] |

[a] Isolated yield of azide-alkyne click chemistry; [b] ESI-MS (negative mode) m/z; [c] Total interaction energy between virtual compound and mPGES-1; [d] $IC_{50}$ value is the compound concentration required to produce 50% inhibition of mPGES-1; [e] Positive control for mPGES-1 used; [f] Reported value.[13]

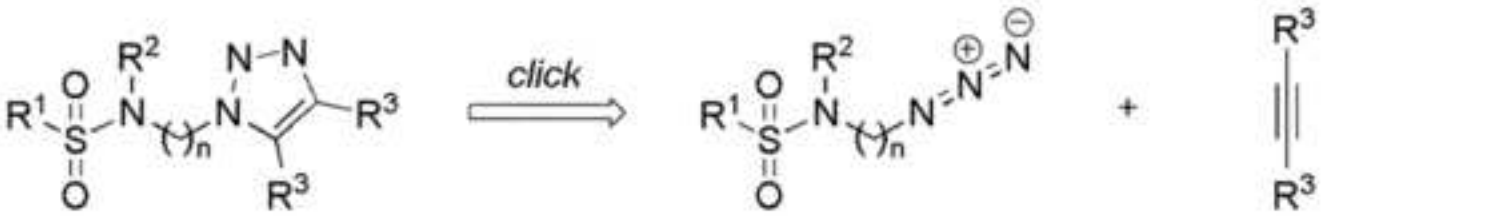
Graphical Abstract



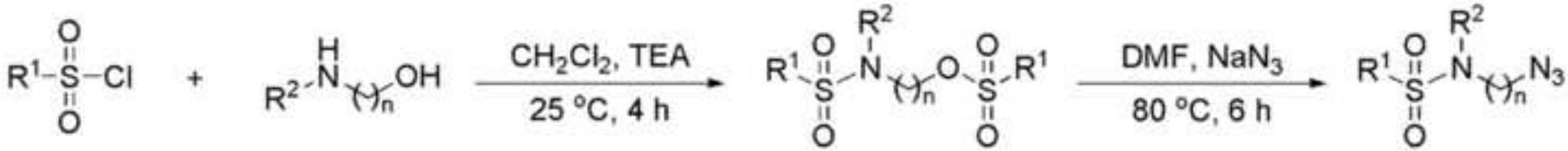


Scheme 1

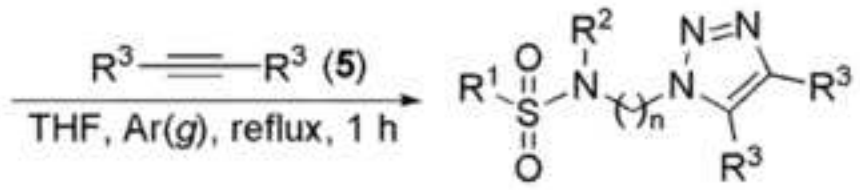





Target compound (**6**) Azide compound (**4**) Alkyne compound (**5**)




**1** **2** (n = 1-3) **3** **4**




**6**

Table 1



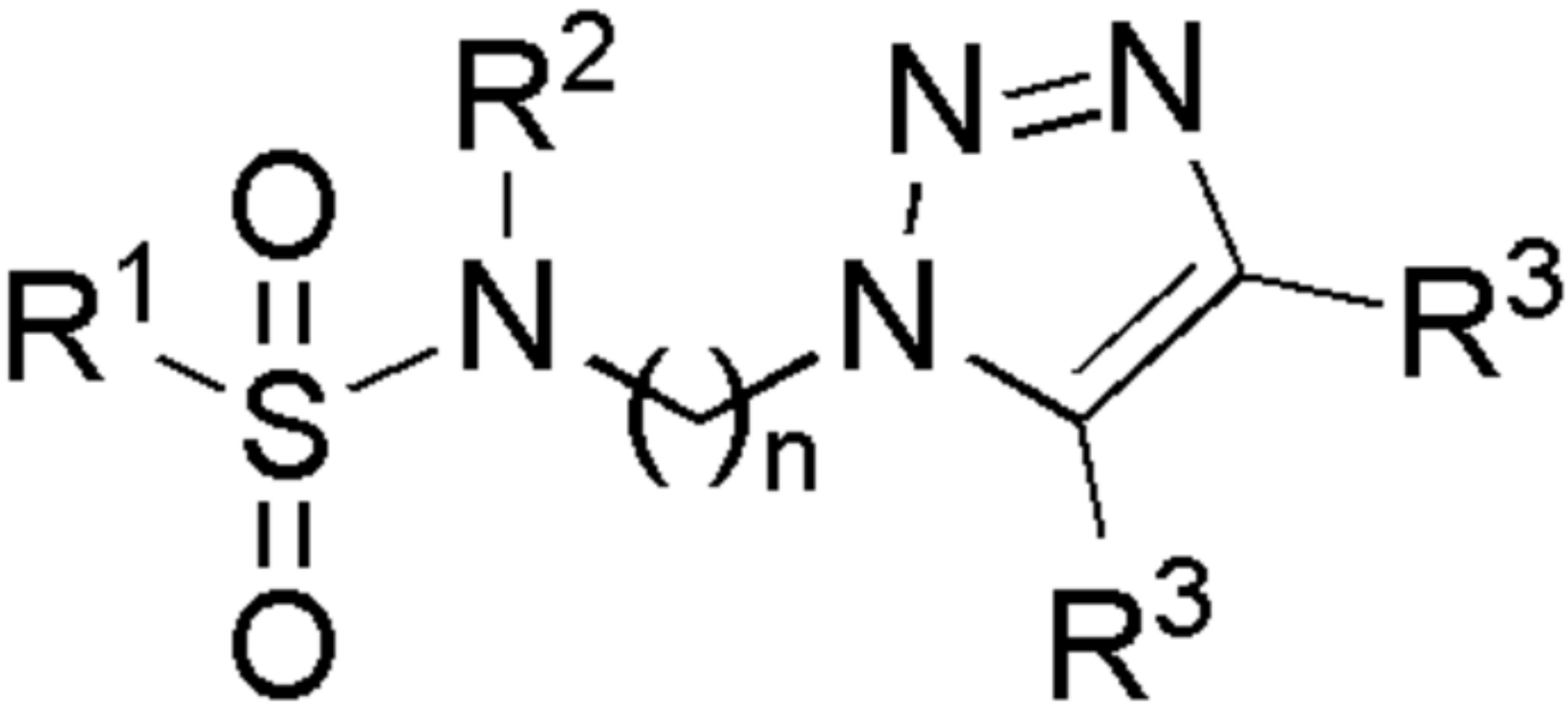


Figure 1



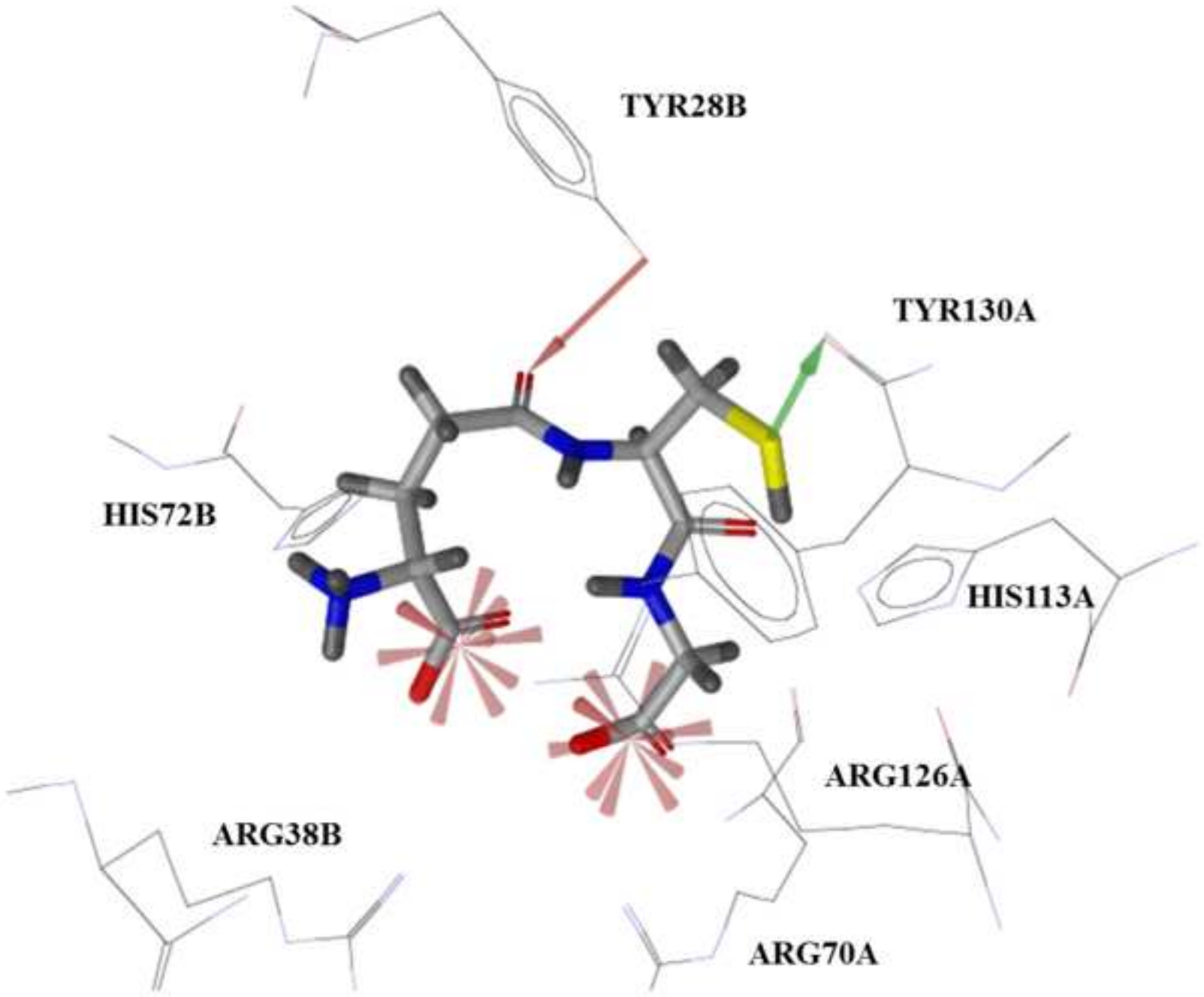


Figure 2



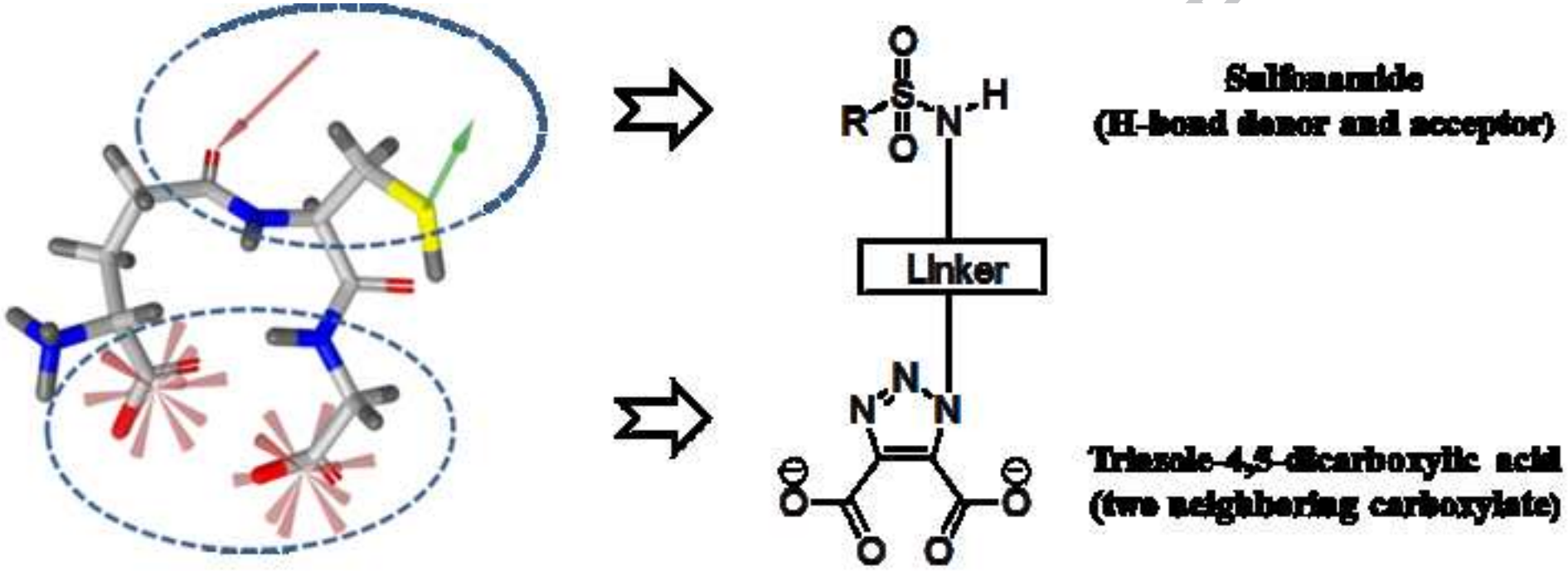


Figure 3



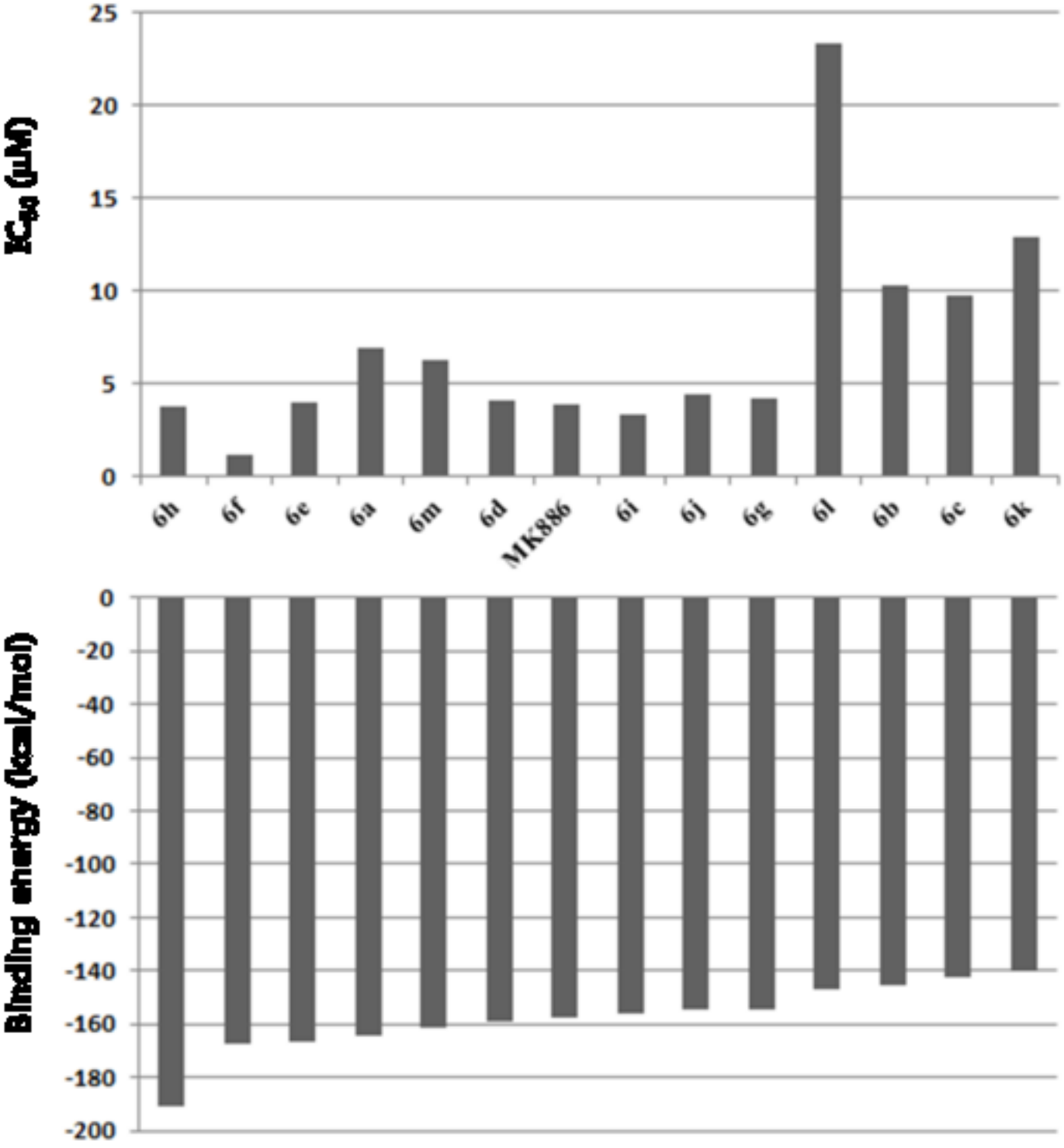


Figure 4



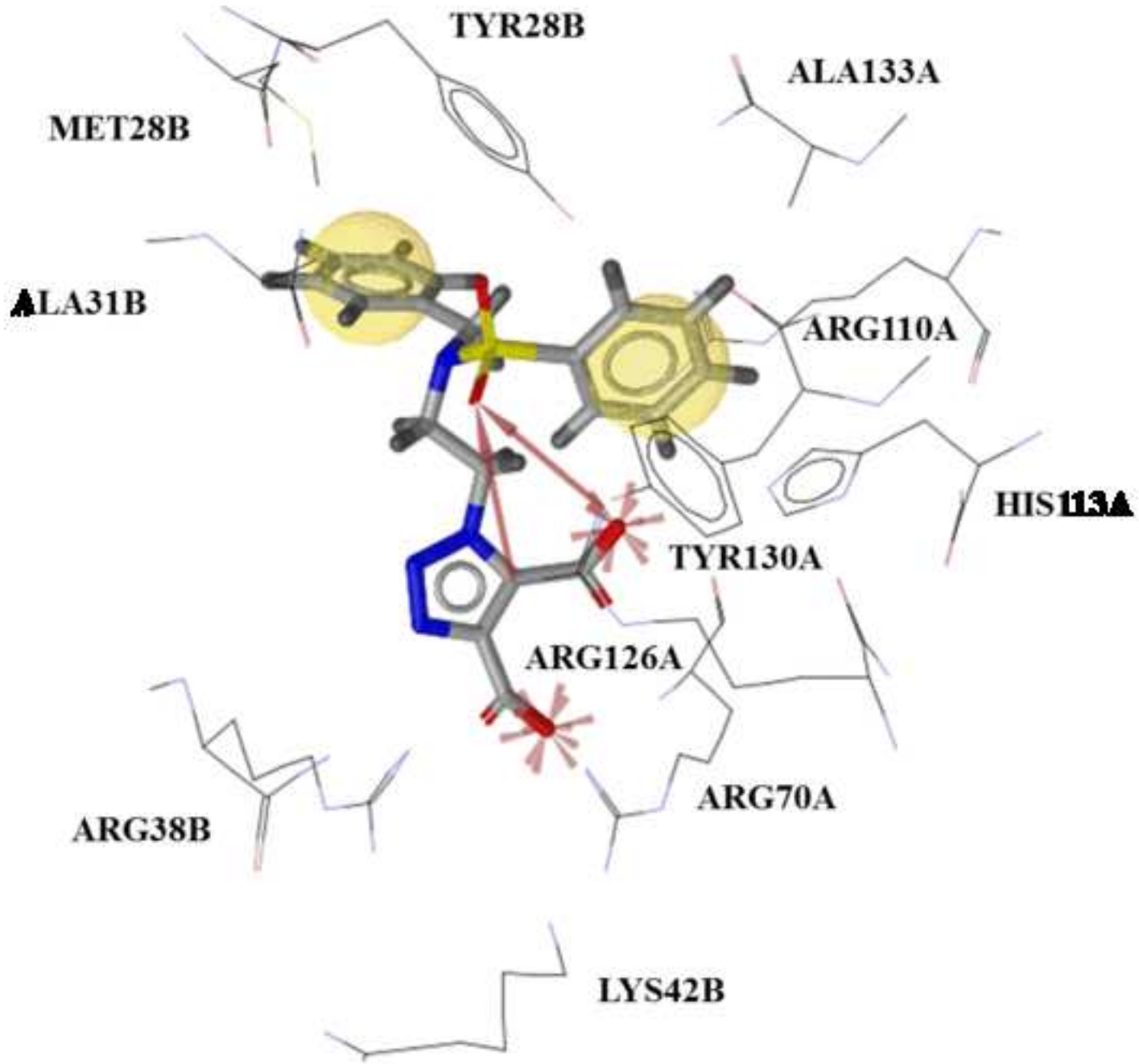
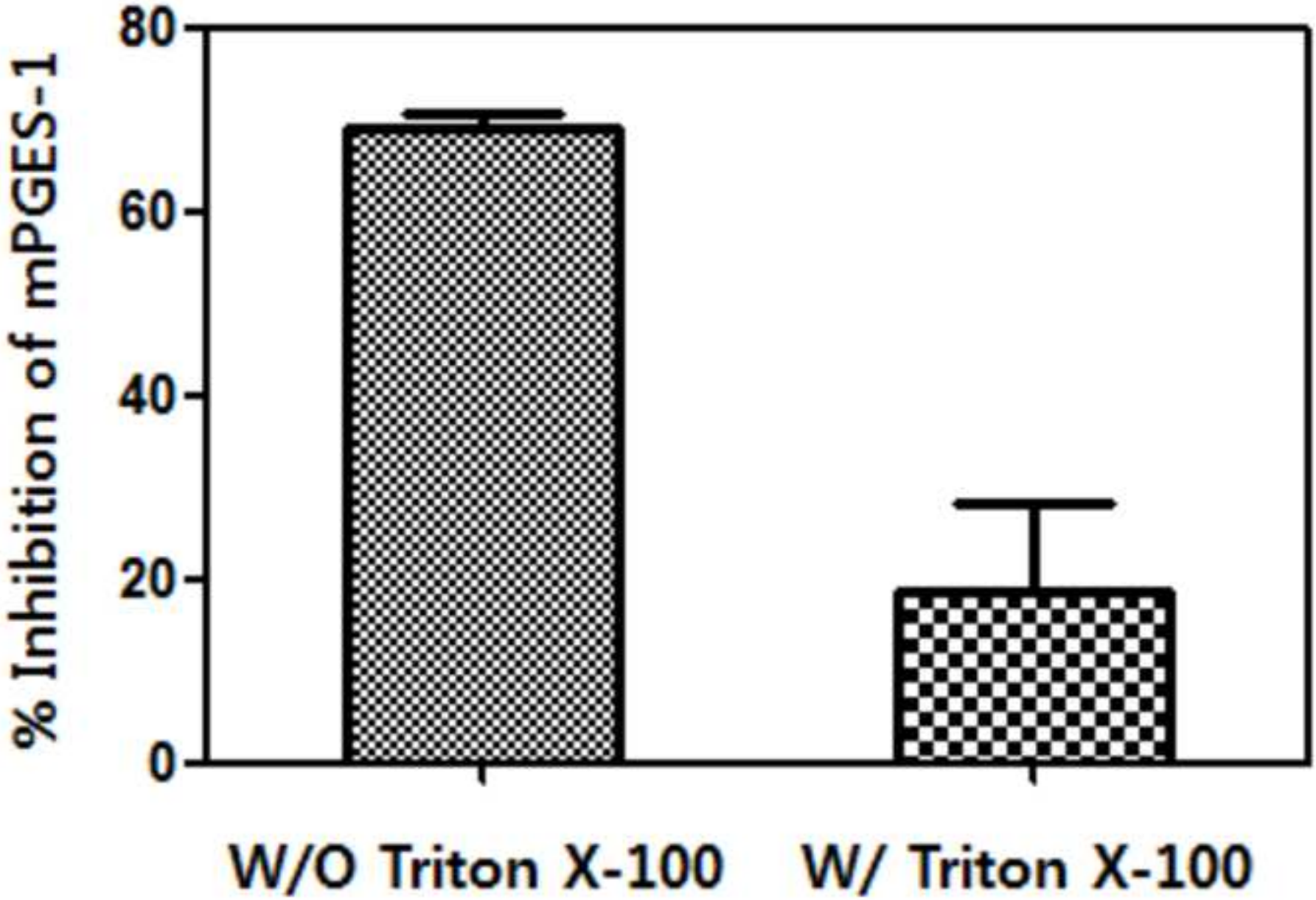


Figure 5





## Graphical Abstract

**Fragment-Based Discovery of Novel and Selective mPGES-1 Inhibitors Part 1: Identification of Sulfonamido-1,2,3-Triazole-4,5-Dicarboxlic acid**

Kijae Lee, Van Chung Pham, Min Ji Choi, Kyung Ju Kim, Kyung-Tae Lee, Seong-Gu Han, Yeon Gyu Yu, Jae Yeol Lee*