Short Communication

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Future Medicinal Chemistry

Common structural and pharmacophoric features of mPGES-1 and LTC4S

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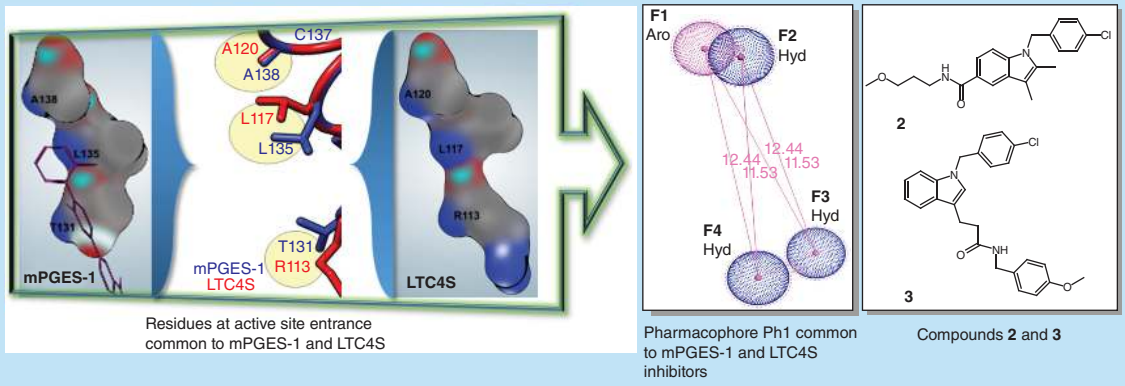
Prostaglandins and leukotrienes are produced in the COX and 5-LOX pathways of the inflammatory process. The current drugs target the upstream enzymes of either of the two pathways, leading to side effects. We have attempted to target the downstream enzymes simultaneously. Two compounds **2** and **3** (10 μM), identified by virtual screening, inhibited mPGES-1 activity by 53.4 ± 4.0 and 53.9 ± 8.1%, respectively. Structural and pharmacophore studies revealed a set of common residues between LTC4S and mPGES-1 as well as four-point pharmacophore mapping onto the inhibitors of both these enzymes as well as **2** and **3**. These structural and pharmacophoric features may be exploited for ligand- and structure-based screening of inhibitors and designing of dual inhibitors.

Graphical abstract:



First draft submitted: 15 May 2017; Accepted for publication: 13 September 2017; Published online: 22 January 2018

Keywords: anti-inflammatory • drug • dual inhibition • leukotriene • LTC4S • mPGES-1 • pharmacophore • prostaglandin

The inflammatory mediators, prostaglandins and leukotrienes (Supplementary Figure 1), produced in the arachidonic acid pathway are responsible for the various processes associated with pain, changes in vascular permeability, chemotaxis of immune cells and bronchiolar smooth muscle cell contraction [1]. Hence, the enzymes constituting these pathways are important targets for the action of anti-inflammatory drugs. The current drugs in the market target COX-1/2, although studies show the importance of other downstream enzymes in the process of inflammation. LTC4S, which converts leukotriene A4 to leukotriene C4, is overexpressed in aspirin-intolerant asthma, rheumatoid arthritis and allergies while mPGES-1, catalyzing the conversion of prostaglandin H2 (PGH2) to

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prostaglandin E2 (PGE2) is implicated in a number of pathological conditions including rheumatoid arthritis, fever and pain [2,3]. Thus, it becomes apparent that from the therapeutic side as well inhibition of the downstream enzymes in the arachidonic acid pathway is a promising strategy for the amelioration of the disease conditions involving inflammatory components.

Nonsteroidal anti-inflammatory drugs are currently the most commonly used therapy for the treatment of inflammation but are fraught with many side effects including gastric toxicity [4]. To overcome this, specific COX-2 inhibitors were developed with the hope that targeting this enzyme would eliminate some of the side effects. However, such a strategy was found to be associated with increased risk of cardiovascular side effects, leading to the withdrawal of these selective COX-2 inhibitors from the market [5]. In recent times, dual inhibition of enzymes in the 5-LOX/COX pathway as been proposed as a therapeutic avenue, given the existing evidence of shunting in cases where only a single pathway is targeted [6]. COX inhibition was shown to decrease PGE2 production and, on the other hand, increase leukotriene production, thus lowering the anticancer effects of the therapy [7]. Also theoretical network models such as those developed by Csermely *et al.* have showed that partial inhibition of many targets might be a better treatment strategy than complete inhibition of a single target, both relating to increased efficiency and an improved side effect profile [8].

Rationale for the present study

The development of dual inhibitors for the arachidonic acid pathway has until now mainly focused on 5-LOX/COX inhibitors. Their most noticeable advantage is the lack of gastric toxicity, which is prevalent in the COX-specific inhibitors [9].

However, as COX and 5-LOX are present upstream in the arachidonic acid pathway, they also have a role to play in the production of other mediators such as lipoxins [10]. Inhibiting them can disrupt various biological processes, therefore, subsequent research has focused on 5-LOX/mPGES-1 dual inhibitors. However, an inherent drawback of this strategy is that these two enzymes belong to two different protein families, and hence designing molecules that are able to modulate both the targets is a challenging task [11]. Also, the mode of action of several 5-LOX inhibitors is iron chelation or antioxidant activity, which are nonspecific and can lead to side effects as seen in the case of Zileuton, which is associated with hepatotoxicity [12].

In contrast, LTC4S and mPGES-1, the pair of targets which we investigate here for dual inhibition, belong to the same protein superfamily, the membrane associated proteins in eicosanoid and glutathione metabolism (MAPEG) [13]. This makes them more amenable to simultaneous inhibition owing to their inherent similarity. However, till date, only a few LTC4S inhibitors are reported due to the instability of LTC4S, a transmembrane enzyme, as well as its substrate leukotriene A4, which has a half-life of 3 s [14,15]. Due to this, the potential of LTC4S as an anti-inflammatory drug target or of LTC4S-mPGES-1 dual inhibition has not yet been fully explored. However, the elucidation of high-resolution crystal structures of both the enzymes in the recent past has opened up the possibility of structure-based drug design [16,17]. Ago *et. al.* reported a structure-based screening for LTC4S inhibitors where screening of 6 million molecules yielded only seven molecules with more than 50% inhibition at 100 μM [18]. This led us to postulate that exploring the common features (pharmacophore) of currently known LTC4S inhibitors can aid in the development a ligand-based screening approach. It can be used in conjunction with the structure-based one to increase the accuracy of computational methods of hit identification. This will ensure that compounds shortlisted for eventual testing on this challenging assay system have a better chance of succeeding. We also wanted to explore some structural and pharmacophoric features common to the mPGES-1 and LTC4S enzymes and their inhibitors, which may be targeted for dual inhibition. This lead us to identify the broad objectives for the present work as: identification of common structural features of mPGES-1 and LTC4S enzymes; elucidation of a pharmacophore common to both mPGES-1 and LTC4S inhibitors. In this study, several computational approaches have been combined together to identify these common features which may be used for the development of dual inhibitors in future. This was followed by experimental studies of some identified compounds against mPGES-1 as a validation of the hypothesis from the computational studies. It explored the relevance of using the common pharmacophore as a guide while designing inhibitors against these enzymes.

Materials & methods

Human recombinant mPGES-1, PGH2 and PGE2 EIA kit were sourced from Cayman Chemical (MI, USA). Other chemicals were obtained from SRL (Mumbai, India) and HiMedia Laboratories Pvt Ltd. (Bangalore, India). A detailed description of the methodology followed is given in the Supplementary Information.

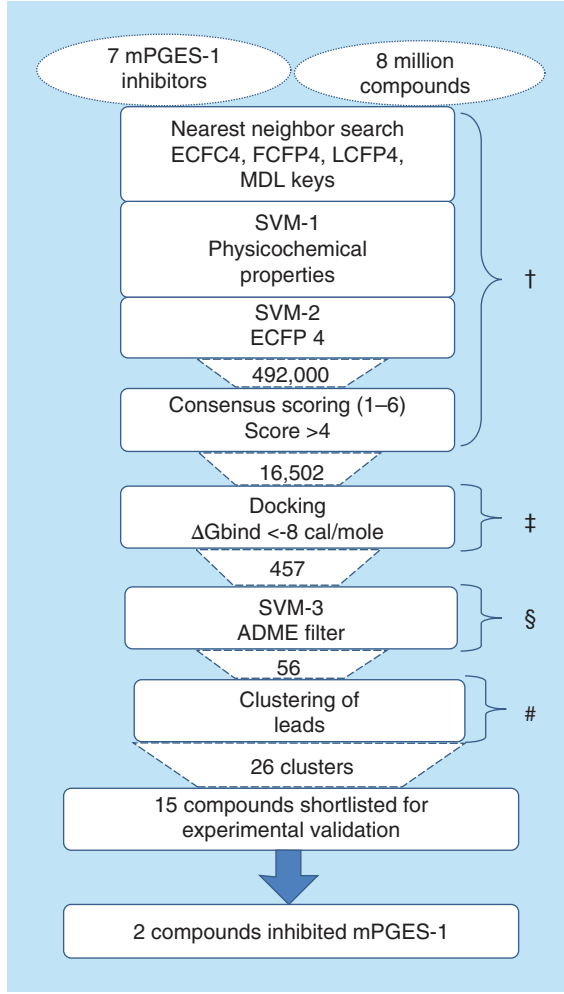

Figure 1. Virtual screening protocol for identifying mPGES-1 inhibitors. The screening led to two compounds with significant inhibition of mPGES-1 activity (53.4 ± 4.0 and 53.9 ± 8.1% at 10 μM).
†PipelinePilot 6.1.
‡Autodock 4.0 and DOVIS 2.0.
§QikProp.
#Ligand-Info.

Virtual screening for mPGES-1 inhibitors

The virtual screening protocol is summarized in Figure 1. Briefly, the 'all-purchasable' subset of ZINC8 containing 8,204,014 ligands was subjected to ligand-based virtual screening using seven known mPGES-1 inhibitors (Supplementary Figure 2) as reference compounds [11,19–24]. The screening consisted of nearest neighbor search using 2D fingerprints followed by further shortlisting using physicochemical properties and extended connectivity fingerprints (ECFP4) [25,26]. The shortlisted compounds which satisfied more than four of these criteria (consensus scoring) were used for structure-based screening. The whole process was implemented in PipelinePilot 6.1 [27] (Accelrys, Inc., CA, USA, 2007).

Then, the shortlisted compounds were docked into the active site of the modeled open conformation of mPGES-1 trimer (Protein Data Bank [PDB] ID:3DWW) using Autodock 4.0 and the DOVIS 2.0 package in the High-Performance Computing Environment at the Indian Institute of Technology, Madras [28,29]. Compounds having binding energy lower than that of the substrate, PGH2 are shortlisted and further filtered using the absorption, distribution, metabolism and excretion (ADME) criteria (QikProp, Schrodinger, NY, USA). The shortlisted hits were clustered using ligand-info and 15 compounds were chosen for experimental validation [30].

The *in vitro* mPGES-1 inhibitory activity of these compounds was established as previously described [31]. Here, the formation of the product, PGE2, was measured using the enzyme-linked immunosorbent assay kit as per the manufacturer's protocol (PGE2 EIA kit, Cayman Chemical).

Protein structural similarity & pharmacophore studies

Structure and pharmacophore comparison studies are summarized in Figure 2. The comparison between mPGES-1 (PDB:4YL0, human) and LTC4S (PDB:4JCZ, human and PDB:4NTF, mouse) was carried out using the Probis

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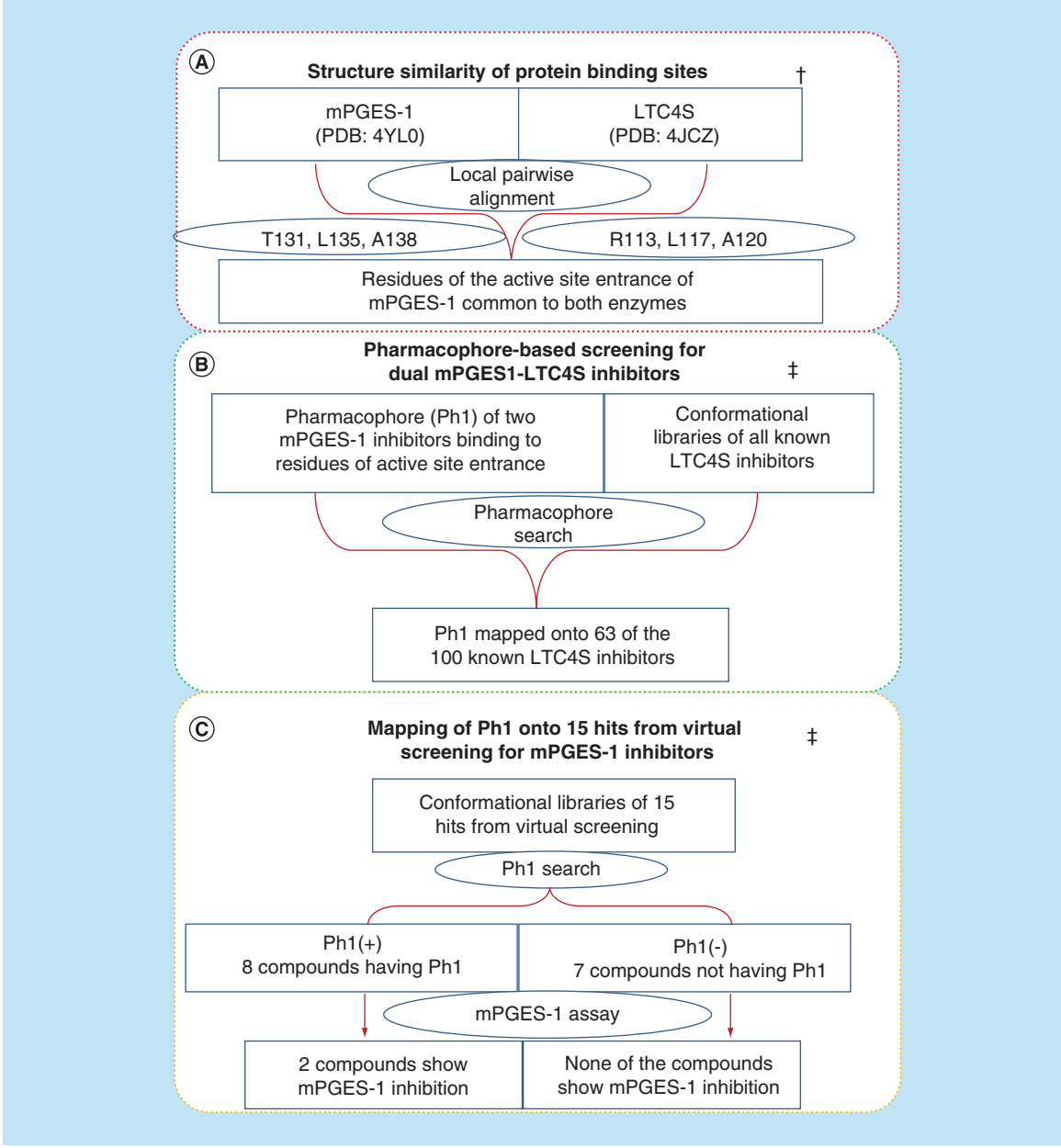

Figure 2. Structural and pharmacophore similarity between mPGES-1 and LTC4S. (A) Structural similarity studies of mPGES-1 and LTC4S identified residues at the active site entrance common to both enzymes. **(B)** Pharmacophore Ph1 elucidated from two mPGES-1 inhibitors binding to the these residues was present in 63 known LTC4S inhibitors. **(C)** Fifteen compounds obtained from virtual screening for mPGES-1 inhibitors were divided into two categories based on the presence [Ph1(+)] or absence [Ph1(-)] of Ph1. Both compounds showing mPGES-1 inhibition (10 μM) belonged to the group Ph1(+).
†Probis.
‡Molecular Operating Environment 2015.1001.

webserver [16,17,32,33]. Pharmacophore elucidation was performed by the Low Mode Molecular Dynamics Method using Molecular Operating Environment 2015.1001 (Chemical Computing Group Inc., QC, Canada) [34]. A library of LTC4S inhibitors was created from the previously reported compounds in the literature [18,35–45]. The results were visualized in UCSF (University of Californis, San Francisco) chimera[6] [46].

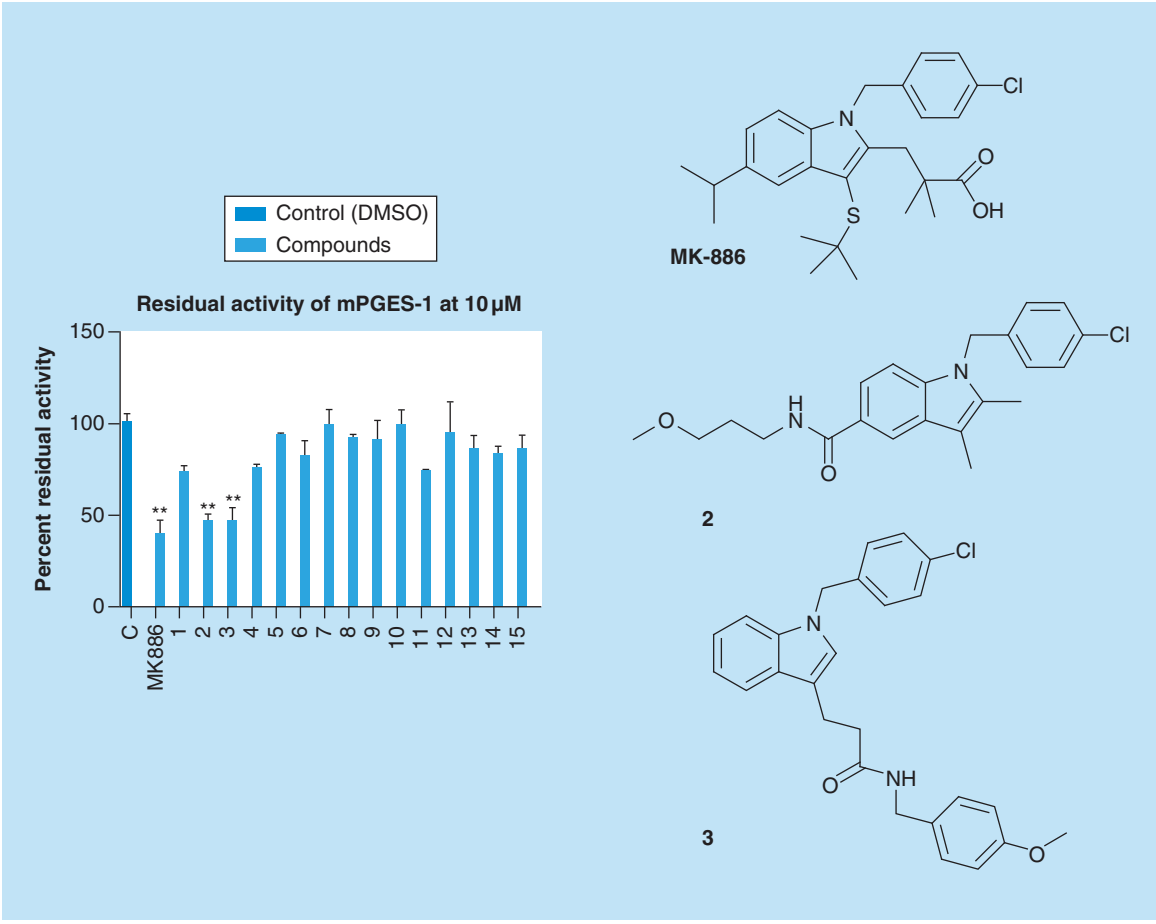

Figure 3. Inhibition of mPGES-1 activity. Percent activity of mPGES-1 in the presence of compounds 1–15 (10 μM) when compared with control (DMSO). The positive control is MK-886 (61.1 ± 8.2% inhibition at 2 μM). Compounds **2** and **3** inhibited mPGES-1 activity by 53.4 ± 4.0 and 53.9 ± 8.1%, respectively. Experiments were performed in duplicates.
**$p \leq 0.01$.

Results & discussion

Virtual screening of mPGES-1 inhibitors

Based on consensus scoring of the ZINC library to known mPGES-1 inhibitors using different types of fingerprints and physicochemical properties, 16,502 molecules which have a metric of four or more in this search space were selected. Docking of these molecules into the active site of human mPGES-1 resulted in 457 hits which had an energy of binding lower than that of PGH2. Further screening based on ADME properties resulted in 56 compounds. These were grouped into 26 clusters, which were seen to predominantly consist of indole cores decorated with different side chains. Fifteen compounds from these clusters were shortlisted, purchased and their activity against mPGES-1 was experimentally determined at 10 μM with cell-free mPGES-1 assay using the human recombinant mPGES-1.

The results of this test are displayed in Figure 3, expressed as percentage of the PGE2 formed from mPGES-1 with respect to the vehicle control (DMSO) – that is, the residual activity. MK886 was used as the positive control which showed 61.1 ± 8.2% inhibition at a concentration of 2 μM. Two compounds, **2** and **3** (Figure 3), showed 53.4 ± 4 and 53.9 ± 8.09% inhibition, respectively, at a concentration of 10 μM ($p \leq 0.01$).

Similarity in the active site entrances of mPGES-1 & LTC4S

Pawelzik *et al.* noticed that a dicarboxamide derivative, which inhibited human mPGES-1 with an IC_{50} of 58 nM, had no effect on rat mPGES-1 and subsequent mutagenesis studies showed that three residues (T131, L135 and A138 in human mPGES-1) located in the transmembrane helix 4 (TM4) were responsible for those differences

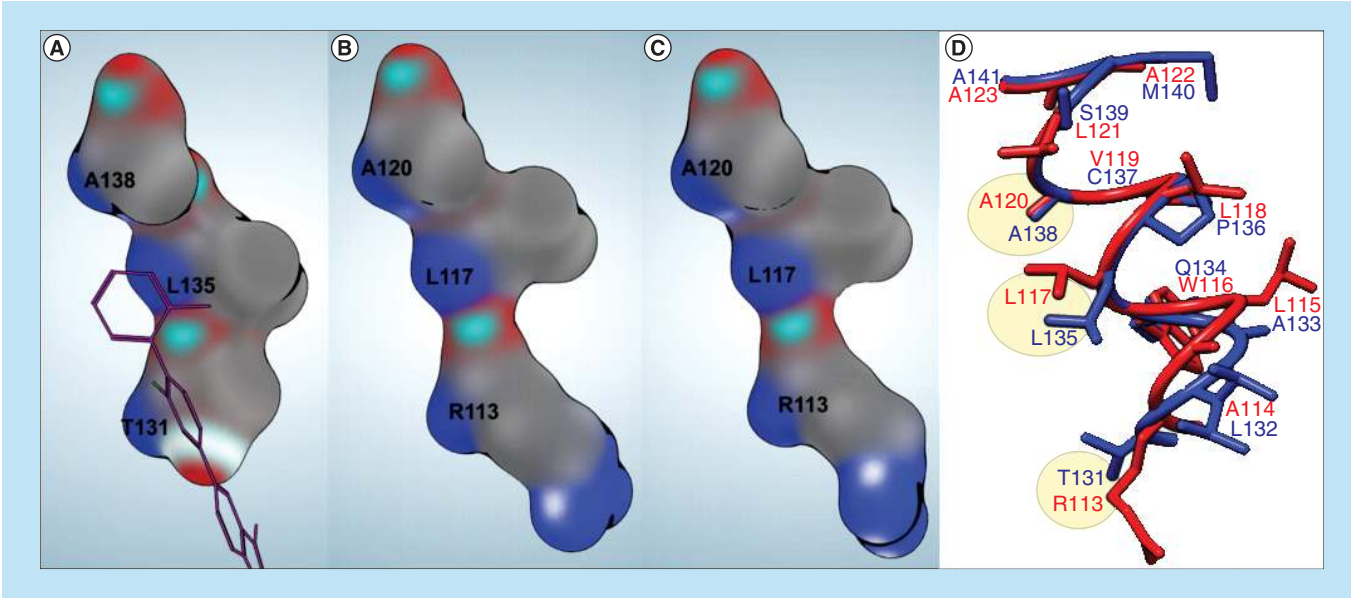

Figure 4. Similarity in active site entrances. (A) Human mPGES-1, **(B)** human LTC4S and **(C)** mouse LTC4S showing electrostatically positive (blue), negative (red) and neutral (green) regions. **(D)** Overlapping residues of human mPGES-1 (blue) and human LTC4S (red). It can be seen that the residues of mPGES-1, namely, T131, L135 and A138 correspond to the residues R113, L117 and A120 of LTC4S, having similar electrostatic distributions.

and they were replaced by bulkier aromatic residues, namely, V131, F135, F138, in rat [47]. These residues line the entrance to the substrate-binding site located at the crevice of TM4 and TM1 of the neighboring monomer, hence leading to a more sterically restricted entrance channel in rat. The importance of these three residues in binding of the inhibitor was reinforced by hydrogen/deuterium exchange mass spectroscopy studies on mPGES-1 and from the availability of crystal structures [17,48]. The biarylimidazole inhibitor in those cocrystals forms a hydrogen bond with T131 as well as hydrophobic interactions with the P124, S127, V128, T131, L132 and L135 side chains. The MK-886 derivative, a biarylindole, forms two bonds with T131 and has van der Waals interactions with Q134, L135 and A138.

When a Probis pairwise similarity search was conducted between human mPGES-1 and LTC4S enzymes, it was seen that the hydrophobic duo, L135 and A138 (Figure 4A & D) in the former aligned with L117 and A120 (Figure 4B & D) in the latter (with a Z-score of 1.24, a root mean square deviation [RMSD] of 0.6 and an E-value of 0.016), indicating a reasonably high degree of similarity between them. Since R113 in LTC4S was previously believed to be located at the entrance of the active site, the location of this residue was also determined in mPGES-1 and it was found to map to T131 [49]. An analysis of the mouse LTC4S (Figure 4C) showed a similar positioning of the three residues, viz, R113, L117 and A120. Previous studies had predicted, based on sequence similarity, that the residues Tyr-109, Arg-113 and Trp-116 in LTC4S corresponded to Thr-131, Leu-135 and Ala-138 in mPGES-1 [13,47]. Our method is based on the search for 3D structurally similar regions in the two, namely, residues which may have similar placements in the 3D protein structure despite being far apart in the sequence. Thus, the local similarity search method adopted in this study has helped us to identify similar architectures between these two proteins especially at the active site entrance, which may not have been apparent from sequence alignment studies.

Pharmacophore similarity among & between mPGES-1 & LTC4S inhibitors

Since our studies had found a similarity in the active site entrances of both mPGES-1 and LTC4S, we next performed a pharmacophore analysis of within and between the groups of mPGES-1 and LTC4S inhibitors. We selected two mPGES-1 inhibitors that have been proven to be interacting with the three crucial residues (T-131, L135 and A138) of mPGES-1. The first compound is a biarylindole (Figure 4A &Supplementary Figure 3A) and is shown to bind to the three residues of mPGES-1 (PDB ID:4YK5) [17]. The second is a dicarboxamide derivative (Supplementary Figure 3B) which has been shown, based on site-directed mutagenesis, to be binding

to the three residues at the active site entrance [47]. These two inhibitors were then used for elucidating a 4-point pharmacophore (Ph1; Supplementary Figure 4A). Ph1 comprises of an aromatic center (F1) and three hydrophobic centroid features (F2–4). From the crystal structure of the biarylindole inhibitor bound to mPGES-1, F1 and F2 were found to be located near the three residues lining the active site entrance of the mPGES-1.

For discriminating from other binding modes, conformational libraries were prepared with two inhibitors that are reported not to interact with the three residues of the active site entrance. The first inhibitor is a phenanthrene imidazole (Supplementary Figure 3C) that does not interact with the three residues in the cocyrstal (PDB ID:4YL0) [17]. The second one is a pyrazole-1-carbothioamide derivative (Supplementary Figure 3D) that has been shown to inhibit both human and rat mPGES-1, implying that it does not bind to the three residues [47]. Ph1 was then tested against the conformational library of these two compounds to ensure that it did not match these compounds which served as the negative control. We next performed a cross-comparison of the mPGES-1-derived pharmacophore, Ph1, with database of the conformational ensembles of all known LTC4S inhibitors to evaluate the potential of identifying compounds, which can inhibit both the enzymes simultaneously.

The pharmacophore search showed that 63 of the 100 known LTC4S inhibitors possessed the Ph1 pharmacophore. The complete list of the inhibitors having the pharmacophore is given in Supplementary Information. As already discussed, Ph1 has two features, an aromatic center F1 and a hydrophobic centroid F2, which interact with the three residues in the entrance of mPGES-1. Since the inhibitors of both the enzymes share the F1–F2 pharmacophoric features, it suggests the possibility of similar binding modes of the inhibitors to these two enzymes.

Since Ph1 is common to both the mPGES-1 inhibitors that bind at the active site entrance as well as several LTC4S inhibitors, the presence of this pharmacophore is checked on compounds shortlisted from virtual screening of the ZINC database toward mPGES-1 inhibition. It was found that compounds **2** and **3** which inhibited approximately 50% of mPGES-1 activity at 10 μM also possessed this pharmacophore (Supplementary Figure 4B & C). This indicates that the features identified in the Ph1 pharmacophore are important determinants of mPGES-1 inhibition as well as these features enable interaction with the three active site entrance residues. Pawelzik *et al.* showed that a pyrazole-1-carbothioamide derivative (Supplementary Figure 3D) that does not interact with the three residues of mPGES-1 still inhibits the enzyme with a tenfold less potency when compared with the dicarboxamide derivative (Supplementary Figure 3B), which interacts with these residues [47]. This independent reported study also corroborates with our current finding.

Conclusion

A strategy consisting of a ligand- and structure-based virtual screening approach, followed by experimental validation led to the identification of two novel mPGES-1 inhibitors with an indole scaffold. Our study also found similarity in the active site entrance between mPGES-1 and LTC4S, rendering them amenable to dual targeting. This was confirmed by an inhibitor-based pharmacophore analysis of both the targets, which established the presence of a four-point pharmacophore, present in majority of the known LTC4S inhibitors.

To our knowledge, this is the first study which has elucidated a pharmacophore common to several LTC4S inhibitors as well as features responsible for activity against both mPGES-1 and LTC4S. Targeting the active site entrance of these enzymes has an added advantage as inhibitors that target the highly hydrophobic substrate-binding site of mPGES-1 tend to be very lipophilic in nature, resulting in a strong albumin-binding feature and hence reducing their efficacy *in vivo* [48–50]. The findings open up the possibility of designing novel compounds, which will act as dual mPGES-1-LTC4S inhibitors. This would possibly prove to be more advantageous than designing a dual 5-LOX/COX inhibitor since mPGES-1 and LTC4S are downstream enzymes and hence the likelihood of undesired biological consequences leading to side effects is lessened.

The present study elucidated features which can aid in the design of novel scaffolds active against both enzymes. Its importance lies in the fact that experimental screening is a challenge because of the complexity of LTC4S assay. The current findings would help in the structure- and ligand-based screening of compounds for development of both LTC4S and mPGES-1-LTC4S dual inhibitors.

Future perspective

Drug development based on the dual inhibition concept has been shown to be an effective but underutilized strategy in inflammation. It is shown to result in drugs with higher therapeutic potential, but the challenge of targeting different enzymes simultaneously has been a major deterrent. Exploration of the features shared between the different drug targets of the inflammation pathway will aid in exploring this avenue. The advances in the

elucidation of crystal structure of proteins have greatly aided the development of new drugs and targeting strategies. The detailed studies on the inhibition of mPGES-1 and LTC4S by the two molecules identified in this study as well as their *in vivo* efficacy are currently underway. Further modifications of the molecule based on the experimental studies are expected to aid in identifying scaffolds, which can be developed as dual inhibitors of the arachidonic acid pathway in future.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: https://www.futuremedicine.com/doi/suppl/10.415/fmc-2017-0123

Author contributions

NS Devi performed the active site similarity and pharmacophore studies for the two enzymes. Structure-based virtual screening and in vitro enzyme assay was carried out by P Paragi-Vedanthi. A Bender performed the ligand-based virtual screening. Analysis of results and manuscript preparation was carried out by M Doble in conjunction with the other authors.

Acknowledgements

The authors thank the High Performance Computing Environment (HPCE), IIT Madras for making available the computing resources to carry out high-throughput structure-based virtual screening.

Financial & competing interests disclosure

NS Devi received a student fellowship (09/084[0481]2009-EMR-I) from the Council of Scientific and Industrial Research (CSIR) India. P Paragi-Vedanthi acknowledges WOS-A from Department of Science and Technology (DST), India for fellowship (SR/WOS-A/LS- 34/2006). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

Background

- Single target anti-inflammatory drugs can cause undesirable side effects by shunting the substrate.
- Network models have shown that multitargeting can aid in the development of more efficient drugs.

Current findings

- Virtual screening identified compounds likely to inhibit mPGES-1 enzyme.
- Compounds **2** and **3** showed approximately 50% inhibition of mPGES-1 at 10 μM *in vitro*.
- 3D similarity showed that LTC4S and mPGES-1 shared common residues binding to inhibitors.
- Pharmacophore was derived from reported mPGES-1 inhibitors binding to these residues.
- Pharmacophore mapped onto several known LTC4S inhibitors as well as to compounds **2** and **3**.

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