JOURNAL OF CHEMICAL INFORMATION AND MODELING

ARTICLE

pubs.acs.org/jcim

Molecular Docking and Competitive Binding Study Discovered Different Binding Modes of Microsomal Prostaglandin E Synthase-1 Inhibitors

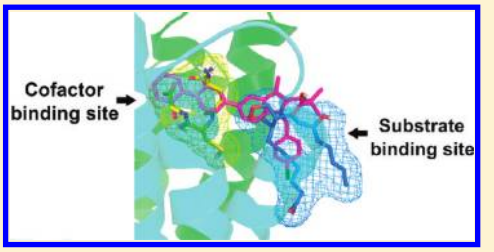
Shan He[†] and Luhua Lai[*,‡,†]

[‡]Beijing National Laboratory for Molecular Sciences, State Key Laboratory for Structural Chemistry of Unstable and Stable Species, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China

[†]Center for Theoretical Biology, Peking University, Beijing 100871, China

Supporting Information

ABSTRACT: Microsomal prostaglandin E synthase-1 (mPGES-1) is a newly recognized therapeutic target for the treatment of inflammation, pain, cancer, atherosclerosis, and stroke. Many mPGES-1 inhibitors have been discovered. However, as the structure of the binding site is not well-characterized, none of these inhibitors was designed based on the mPGES-1 structure, and their inhibition mechanism remains to be fully disclosed. Recently, we built a new structural model of mPGES-1 which was well supported by experimental data. Based on this model, molecular docking and competition experiments were used to investigate the binding modes of four representive mPGES-1 inhibitors. As the inhibitor binding sites predicted by docking overlapped with both the substrate and the cofactor binding sites, mPGES-1 inhibitors might act as dual-site inhibitors. This inhibitory mechanism was further verified by inhibitor-cofactor and inhibitor-substrate competition experiments. To investigate the potency-binding site relationships of mPGES-1 inhibitors, we also carried out molecular docking studies for another series of compounds. The docking results correlated well with the different inhibitory effects observed experimentally. Our data revealed that mPGES-1 inhibitors could bind to the substrate and the cofactor binding sites simultaneously, and this dual-site binding mode improved their potency. Future rational design and optimization of mPGES-1 inhibitors can be carried out based on this binding mechanism.



INTRODUCTION

Microsomal prostaglandin E synthase-1 (mPGES-1) is a novel therapeutic target first identified in 1999.[1] Recent studies, mainly from disruption of the mPGES-1 gene in mice, demonstrate the key role of mPGES-1-generated PGE_2 in female reproduction and pathological conditions such as inflammation, pain, fever, anorexia, atherosclerosis, stroke, tumorigenesis, and Alzheimer's disease.[2−4] These findings suggest that mPGES-1 is a potential drug target for treatment of related diseases. mPGES-1 is one of the membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG) protein family, which also includes microsomal glutathione transferase-1 (MGST-1), MGST-2, MGST-3, leukotriene C4 synthase (LTC_4S), and 5-lipoxygenase-activation protein (FLAP).[5] It catalyzes the isomerization of PGH_2 to PGE_2 and requires glutathione (GSH) as an essential cofactor for its activity.

As mPGES-1 has been identified as a promising drug target, much has been done to develop mPGES-1 inhibitors.[6] Several types of inhibitors have been discovered (see Table S1). However, as a membrane protein, the crystal structure of mPGES-1 was determined only recently by electron crystallography to a resolution of 3.5 Å (PDB code 3DWW).[7] The mPGES-1 molecule in this structure takes a closed inactive conformation that is not accessible by the substrate PGH_2. Due to the lack of a well-characterized binding site structure, none of the published inhibitors were designed based on the mPGES-1 structure, and their inhibition mechanism remains to be fully disclosed.

Among the published mPGES-1 inhibitors, the structure−activity relationships (SAR) of 5 types (inhibitors **1**−**5**, Figure 1) have been extensively studied.[8−12] The inhibitors **2**−**4** were first discovered by Koeberle et al.. They have performed Lineweaver−Burk analysis and simplified competition experiments (using only two substrate concentrations) and demonstrated that inhibitors **2**−**5** might be noncompetitive to substrate PGH_2.[9−11,13] However, with the substrate binding site excluded, it was difficult to explain where the inhibitors bind to mPGES-1. Pawelzik et al. investigated the key residues responsible for the species differences in inhibitor binding of mPGES-1.[14] The inhibitors examined were verified as competitive inhibitors of the substrate, and both the inhibitor binding site and the substrate binding site were located in the same environment. Furthermore, the structure of a homologous protein, FLAP, was solved recently in complex with the inhibitor MK-591 (analogue of inhibitor **5**, named inhibitor **6**).[15] MK-591 was found to bind to the active site, and the binding mode might be conserved throughout the MAPEG superfamily.

Received: September 11, 2011
Published: November 12, 2011

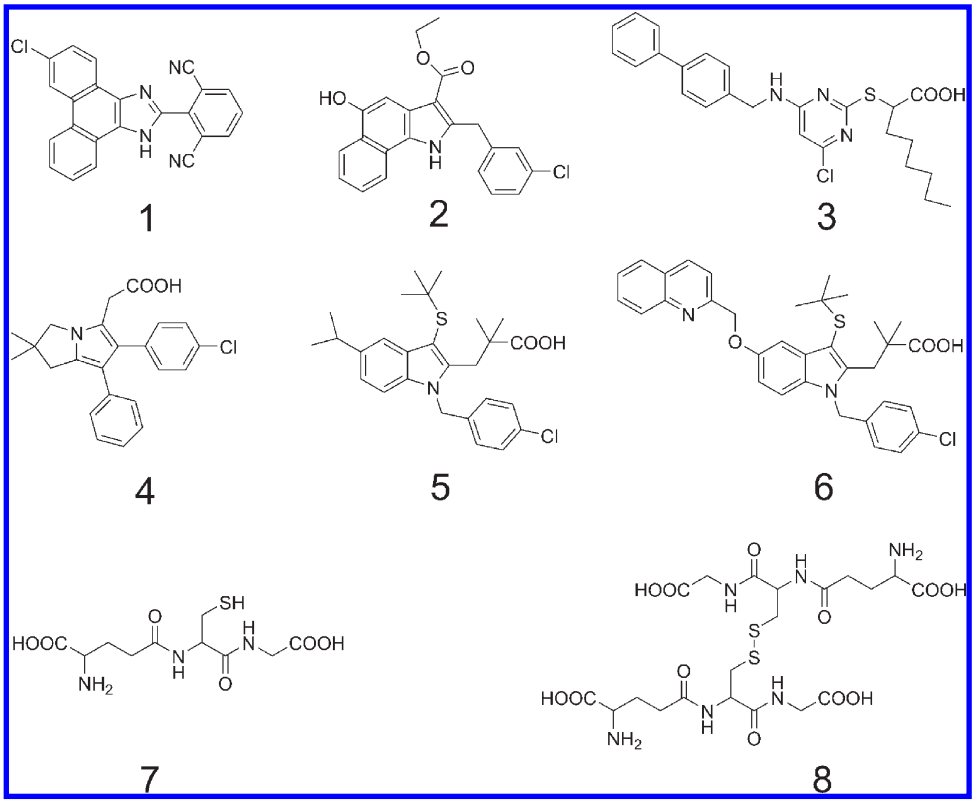


Figure 1. mPGES-1 inhibitors: (1) MF63, (2) **7a**, (3) **7b**, (4) Licofelone, and (5) MK-886. FLAP inhibitor: (6) MK-591. The inhibitor names are in accordance with those in the original literature.[8−12,15] The literature IC_{50} values for the five mPGES-1 inhibitors are 0.0013 μM, 0.6 μM, 1.3 μM, 6.7 μM and 1.6 μM., respectively. (7) Cofactor GSH, (8) control molecule GSSG.

Therefore, the inhibitory mechanism of mPGES-1 inhibitors remains to be fully disclosed. Recently, we built an active conformation structural model of mPGES-1 which enabled us to explore the binding modes of mPGES-1 inhibitors (see ref 16 for modeling details). Briefly, we compared the crystal structures of mPGES-1 (PDB code 3DWW) and LTC_4S (PDB code 2UUH) and manually 'open' the substrate-binding pocket of mPGES-1. Then we docked the substrate PGH_2 into the binding pocket and optimized the structure by MD simulation.[16] The molecular docking studies based on our model revealed that inhibitors occupy not only the substrate site but also the cofactor GSH binding site. This dual-site binding mode was further verified by competition experiments and published SAR results.

MATERIALS AND METHODS

Materials. The plasmid DNA harboring the full-length cDNA of the PIG12 gene was from Addgene (Addgene plasmid 16506: pBK-CMV Pig12). PGH_2, PGE_2 EIA kit, and inhibitors **2**−**4** were from Cayman Chemical. Inhibitor **1** was from Manganna Biochem. 1-Hexadecanoyl-2-(9Z-octadecenoyl)-*sn*-glycero-3-phosphocholine (POPC) was from Avanti Polar Lipids. Other reagents were from Sigma Aldrich unless indicated otherwise.

Molecular Cloning and Protein Expression. The plasmid construction and protein expression were performed as described previously.[16] Briefly, the mPGES-1 coding region was ligated into pET28a(+) vector and transformed to the Rosetta(DE3) strain of *Escherichia coli*. Recombinant cells were cultivated at 37 °C until the OD_{600} reached 1.0. Then mPGES-1 expression was induced, and the cells were grown for another 12 h at 25 °C.

Microsome Preparation and Enzyme Activity Assay. The microsome preparation was performed as described previously.[16] Cells were harvested by centrifugation (5,000 g, 20 min) and broken by sonication. Insoluble material was separated by centrifugation (12,000 g, 30 min), and the supernatant was then ultracentrifuged at 174,000 g for 1 h. The membrane pellet was washed in activity assay buffer (2.5 mM GSH, 0.1 M potassium phosphate buffer (pH = 7.4) and 1% (v/v) glycerol) once and then resuspended in 2 mL of solubilizing buffer (2.5% (w/v) POPC in activity assay buffer). Protein concentrations were determined using a bicinchoninic acid protein assay kit (Biomed).

Enzyme activity was measured by assessment of PGH_2 conversion to PGE_2 according to ref 17. Briefly, PGH_2 was added to each well of a 96-well plate, and the reaction was started by adding a microsome sample. After reacting at 4 °C for 1 min, the reaction was terminated by adding stop solution (50 mM $FeCl_2$ and 100 mM citric acid). PGE_2 in the reaction mixture was quantified using the PGE_2 EIA kit (Cayman).

To evaluate the effects of inhibitors on the mPGES-1 activity (IC_{50}), enzyme samples were preincubated with inhibitor for 15 min at 4 °C. The cofactor concentration in the assay buffer was 2.5 mM and the PGH_2 concentration was 17 μM.[9−11] IC_{50} values were obtained from fitting the data to a four-parameter logistical model of the graph of log dose against percentage inhibition. Data are expressed as mean ± SEM (n = 3).

In Figure 3A and 3B, results are reported as a percentage of inhibition or activity. The inhibitory effect measured using a vehicle (2% DMSO)-treated microsome sample (cofactor concentration in assay buffer, 2.5 mM; PGH_2 concentration, 17 μM) was set as 0% inhibition (or 100% activity). The inhibitory effect

dx.doi.org/10.1021/ci200427k |*J. Chem. Inf. Model.* 2011, 51, 3254–3261

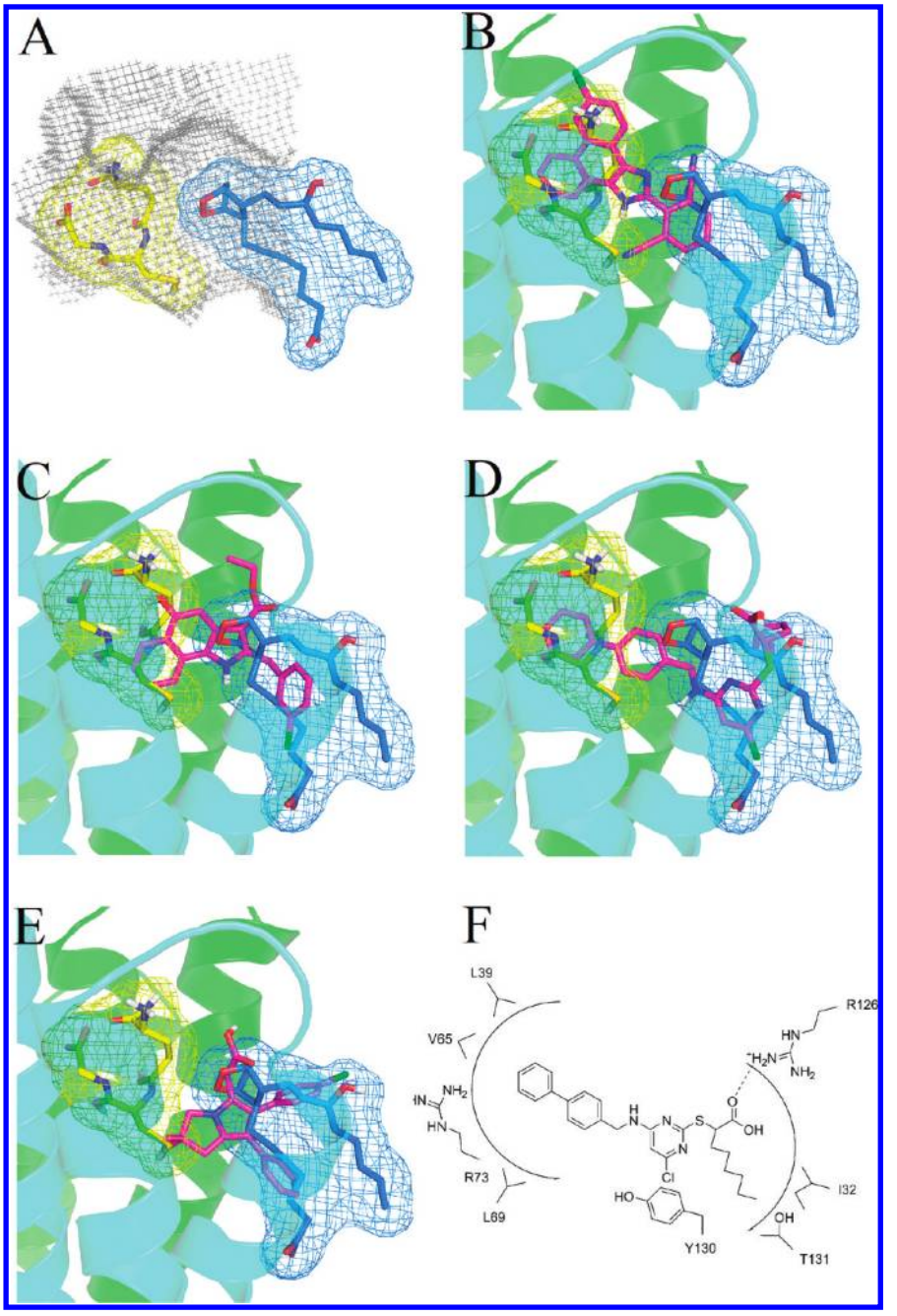


Figure 2. (A) mPGES-1 pocket (gray dot) includes two binding sites: cofactor (yellow) and substrate (blue) binding sites (the binding pocket was calculated using the program Cavity). The cofactor and substrate are also shown in mesh representation. (B)-(E) Inhibitors **1**−**4** (magenta) binding modes calculated using Autodock 4. Two subunits (green and cyan) of the mPGES-1 trimer are shown to represent the binding pocket. (F) Schematic representation of the interaction between mPGES-1 and inhibitor **3**. Important residues involved in the interaction are labeled.

measured using vehicle (2% DMSO)-treated negative control (microsome sample prepared with Rosetta cells without IPTG induction; cofactor concentration in assay buffer, 2.5 mM; the PGH_2 concentration, 17 μM) was set as 100% inhibition (or 0% activity).

Competition Experiments. In order to investigate the interactions between inhibitor, cofactor GSH, and substrate PGH_2, we performed inhibitor-cofactor and inhibitor-substrate competition experiments as follows:

Inhibitor-Cofactor Competition Experiment. Prior to the activity assay, the enzyme sample was copreincubated with inhibitor and cofactor for 15 min at 4 °C. The inhibitor concentration was kept constant while gradually increasing the cofactor concentration from 2.5 to 25 mM. The concentrations of inhibitors **1**−**4** were selected as 0.01, 1, 5, and 50 μM, which inhibited the mPGES-1 activity 100% when the cofactor concentration was 2.5 mM. The substrate PGH_2 concentration was kept constant as 17 μM. A control experiment was performed under the same conditions except that the GSH was replaced by GSSG with the same final mass-volume percentage (therefore the molarity of GSH equaled that of GSSH × 2, as the molecular weight of GSSH is double that of GSH). To ensure the enzyme activity of mPGES-1 (mPGES-1 requires GSH as an essential cofactor for its activity),

2.5 mM GSH was always present in the assay buffer. For calculation of the percentage of inhibition in Figure 3C, the activity curve shown in Figure 3B (the upper curve for GSH) was set as 0% inhibition.

Inhibitor-Substrate Competition Experiment. As the enzyme sample could not be preincubated with the substrate, it was preincubated with the inhibitor for 5 min at 4 °C, and then PGH_2 was added to start the reaction. The inhibitor concentration was kept constant while gradually increasing the substrate concentration from 2.8 to 200 μM. As we need to determine the effect of inhibitors on the mPGES-1 activity at a lower substrate concentration (2.8 μM), the concentrations of inhibitors **1**−**4** were selected as 0.003, 0.2, 0.4, and 4 μM, respectively, which inhibited mPGES-1 activity by 50% (IC_{50}) when substrate concentration was 17 μM. The cofactor GSH concentration was kept constant as 2.5 mM. To calculate the percentage inhibition in Figure 3D, the inhibitory effect when the substrate concentration was 17 μM for each inhibitor (inhibitors **1**−**4**) was set as 50% inhibition.

Molecular Docking. Molecular docking studies were carried out using Autodock 4.[18] Inhibitor structures were constructed and optimized using Schrödinger Suite 2009.[19] Our newly developed active conformation structural model of mPGES-1 was used as the receptor structure.[16] Molecular docking was carried out using the empirical free-energy function and the GALS algorithm (genetic algorithm with local search).[18] For all dockings, 50 independent runs were performed with an initial population of 300 randomly placed individuals, 50,000,000 energy evaluations, and a maximum number of generations of 270,000. All the residues of the enzyme were kept rigid.

A two-step docking protocol was used. In the first step, a large grid box with 72 × 90 × 72 points and a grid spacing of 0.375 Å was used to explore the potential inhibitor binding sites. The docking results were clustered based on a 2.0 Å rmsd criterion, and the region where the largest cluster was located was selected as the preferred binding site. In the second step, a grid box with 48 × 56 × 56 points around the preferred binding site was used to find the best binding conformation. The final docking result was selected as the structure with the lowest energy in the largest cluster.

RESULTS

Molecular Docking of Inhibitors 1−4. To explore the binding modes of mPGES-1 inhibitors, molecular docking studies of inhibitors **1**−**4** were carried out using Autodock 4 based on our newly developed structural model of mPGES-1.[16] The docking results revealed that all the four inhibitors bound to the same binding pocket. For inhibitors **1**−**4**, the calculated K_d values (given by the program Autodock 4) were 0.038 μM, 0.8 μM, 0.16 μM, and 2.7 μM, respectively, which were in good agreement with our experimentally determined IC_{50} values: 0.0029 ± 0.0001 μM, 0.16 ± 0.03 μM, 0.41 ± 0.08 μM, and 3.9 ± 0.5 μM, respectively (Figure 3A).

We further examined the binding pocket using the program Cavity.[20] The mPGES-1 pocket lay in the interface of two subunits and had a dumbbell shape (Figure 2). The cofactor and substrate bound to two adjacent sites with a narrow passage connecting them. Jegerschöld et al. proposed that the reaction mechanism of mPGES-1 involves the participation of cofactor GSH and Arg126.[7] Therefore, the close interaction between the cofactor and substrate is important for the enzyme reaction.

For the inhibitors **1**−**4**, the calculated binding sites overlapped with both the cofactor and substrate binding sites. Superimposition

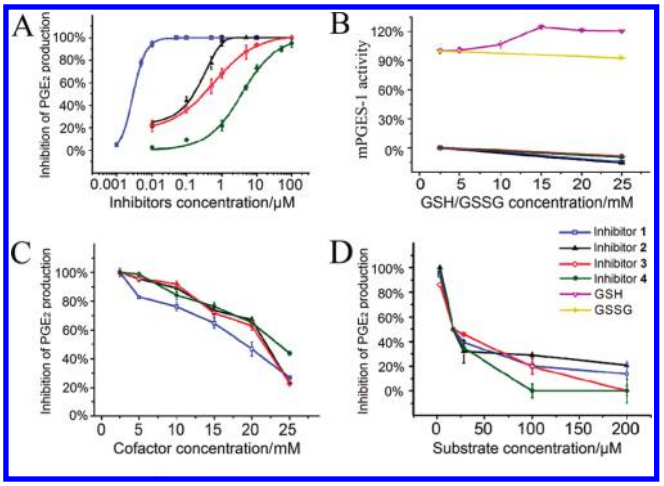


Figure 3. Competition experiments. (A) Effects of inhibitors **1**−**4** on mPGES-1 activity. (B) Effects of cofactor GSH and control compound GSSG on mPGES-1 activity. The *x*-axis represents the molarity concentration of GSH or GSSG × 2 (GSH and GSSG have the same mass-volume percentage). The mPGES-1 activity increased with increased GSH concentration as previously reported.[17] Increasing GSSG concentration had no significant effect on the enzyme activity or inhibitory effect (The lower lines demonstrate that with preincubation of the enzyme with inhibitor and GSSG, the enzyme remained 100% inhibited. All four inhibitors gave similar results and the four curves overlapped). (C) Inhibitor-cofactor competition experimental result. (D) Inhibitor-substrate competition experimental results. Results are reported as percentage of inhibition or activity. Data shown represent the mean ± SEM (n = 3).

of the four inhibitors demonstrated that they had similar binding modes. As inhibitor **3** fit perfectly to the entire binding pocket, we used it as an example to illustrate the important residues involved in the enzyme−inhibitor interaction (see Figure S1 for other inhibitors). Inhibitor **3** formed two characteristic contacts to the enzyme: 1) a hydrogen bond between Arg126 and the carboxylic acid group and 2) a π−π stacking interaction between Tyr130 and the pyrimidine ring. In addition, the biphenyl group and the hexyl group of inhibitor **3** were located in the vicinity of residues Ile 32, Leu39, Val65, Arg73, Leu69, and Thr131, forming mainly hydrophobic interactions with the enzyme.

Inhibitors 1−4 were competitive with both the substrate and the cofactor. To verify the inhibitory mechanism suggested by the molecular docking studies, we performed inhibitor-cofactor and inhibitor-substrate competition experiments for inhibitors **1**−**4** (Figure 3).

Inhibitor-Cofactor Competition Experiment. If the inhibitor and the cofactor competitively bind to the same site, at constant inhibitor concentration, increasing cofactor concentration should impair the inhibitor's binding ability, therefore decreasing its inhibitory effect. The experimental results supported this hypothesis as the inhibitory effect of all four inhibitors decreased from 100% inhibition to below 50% with a ten-times increase in cofactor concentration. To further confirm that the inhibitors' impaired effect was specifically caused by the competitive binding of the cofactor GSH, we performed a control experiment using glutathione disulfide (GSSG) which is derived from two GSH molecules (Figure 1). The GSSG molecule has properties similar to the GSH molecule but cannot bind to the mPGES-1 pocket due to its bigger size. Our enzyme activity essay also revealed that increasing GSSG concentration had no significant

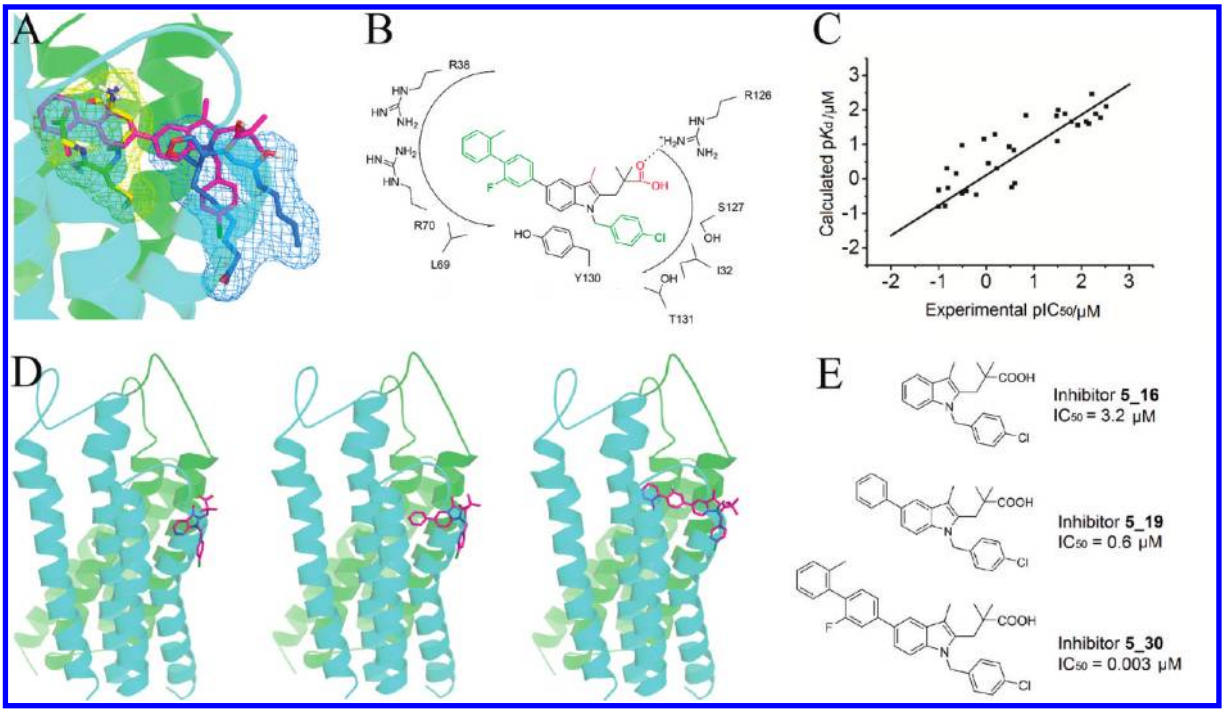


Figure 4. Molecular docking studies of inhibitor **series 5** compounds. (A) Binding mode of inhibitor **5_30** (magenta) calculated using Autodock 4. mPGES-1, cofactor, and substrate are colored following Figure 2. (B) Schematic representation of the interaction between mPGES-1 and inhibitor **5_30**. The substituent groups are colored according to the SAR studies.[12] Green represents positions where the sterically larger substituent is favorable. Red represents positions where the hydrogen acceptor group is favorable. Magenta indicates tolerant positions. Important residues involved in the interaction are labeled. (C) Correlation analysis between experimental pIC_{50} and calculated pK_d for inhibitor **series 5** compounds. The correlation coefficient R^2 was 0.74. (D),(E) Binding modes, structures, and IC_{50} values of inhibitors **5_16**, **5_19**, and **5_30**.

effect on the enzyme activity (Figure 3B). To ensure the enzyme activity of mPGES-1 (mPGES-1 requires GSH as an essential cofactor for its activity), 2.5 mM GSH was always present in the assay buffer (see the Supporting Information for experimental details). We repeated the competition experiment under the same conditions except that the additional GSH was replaced by GSSG. The experimental results revealed that, with increased GSSG concentration, no decrease in the inhibitory effect was measured. Therefore, the decrease in the inhibitor-GSH competition result was caused by the competitive binding of GSH. This result provided additional support for the inhibitor-cofactor competitive binding mode.

Inhibitor-Substrate Competition Experiment. Similar to the inhibitor-cofactor competition experiment, if the inhibitor and substrate competitively bind to the same site, at constant inhibitor concentration, increasing the substrate concentration should reduce the effect of the inhibitor. The experimental observations confirmed this hypothesis as the inhibitory effect of all four inhibitors decreased from nearly 100% to below 30% with increased substrate concentration. This result also supported the inhibitor-substrate competitive binding mode.

In previous studies, Koeberle et al. performed Lineweaver–Burk analysis and found that inhibitor **3** is noncompetitive with substrate PGH_2.[10] However, the Michaelis–Menten kinetics model is derived from the assumption of free diffusion. For heterogeneous enzymatic reactions, such as membrane enzymes like mPGES-1, molecular mobility of the enzyme or substrate is severely restricted. The limitation on molecular mobility demands modifications of the conventional Michaelis–Menten kinetics and directly applying it to membrane enzymes might not reflect the real situation.[21] Our enzyme activity assay also demonstrated that the mPGES-1 activity curve did not have a typical shape and determination of K_m and V_{max} was difficult (data not shown). Koeberle et al. also performed simplified competition experiments to verify that inhibitors **2, 4**, and **5** were not competitive with substrate.[9,11,13] Due to the labile property and high cost of the substrate PGH_2, they only used two low concentrations of PGH_2 (1 and 20 μM). However, as the K_m of PGH_2 is approximately 170 μM,[17] at low concentrations like 1 or 20 μM, PGH_2 is not able to compete with inhibitor binding to the enzyme. We have managed to increase the PGH_2 concentration to 200 μM, and the experimental result revealed that the inhibitory effect dramatically decreased to below 30%, supporting the inhibitor-substrate competitive binding mode.

Comprehensive analysis of both the inhibitor-cofactor and inhibitor-substrate competition experiments revealed that the behavior of inhibitor **4** was different in the two experiments. While its inhibitory effect decreased more slowly than other inhibitors with increased cofactor concentration, its inhibition decreased rapidly with increased substrate concentration. This suggested that inhibitor **4** mainly competed with the substrate. The calculated binding modes of inhibitors revealed that, unlike inhibitors **1**–**3** which equally occupied the cofactor and substrate binding sites, inhibitor **4** mainly took the substrate binding site (Figure 2). Therefore, the calculated binding modes were in good agreement with the experimental results.

Occupying both sites improved the potency of mPGES-1 inhibitors. Among inhibitors **1**–**4**, inhibitor **4** mainly bound to the substrate binding site with the lowest inhibitory effect (IC_{50} = 4 μM). This suggests that occupying both the substrate and the

cofactor binding sites, like inhibitors **1**−**3**, improves the inhibitors' potency. In order to verify this hypothesis, we then carried out further molecular docking studies on another series of inhibitors (inhibitor **series 5** of 32 compounds; Table S2) to investigate the potency-binding sites relationships of mPGES-1 inhibitors. The lead compound (MK-886 named inhibitor **5**) was the first mPGES-1 inhibitor discovered, and the SAR of this series was explored to a great extent previously.[12,22] We docked all 32 compounds to the enzyme and analyzed the correlation between experimental pIC_{50} and predicted pK_d (Figure 4C). The correlation coefficient R^2 reached 0.74.

In the previous SAR studies undertaken by Riendeau et al., MK-886 was used as the lead structure, and the effects of N1, C2, C3, C5, and C7 substitution of the indole ring on potency were explored.[12] Their SAR can be well explained by our molecular docking studies. We used the most potent inhibitor **5_30** as an example to illustrate the binding modes of inhibitor **series 5** compounds (Figure 4):

1) The N1 region was located in the mPGES-1 substrate binding site where inhibitor **5_30** formed mainly hydrophobic interactions with the enzyme. Therefore, substituent groups of moderate size like N-p-chlorobenzyl were favorable.
2) At the C2 region, the carboxylic acid group formed a hydrogen bond with the key catalytic residue Arg126. Therefore, this carboxylic acid group was important for potency.
3) The C3 region was located at the entrance of the mPGES-1 pocket and pointed to the outside. Therefore, this region tolerated modification.
4) The C5 region was located in the mPGES-1 cofactor binding site which was also dominated by hydrophobic interactions. As the mPGES-1 pocket had a dumbbell shape (Figure 2) and there was a narrow passage connecting the cofactor and substrate binding sites, long and narrow substituent groups like biphenyl were favorable.
5) At the C7 region, the indole ring of inhibitor **series 5** compounds formed a $\pi-\pi$ stacking interaction with Tyr130. Therefore, it was better to have no modification at this region.

The SAR studies of Riendeau et al. revealed that modification at the C5 position had the greatest impact on potency. Additional substitution of the biphenyl group yielded the most potent inhibitor in this series (inhibitor **5_30**, IC_{50} = 3 nM). Examination of the optimization process from inhibitor **5_16** to inhibitor **5_30** revealed that as the inhibitor's binding mode changed from occupying only the substrate site to occupying both the substrate and the cofactor sites, dramatic potency enhancement of 1000-fold resulted (Figure 4D,E). Therefore, the molecular docking studies of inhibitor **series 5** compounds revealed that occupying both the substrate and the cofactor binding sites improved the inhibitors' potency against mPGES-1.

■ DISCUSSION

In drug discovery and development, it is a long-term goal to pursue inhibitors with enhanced potency and minimized side effects. Dual-site inhibition is a newly developed strategy which has been successfully applied in the acetylcholinesterase (AChE) and anthrax dihydrofolate reductase (DHFR) inhibitor discovery.[23,24] The first generation of AChE inhibitors only bound to the catalytic site of the enzyme. Recent studies reveal that besides catalytic site, AChE also has a peripheral site. The new AChE inhibitors which bind to both the catalytic and peripheral sites have much greater inhibitory activity than all previously examined inhibitors.[23] In DHFR inhibitor studies, the development of anthrax DHFR inhibitors was hampered by insufficient species selectivity and host resistance. As the cofactor binding site region between baDHFR and human DHFR reveals significant differences, dual-site inhibitors of DHFR which additionally occupy a second pharmacophore region (cofactor binding site) display enhanced species selectivity and help evade host resistance.

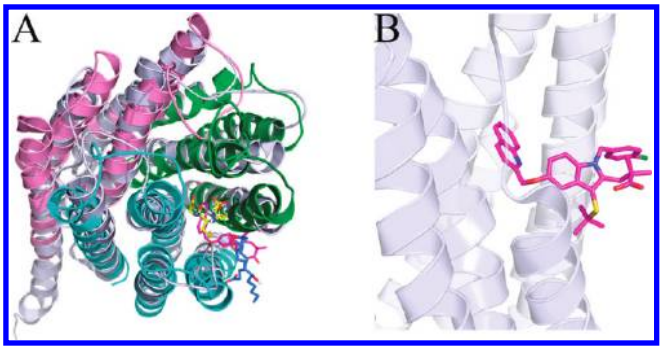


Figure 5. (A) Superimposition of FLAP (gray, PDB code 2Q7M) and our structural model of mPGES-1 (the three monomers are colored green, cyan, and pink). Inhibitor 6, cofactor, and substrate of mPGES-1 are colored magenta, yellow, and blue, respectively. (B) Binding mode of inhibitor **6** (magenta) in FLAP (gray) as determined in the crystal structure.

The development of these dual-site inhibitors were enabled by well-characterized complex crystal structures (PDB code 1ACJ, 1E66, and 1EVE for AChE and 3DAT, 3DAU, and 1U72 for DHFR). Due to the lack of a well-characterized binding site structure, the binding modes of mPGES-1 inhibitors were unclear. Our results revealed that mPGES-1 inhibitors bound to both the substrate and the cofactor binding sites and this dual-site binding mode improved its potency. Like the two successful cases of AChE and DHFR, the dual-site inhibitory mechanism can be used for future rational design and optimization of mPGES-1 inhibitors.

MAPEG proteins constitute a novel integral membrane enzyme superfamily and are involved in the protection against oxidative stress, xenobiotic detoxification, and the production of arachidonic acid-derived mediators.[25] Recent advances in membrane protein studies and structural biology produced an explosion of crystal structures for MAPEG family proteins including MGST-1 (PDB code 2H8A), LTC_4S (PDB code 2UUH), FLAP (PDB code 2Q7M), and mPGES-1 (in a closed inactive conformation, PDB code 3DWW).[7,15,26,27] All theses enzymes share the same overall fold and oligomerization state as homotrimers. Among them, only one complex structure with inhibitor bound was solved, which was FLAP in complex with inhibitor **6** (structurally similar to inhibitor **5_30**). Superimposition of FLAP and our structural model of mPGES-1 revealed that inhibitor **6** in the FLAP structure overlapped with the cofactor and substrate from mPGES-1 (Figure 5). The binding mode of inhibitor **6** in FLAP was also similar to the calculated binding mode of inhibitor **5_30** in mPGES-1. This provides additional support to our molecular docking studies and indicates that the dual-site binding mode of inhibitors might be conserved throughout the MAPEG superfamily.

Prage et al. recently reported their H/D exchange experiments with inhibitor **1** and inhibitor **5**.[28] Their results revealed that the locations of these two inhibitors include the GSH binding site and the substrate binding cleft. They also introduced the studies

of Spahiu et al. which showed that inhibitor **5** exhibits mixed-type inhibition with a predominant competitive component versus the substrate.[29] Our docking studies revealed that inhibitor **5** mainly took the substrate binding site (Figure S1). Therefore, the results of Prage et al.[28] and Spahiu et al.[29] agrees well with the results of our own investigation, and this dual-site binding mechanism can be used for future rational design and optimization of mPGES-1 inhibitors.

■ ASSOCIATED CONTENT

Supporting Information. A selection of published mPGES-1 inhibitors and the compounds used in the molecular docking studies and a schematic representation of the interaction between mPGES-1 and inhibitors. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 86-10-62757486. Fax: 86-10-62751725. E-mail: lhlai@pku.edu.cn.

■ ACKNOWLEDGMENT

This research was supported, in part, by the Ministry of Science and Technology of China and the National Natural Science Foundation of China. We thank the Shanghai Supercomputer Center of China for providing high-performance computing resources. The authors are grateful to Ning Yin and Dr. I. C. Bruce for revision of the manuscript and Prof. Dr. Jianfeng Pei, Dr. Fangjin Chen, Erchang Shang, and others from the Lai lab for helpful discussions.

■ REFERENCES

(1) Jakobsson, P. J.; Thoren, S.; Morgenstern, R.; Samuelsson, B. Identification of human prostaglandin E synthase: A microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96* (13), 7220–7225.

(2) Samuelsson, B.; Morgenstern, R.; Jakobsson, P. J. Membrane prostaglandin E synthase-1: A novel therapeutic target. *Pharmacol. Rev.* **2007**, *59* (3), 207–224.

(3) Hara, S.; Kamei, D.; Sasaki, Y.; Tanemoto, A.; Nakatani, Y.; Murakami, M. Prostaglandin E synthases: Understanding their pathophysiological roles through mouse genetic models. *Biochimie* **2010**, *92* (6), 651–659.

(4) Satoh, K.; Nagano, Y.; Shimomura, C.; Suzuki, N.; Saeki, Y.; Yokota, H. Expression of prostaglandin E synthase mRNA is induced in beta-amyloid treated rat astrocytes. *Neurosci. Lett.* **2000**, *283* (3), 221–223.

(5) Jakobsson, P. J.; Morgenstern, R.; Mancini, J.; Ford-Hutchinson, A.; Persson, B. Membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG) - A widespread protein superfamily. *Am. J. Resp. Crit. Care* **2000**, *161* (2), S20–S24.

(6) Friesen, R. W.; Mancini, J. A. Microsomal prostaglandin E-2 synthase-1 (mPGES-1): A novel anti-inflammatory therapeutic target. *J. Med. Chem.* **2008**, *51* (14), 4059–4067.

(7) Jegerschöld, C.; Paweizik, S. C.; Purhonen, P.; Bhakat, P.; Gheorghe, K. R.; Gyobu, N.; Mitsuoka, K.; Morgenstern, R.; Jakobsson, P. J.; Hebert, H. Structural basis for induced formation of the inflammatory mediator prostaglandin E-2. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105* (32), 11110–11115.

(8) Cote, B.; Boulet, L.; Brideau, C.; Claveau, D.; Ethier, D.; Frenette, R.; Gagnon, M.; Giroux, A.; Guay, J.; Guiral, S.; Mancini, J.; Martins, E.; Masse, F.; Methot, N.; Riendeau, D.; Rubin, J.; Xu, D. G.; Yu, H. P.; Ducharme, Y.; Friesen, R. W. Substituted phenanthrene imidazoles as potent, selective, and orally active mPGES-1 inhibitors. *Bioorg. Med. Chem. Lett.* **2007**, *17* (24), 6816–6820.

(9) Koeberle, A.; Haberl, E. M.; Rossi, A.; Pergola, C.; Dehm, F.; Northoff, H.; Troschuetz, R.; Sautebin, L.; Werz, O. Discovery of benzo[g]indol-3-carboxylates as potent inhibitors of microsomal prostaglandin E-2 synthase-1. *Bioorg. Med. Chem.* **2009**, *17* (23), 7924–7932.

(10) Koeberle, A.; Rossi, A.; Zettl, H.; Pergola, C.; Dehm, F.; Bauer, J.; Greiner, C.; Reckel, S.; Hoernig, C.; Northoff, H.; Bernhard, F.; Dotsch, V.; Sautebin, L.; Schubert-Zsilavecz, M.; Werz, O. The Molecular Pharmacology and In Vivo Activity of 2-(4-Chloro-6-(2,3-dimethylphenylamino)pyrimidin-2-ylthio)octanoic acid (YS121), a Dual Inhibitor of Microsomal Prostaglandin E-2 Synthase-1 and 5-Lipoxygenase. *J. Pharmacol. Exp. Ther.* **2010**, *332* (3), 840–848.

(11) Koeberle, A.; Siemoneit, U.; Buhring, U.; Northoff, H.; Laufer, S.; Albrecht, W.; Werz, O. Licofelone suppresses prostaglandin E-2 formation by interference with the inducible microsomal prostaglandin E-2 synthase-1. *J. Pharmacol. Exp. Ther.* **2008**, *326* (3), 975–982.

(12) Riendeau, D.; Aspiotis, R.; Ethier, D.; Gareau, Y.; Grimm, E. L.; Guay, J.; Guiral, S.; Juteau, H.; Mancini, J. A.; Methot, N.; Rubin, J.; Friesen, R. W. Inhibitors of the inducible microsomal prostaglandin E-2 synthase (mPGES-1) derived from MK-886. *Bioorg. Med. Chem. Lett.* **2005**, *15* (14), 3352–3355.

(13) Koeberle, A.; Pollastro, F.; Northoff, H.; Werz, O. Myrtucommulone, a natural acylphloroglucinol, inhibits microsomal prostaglandin E-2 synthase-1. *Br. J. Pharmacol.* **2009**, *156* (6), 952–961.

(14) Pawelzik, S. C.; Uda, N. R.; Spahiu, L.; Jegerschöld, C.; Stenberg, P.; Hebert, H.; Morgenstern, R.; Jakobsson, P. J. Identification of Key Residues Determining Species Differences in Inhibitor Binding of Microsomal Prostaglandin E Synthase-1. *J. Biol. Chem.* **2010**, *285* (38), 29254–29261.

(15) Ferguson, A. D.; McKeever, B. M.; Xu, S. H.; Wisniewski, D.; Miller, D. K.; Yamin, T. T.; Spencer, R. H.; Chu, L.; Ujjainwalla, F.; Cunningham, B. R.; Evans, J. F.; Becker, J. W. Crystal structure of inhibitor-bound human 5-lipoxygenase-activating protein. *Science* **2007**, *317* (5837), 510–512.

(16) He, S.; Wu, E. R.; Yu, D. Q.; Lai, L. H. Microsomal prostaglandin E synthase-1 exhibits one-third-of-the-sites reactivity. 2011, doi:10.1042/BJ20110977. *Biochem. J.* Immediate Publication. http://www.biochemj.org/bj/imps/abs/BJ20110977.htm (accessed Oct 11, 2011).

(17) Thoren, S.; Weinander, R.; Saha, S.; Jegerschöld, C.; Pettersson, P. L.; Samuelsson, B.; Hebert, H.; Hamberg, M.; Morgenstern, R.; Jakobsson, P. J. Human microsomal prostaglandin E synthase-1 - Purification, functional characterization, and projection structure determination. *J. Biol. Chem.* **2003**, *278* (25), 22199–22209.

(18) Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J. Comput. Chem.* **1998**, *19* (14), 1639–1662.

(19) Maestro, version 9.0; Schrödinger, Inc.: New York, 2009.

(20) Yuan, Y. X.; Pei, J. F.; Lai, L. H. LigBuilder 2: A Practical de Novo Drug Design Approach. *J. Chem. Inf. Model.* **2011**, *51* (5), 1083–1091.

(21) Xu, F.; Ding, H. S. A new kinetic model for heterogeneous (or spatially confined) enzymatic catalysis: Contributions from the fractal and jamming (overcrowding) effects. *Appl. Catal., A* **2007**, *317* (1), 70–81.

(22) AbdulHameed, M. D. M.; Hamza, A.; Liu, J. J.; Huang, X. Q.; Zhan, C. G. Human microsomal prostaglandin E synthase-1 (mPGES-1) binding with inhibitors and the quantitative structure-activity correlation. *J. Chem. Inf. Model.* **2008**, *48* (1), 179–185.

(23) Munoz-Ruiz, P.; Rubio, L.; Garcia-Palomero, E.; Dorronsoro, I.; del Monte-Millan, M.; Valenzuela, R.; Usan, P.; de Austria, C.; Bartolini, M.; Andrisano, V.; Bidon-Chanal, A.; Orozco, M.; Luque, F. J.; Medina, M.; Martinez, A. Design, synthesis, and biological evaluation of dual binding site acetylcholinesterase inhibitors: New disease-modifying agents for Alzheimer's disease. *J. Med. Chem.* **2005**, *48* (23), 7223–7233.

(24) Bennett, B. C.; Wan, Q.; Ahmad, M. F.; Langan, P.; Dealwis, C. G. X-ray structure of the ternary MTX.NADPH complex of the

anthrax dihydrofolate reductase: A pharmacophore for dual-site inhibitor design. *J. Struct. Biol.* **2009**, *166* (2), 162–171.

(25) Molina, D. M.; Eshaghi, S.; Nordlund, P. Catalysis within the lipid bilayer - structure and mechanism of the MAPEG family of integral membrane proteins. *Curr. Opin. Struct. Biol.* **2008**, *18* (4), 442–449.

(26) Holm, P. J.; Bhakat, P.; Jegerschöld, C.; Gyobu, N.; Mitsuoka, K.; Fujiyoshi, Y.; Morgenstern, R.; Hebert, H. Structural basis for detoxification and oxidative stress protection in membranes. *J. Mol. Biol.* **2006**, *360* (5), 934–945.

(27) Molina, D. M.; Wetterholm, A.; Kohl, A.; McCarthy, A. A.; Niegowski, D.; Ohlson, E.; Hammarberg, T.; Eshaghi, S.; Haeggstrom, J.; Nordlund, P. R. Structural basis for synthesis of inflammatory mediators by human leukotriene C-4 synthase. *Nature* **2007**, *448* (7153), 613–U13.

(28) Prage, E. B.; Pawelzik, S.-C.; Busenlehner, L. S.; Kim, K.; Morgenstern, R.; Jakobsson, P.-J.; Armstrong, R. N. Location of Inhibitor Binding Sites in the Human Inducible Prostaglandin E Synthase, MPGES1. *Biochemistry* **2011**, *50* (35), 7684–7693.

(29) Spahiu, L.; Stenberg, P.; Larsson, C.; Wannberg, J.; Alterman, M.; Kull, B.; Nekhotiaeva, N.; Morgenstern, R. A Facilitated Approach to Evaluate the Inhibitor Mode and Potency of Compounds Targeting Microsomal Prostaglandin E Synthase-1. *Assay Drug Dev. Technol.* **2011**, *9* (5), 487–495.