ACCOUNTS of chemical research

Article

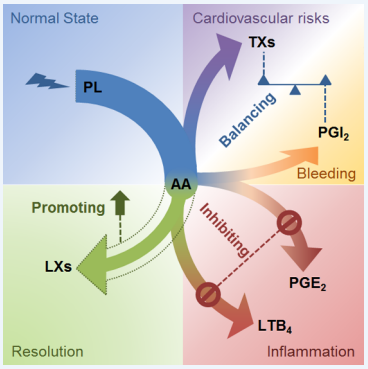
pubs.acs.org/accounts

Diverse Ways of Perturbing the Human Arachidonic Acid Metabolic Network To Control Inflammation

Hu Meng,[†] Ying Liu,*[,†,‡] and Luhua Lai*[,†,‡,§]

[†]BNLMS, State Key Laboratory for Structural Chemistry of Unstable and Stable Species, College of Chemistry and Molecular Engineering, [‡]Center for Quantitative Biology, and [§]Peking−Tsinghua Center for Life Sciences, Peking University, Beijing 100871, China

CONSPECTUS: Inflammation and other common disorders including diabetes, cardiovascular disease, and cancer are often the result of several molecular abnormalities and are not likely to be resolved by a traditional single-target drug discovery approach. Though inflammation is a normal bodily reaction, uncontrolled and misdirected inflammation can cause inflammatory diseases such as rheumatoid arthritis and asthma. Nonsteroidal anti-inflammatory drugs including aspirin, ibuprofen, naproxen, or celecoxib are commonly used to relieve aches and pains, but often these drugs have undesirable and sometimes even fatal side effects. To facilitate safer and more effective anti-inflammatory drug discovery, a balanced treatment strategy should be developed at the biological network level.



In this Account, we focus on our recent progress in modeling the inflammation-related arachidonic acid (AA) metabolic network and subsequent multiple drug design. We first constructed a mathematical model of inflammation based on experimental data and then applied the model to simulate the effects of commonly used anti-inflammatory drugs. Our results indicated that the model correctly reproduced the established bleeding and cardiovascular side effects. Multitarget optimal intervention (MTOI), a Monte Carlo simulated annealing based computational scheme, was then developed to identify key targets and optimal solutions for controlling inflammation. A number of optimal multitarget strategies were discovered that were both effective and safe and had minimal associated side effects. Experimental studies were performed to evaluate these multitarget control solutions further using different combinations of inhibitors to perturb the network. Consequently, simultaneous control of cyclooxygenase-1 and -2 and leukotriene A_4 hydrolase, as well as 5-lipoxygenase and prostaglandin E_2 synthase were found to be among the best solutions.

A single compound that can bind multiple targets presents advantages including low risk of drug−drug interactions and robustness regarding concentration fluctuations. Thus, we developed strategies for multiple-target drug design and successfully discovered several series of multiple-target inhibitors. Optimal solutions for a disease network often involve mild but simultaneous interventions of multiple targets, which is in accord with the philosophy of traditional Chinese medicine (TCM). To this end, our AA network model can aptly explain TCM anti-inflammatory herbs and formulas at the molecular level. We also aimed to identify activators for several enzymes that appeared to have increased activity based on MTOI outcomes. Strategies were then developed to predict potential allosteric sites and to discover enzyme activators based on our hypothesis that combined treatment with the projected activators and inhibitors could balance different AA network pathways, control inflammation, and reduce associated adverse effects.

Our work demonstrates that the integration of network modeling and drug discovery can provide novel solutions for disease control, which also calls for new developments in drug design concepts and methodologies. With the rapid accumulation of quantitative data and knowledge of the molecular networks of disease, we can expect an increase in the development and use of quantitative disease models to facilitate efficient and safe drug discovery.

1. INTRODUCTION

The arachidonic acid (AA) metabolic network produces a large family of inflammatory mediators, including leukotrienes (LTs) and prostaglandins (PGs),[1] which contribute to numerous inflammatory-related diseases such as asthma, rheumatoid arthritis, atherosclerosis, Alzheimer's disease, and cancer.[2,3] Leukotriene B_4 (LTB_4) and prostaglandin E_2 (PGE_2), which are synthesized via two major pathways involving 5-lipoxygenase (5-LOX) and cyclooxygenase (COX), are the major mediators and play crucial roles in the inflammatory response (Figure 1).[1] Other metabolites of the 5-LOX pathway, such as leukotriene C_4 (LTC_4), leukotriene D_4 (LTD_4), and leukotriene E_4 (LTE_4), are slow-acting substances of anaphylaxis.[4] Hence, enzymes involved in LT and PG biosynthesis, such as 5-LOX, 5-LOX activating protein (FLAP), COX, phospholipase A2 (PLA2), LTC_4 synthase (LTCS), leukotriene A_4 hydrolase (LTA4H), and microsomal prostaglandin E synthase-1 (mPGES-1), are key targets for anti-inflammatory drug discovery.[1]

Received: April 22, 2015
Published: August 3, 2015

DOI: 10.1021/acs.accounts.5b00226
Acc. Chem. Res. 2015, 48, 2242−2250

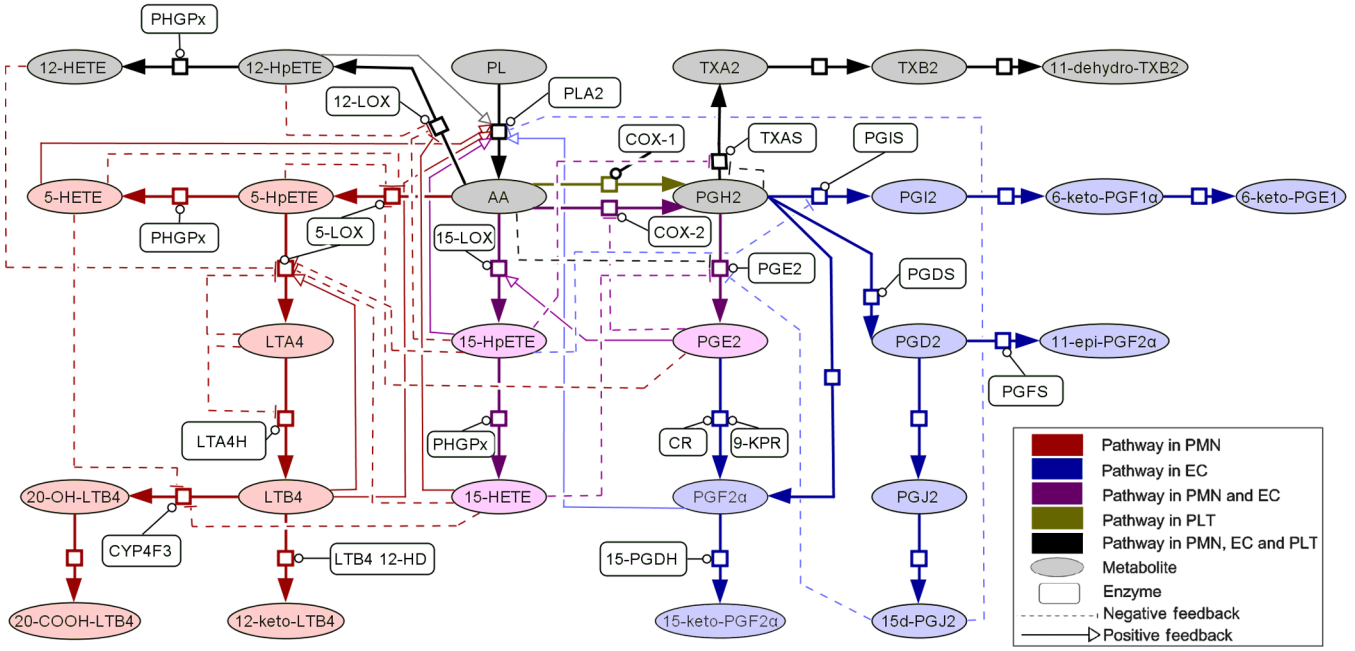


Figure 1. Metabolic network of AA in human PMNs, ECs, and PLTs.

Table 1. Efficacy Simulation of Common NSAIDs in the AA Network[a]

inhibitor	relative IC_{50}	inhibition of PGE_2 (%)	inhibition of LTB_4 (%)	$[PGI_2]/[TXA_2]$
control				0.6817
aspirin	IC_{50}(COX-1/COX-2) = 0.01	90.0		5.1947
ibuprofen	IC_{50}(COX-1/COX-2) = 0.5	90.0		3.5599
naproxen	IC_{50}(COX-1/COX-2) = 0.7	90.0		2.2915
6-MNA	IC_{50}(COX-1/COX-2) = 1.5	90.0		2.2334
acetaminophen	IC_{50}(COX-1/COX-2) = 1.6	90.0		2.1574
indomethacin	IC_{50}(COX-1/COX-2) = 1.9	90.0		1.9613
meloxicam	IC_{50}(COX-1/COX-2) = 18	90.0		0.5335
nimesulide	IC_{50}(COX-1/COX-2) = 19	90.0		0.5207
diclofenac	IC_{50}(COX-1/COX-2) = 29	90.0		0.4399
celecoxib	IC_{50}(COX-1/COX-2) = 30	90.0		0.4347
rofecoxib (Vioxx)	IC_{50}(COX-1/COX-2) = 267	90.0		0.2804
licofelone	IC_{50}(5-LOX) = 0.18 μM IC_{50}(COX-2) = 0.21 μM IC_{50}(COX-1) = 1 μM	96.5	90.0	0.6983

[a]This table was rebuilt from ref 19.

Nonsteroidal anti-inflammatory drugs (NSAIDs), which block the formation of PGs by inhibiting the COX enzymes, have been widely used for many years to treat acute and chronic inflammation. Traditional NSAIDs, such as aspirin, ibuprofen, and indomethacin cause side effects including gastrointestinal toxicity and mild bleeding by inhibiting COX-1.[5] The discovery of COX-2 stimulated hope that selective COX-2 inhibitors could reduce gastrointestinal toxicity. Accordingly, several selective COX-2 inhibitors were developed and approved for clinical use, including celecoxib[6] and rofecoxib.[7] However, cardiovascular side effects were reported following clinical trials[8] of selective COX-2 inhibitors, and Vioxx (rofecoxib) was withdrawn from the market in 2004.[9] Currently, the only approved[10] 5-LOX inhibitor is zileuton (trade name Zyflo). Inhibitors of LTA4H,[11] LTCS,[12] and PGES[13] have also been developed.

Selective COX-2 inhibitors further demonstrated the importance of understanding the molecular mechanisms of disease. Complex inflammatory diseases present significant challenges for drug discovery and are difficult to treat effectively by targeting a single site because of divergent or redundant structures of the underlying disease networks.[14] Therefore, we developed a systems biology approach to study AA metabolic network dynamics, elucidate optimal intervention solutions, and enable the design of compounds that can effectively shift the AA metabolic network from the disease state to the normal state.

2. NETWORK MODELING OF AA METABOLISM

Complex diseases such as inflammation cannot be effectively controlled by selective single-target drugs because diverse arrays of molecules are involved in the development of disease. To overcome the limitations of single-target drugs and to achieve a better understanding of disease development as well as an accurate evaluation of drug efficacy and toxicity, cross-talk between biological responses should be studied.[15,16]

DOI: 10.1021/acs.accounts.5b00226
Acc. Chem. Res. 2015, 48, 2242−2250

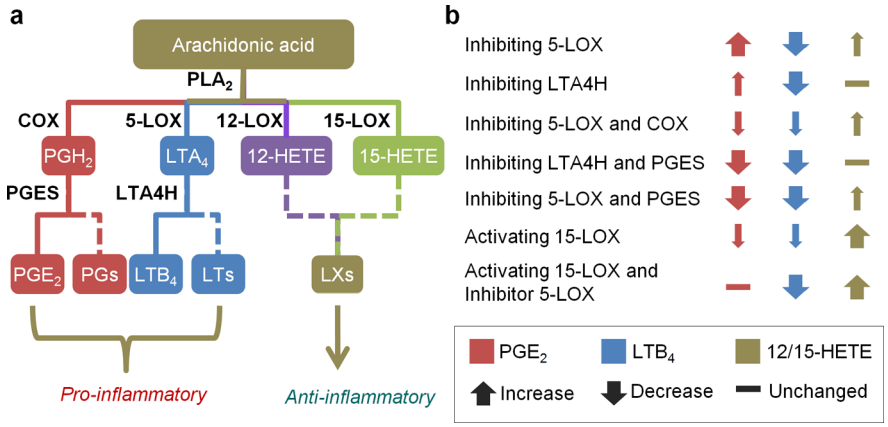


Figure 2. Outline of the influence of different perturbation strategies on the AA network: (a) simplified model of AA network; (b) effectiveness of different intervention strategies.

2.1. Network Model of AA Metabolism

To provide a network-wide analysis for the design of safe anti-inflammatory drugs, we constructed a computational model of the AA metabolic network in human polymorphonuclear leukocytes (PMNs) to analyze flux changes following drug treatment[17] based on the Kyoto Encyclopedia of Genes and Genomes (KEGG)[18] and a survey of available literature.[17] Ordinary differential equations (ODEs) were used to describe enzymatic reactions and feedback inhibition or activation of metabolites. This computational network model accurately predicted flux changes following treatment with exogenous AA, a 5-LOX inhibitor, or a COX inhibitor. Further, network simulations and experiments revealed that treatment with a 5-LOX inhibitor induced peak flux after initiating metabolism in both the COX-2 and the 15-lipoxygenase (15-LOX) pathways. The COX-2 and 5-LOX pathways were blocked when both COX-2 and 5-LOX inhibitors were used, and the flux was mainly through the 15-LOX pathway.

To simulate AA metabolism in human blood vessels, we needed to create a more realistic model. After conducting a literature search, we decided to build a model of human blood vessels that incorporated three cell types including PMNs, platelets (PLTs), and endothelial cells (ECs; Figure 1).[19] We then used the model to predict the potential side effects of common NSAIDs. It is known that prostacyclin (PGI_2) inhibits PLT aggregation and relaxes smooth muscle[20] and that thromboxane A_2 (TXA_2) is a potent inducer of PLT aggregation and vasoconstriction.[21] Under normal conditions, the ratio of PGI_2/TXA_2 is nearly constant. According to our model, the ratio of PGI_2/TXA_2 was 0.68. However, if the PGI_2/TXA_2 ratio exceeds the normal level after NSAID administration, the side effect of bleeding might occur. Conversely, if the ratio of PGI_2/TXA_2 decreases significantly, the risk of cardiovascular side effects will increase. Our model demonstrated the capability to reproduce the known side effects and strengths of 11 common NSAIDs (Table 1).

2.2. Algorithms for Key Target Identification and Optimum Intervention

We established a computational multitarget optimal intervention (MTOI) method to identify key targets and to search for all possible intervention solutions in the biological network.[19] The fundamental concept is that we can define different states of the network, including normal and disease state. MTOI uses a Monte Carlo simulated annealing algorithm to search for all possible solutions that perturb the network to drive the disease state back to the normal state. The MTOI algorithm can also be used to obtain unknown parameters by computational fitting to experimental data. We also developed differential simulated annealing (DSA), a novel global optimization algorithm, for robustly and efficiently estimating the kinetic parameters for biological network models.[22]

2.3. Key Target and Optimum Intervention Analysis of the AA Network

MTOI succeeded in identifying all established anti-inflammatory drug targets, including PLA2, COX-2, 5-LOX, PGES, and LTA4H. In addition, MTOI identified several enzymes for which increased activity is desired for an anti-inflammatory treatment, including 15-LOX and leukotriene-B_4 ω-hydroxylase 2.

MTOI was then used to identify optimal solutions for an AA network intervention. To achieve the goal of reducing inflammatory mediator production while simultaneously maintaining a constant PGI_2/TXA_2 ratio, all of the solutions involved multitarget interventions. For example, the combination of PGES and LTA4H and the combination of COX-2/1 and LTA4H are two promising solutions involving only two targets. However, these inhibitor combinations can only be used in the proper dosing ranges. The range of the valid ratio of drug plasma concentration to inhibition constant ($[I]/K_i$) for LTA4H in the PGES and LTA4H solution is quite narrow, and the ratio of inhibition for COX-1/COX-2 must be maintained in a limited range in the COX-2/1 and LTA4H solution. The dosing ranges are greater, however, when more targets are inhibited, such as the combination of PLA2, 5-LOX, and COX-2/1 or the combination of PLA2, COX-2/1, and LTA4H.

Experimental studies were conducted to investigate the optimal multitarget intervention solutions provided by MTOI. A quantitative study was performed using an LC-MS/MS method to evaluate eicosanoid AA network metabolism responses in calcium ionophore stimulated Sprague–Dawley rat blood samples.[23] As well, selective inhibitors of 5-LOX, COX, LTA4H, PGES, and their binary combinations have been studied for their influences on the AA metabolism dynamics.

Initially, reported selective inhibitors of 5-LOX, LTA4H, COX, and PGES were used to perturb the system, and the modulatory effects of single-target inhibitors on the AA network were examined. Inhibiting 5-LOX or COX caused a switch in AA metabolism to the other respective pathway

DOI: 10.1021/acs.accounts.5b00226
Acc. Chem. Res. 2015, 48, 2242–2250

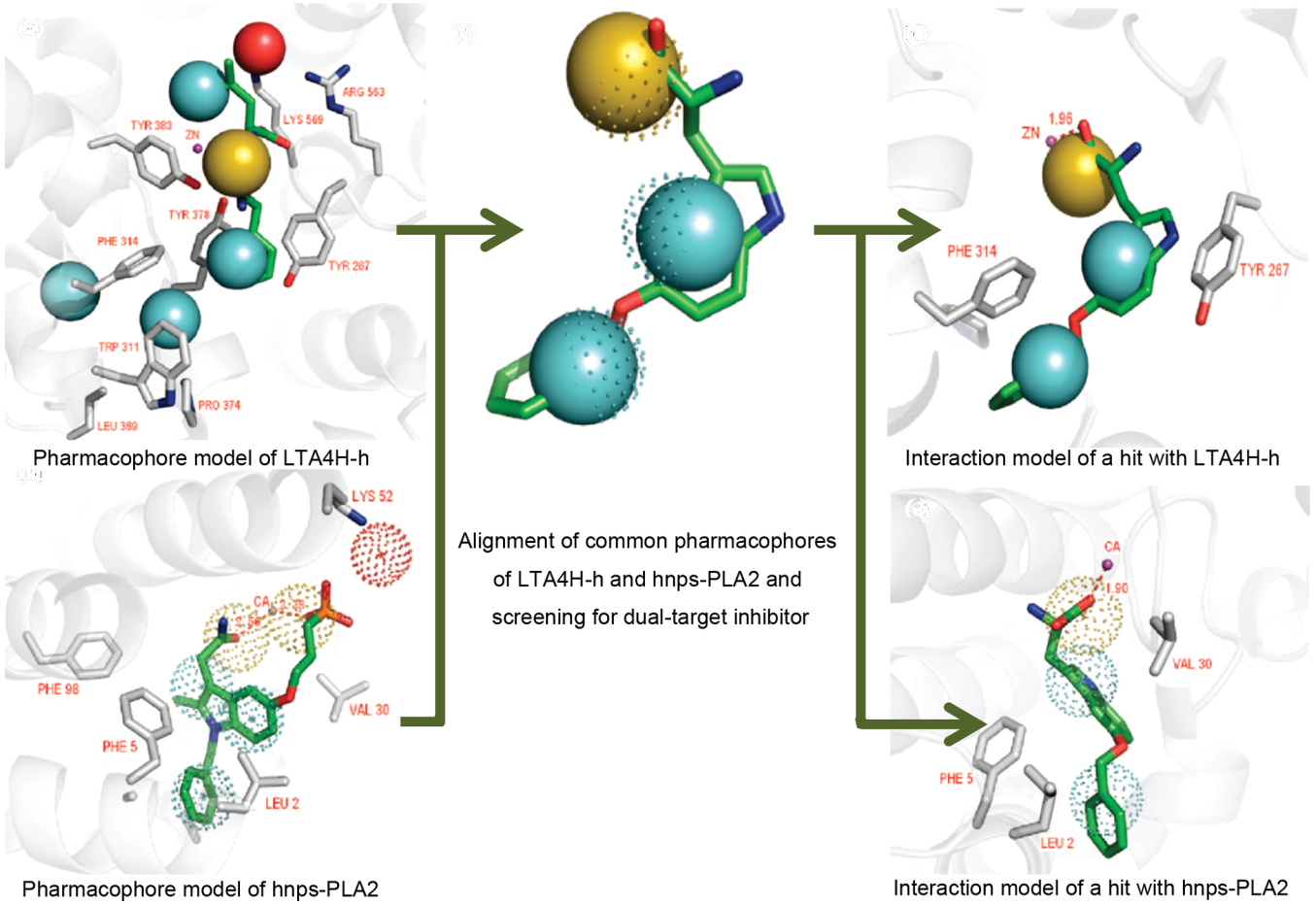


Figure 3. General procedure for the common pharmacophores method.

(Figure 2). These results indicate that when single-target therapies are intended for use as anti-inflammatory treatments, downstream enzymes, including LTA4H and PGES, are better targets compared with upstream enzymes, such as 5-LOX and COX.[11,13]

When inhibitors of the upstream enzymes 5-LOX and COX were used simultaneously, a higher inhibitor concentration was needed to achieve the same reduction in LTB_4 or PGE_2 production. In the current study, however, when one of the binary targets was a downstream enzyme or when both downstream enzymes were inhibited, the 5-LOX and COX pathways had an alternative subpathway just above the point of inhibition, and no obvious redistributing to the other pathway was observed. Moreover, the combination of 5-LOX/PGES or LTA4H/COX can augment the 12/15-LOX pathway, products of which can be further metabolized into endogenous anti-inflammatories (Figure 2). This phenomenon implies that downstream enzymes or combinations with at least one downstream enzyme are better targets than upstream enzymes.[24−26] In fact, licofelone (ML3000), an inhibitor of FLAP, COX, and mPGES-1, has already entered phase III clinical trials.[27,28]

Of course, the AA metabolic network is complicated, and further studies are necessary to discover better intervention solutions. Our model also needs modification before application in other cell types or tissues. Further, full comprehension of the effectiveness of enzymes with more than one function in the network cannot be achieved by analyzing only flux changes. For example, in addition to generating the inflammatory mediator LTB_4, LTA4H also possesses aminopeptidase activity. In 2010, Snelgrove et al. identified the neutrophil chemoattractant Pro-Gly-Pro (PGP) as the physiological substrate of LTA4H.[29] In acute neutrophil driven inflammation, PGP was degraded by LTA4H, which facilitated the resolution of inflammation. Therefore, designing a compound against LTA4H that activates the aminopeptidase activity or selectively inhibits the hydrolase activity or both could be useful for treating pulmonary inflammation. We have found a series of diphenyl ether derivatives that can enhance the aminopeptidase activity of LTA4H without affecting LTB4 formation.[30,31] Shim, Paige, and their co-workers further developed these compounds into 4-methoxydiphenylmethane, a more stable bioavailable molecule that augments the aminopeptidase activity of LTA4H.[32] Preclinical evaluation of this compound revealed protection against intranasal elastase-induced pulmonary emphysema in murine models. Additionally, 5-LOX plays multiple roles in the AA network and is involved in the biosynthesis of anti-inflammatory mediators, including lipoxins (LXs) and other proresolving mediators that are generated from eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[33] Consequently, a LTA4H/COX intervention may provide a better therapeutic solution than interference using 5-LOX/PGES inhibitors.

DOI: 10.1021/acs.accounts.5b00226
Acc. Chem. Res. 2015, 48, 2242−2250

3. MULTIPLE TARGET DRUG DESIGN FOR AA NETWORK INTERVENTION

Three possible pharmacological approaches are available to control multiple targets in a disease network, including drug cocktails, multicomponent drugs, and multiple ligands.[34] Among these strategies, the development of designed multiple ligands (DMLs) has attracted significant attention[35] because DMLs decrease drug−drug interaction risks and may be more robust regarding fluctuations in drug concentration.[17] Several multitarget agents against inflammation have been discovered, especially COX/5-LOX inhibitors, and exhibited significant increases in efficacy both *in vitro* and *in vivo*.[36] However, designing efficient multitarget inhibitors is challenging because interactions with several targets must be considered simultaneously. Hence, we have used four different strategies for the discovery of multitarget inhibitors for AA network intervention.

3.1. Common Pharmacophore Screen

The pharmacophore, which is a group of structural molecular features recognized at binding sites that are responsible for the biological activity of molecules, is widely used in medicinal chemistry. We hypothesized that if several protein targets could bind to the same molecule, all of the binding sites should theoretically share a number of common features. Based on this assumption, a virtual screen can be performed to identify molecules that may bind to these targets after deriving the common pharmacophore.[37] The general procedure for this method (Figure 3) included (a) generating pharmacophore models for each protein using Pocket v.2,[38] (b) identifying the common pharmacophores by comparing the pharmacophore models of multiple proteins, (c) selecting the molecules whose binding conformations accommodated the common pharmacophores, (d) analyzing the binding conformations of the selected compounds using a more rigorous docking approach, and (e) experimentally testing whether the candidates could inhibit the activities of the target proteins.

Dual-target inhibitors against human nonpancreatic secretory PLA2 (hnps-PLA2) and human LTA4H (LTA4H-h) were discovered using the common pharmacophores strategy,[26] and one of the compounds, 1, 2-amino-3-[5-(benzyloxy)-1*H*-indol-3-yl]propanoic acid (JMC08-4), was selected for further optimization. The most potent compound inhibited hnps-PLA2 and LTA4H-h with IC_{50} values of 9.2 ± 0.5 μM and 2.4 ± 1.4 μM, respectively.[39]

3.2. Ligand Merging

For systems with known ligands, multitarget ligands can also be designed by merging the chemical structures,[34] which we employed to devise one series of COX and LTA4H dual inhibitors.[26] We started with 1-(2-(4-phenoxyphenoxy)ethyl)-pyrrolidine, an LTA4H inhibitor reported by Penning et al.,[40] and nimesulide, a COX-2 selective NSAID used clinically, which are compounds that share the same phenoxyphenyl scaffold. Subsequently, a series of 1-(2-(4-phenoxyphenoxy)-ethyl)pyrrolidine derivatives were synthesized and tested. The most potent compound inhibited PGE_2 and LTB_4 production in a human whole blood assay with IC_{50} values of 5.0 and 0.73 μM, respectively (Figure 4).

3.3. Iterative Fragment-Growing and *de Novo* Multitarget Drug Design

We developed a *de novo* multitarget drug design method with an iterative fragment-growing strategy and used it to design a series of dual-target inhibitors for COX and LTA4H.[41] *De novo* design was performed using LigBuilder 3, the third generation of the *de novo* drug design program LigBuilder.[42,43] Fragments from known ligands of single targets were extracted and used as initial structures for further growth using LigBuilder 3. After molecule growth and activity testing, novel dual-target inhibitors for COX-2 and LTA4H were obtained. The most potent compound inhibited PGE_2 and LTB_4 production in a human whole blood assay with IC_{50} values of 7.0 and 7.1 μM, respectively (Figure 5). This method is suitable for designing highly integrated inhibitors.

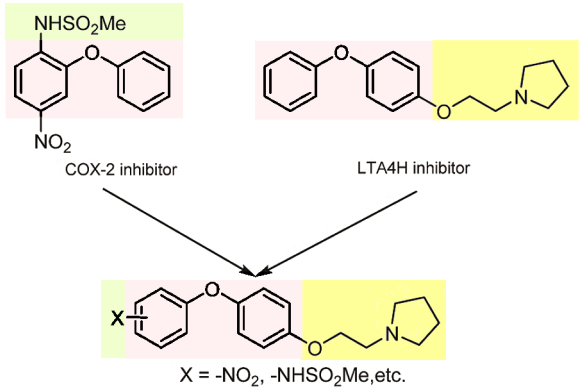


Figure 4. Design strategy of ligand merging for COX-2/LTA4H dual inhibitors.

3.4. Sequential Docking

Sequential docking of compounds to different enzymes is a straightforward method used to identify multitarget inhibitors.[44] Several 5-LOX/mPGES-1 dual inhibitors, such as arylpyrrolizines derivatives,[24] pirinixic acid derivatives,[25] and mercaptohexanoic acid derivatives,[45] have been discovered and were efficient in reducing PGE_2 and LTB_4 *in vivo*. Accordingly, we utilized sequential docking to identify a series of 5-LOX/mPGES-1 dual functional inhibitors.[46] A comparative model for 5-LOX was first constructed based on the closed conformation of 15-LOX. A series of novel 5-LOX inhibitors were identified, of which two appeared to be potential dual-functional inhibitors of 5-LOX and mPGES-1. One of these inhibitors was 6-nitro-3-(*m*-tolylamino)benzo[*d*]isothiazole-1,1-dioxide (JMC-7), which had corresponding IC_{50} values of 1.9 μM for 5-LOX and 6.7 μM for mPGES-1. To identify key interactions between JMC-7 and mPGES-1, we docked the compound to the substrate binding sites of mPGES-1 using the active conformation of mPGES-1 we had previously constructed.[47] A series of JMC-7 analogues were synthesized and evaluated. The most potent inhibitor had approximately 3-folder higher activity than JMC-7 to both 5-LOX (IC_{50} = 0.6 μM) and mPGES-1 (IC_{50} = 2.1 μM)[48] (Figure 6).

4. USING THE AA METABOLIC NETWORK MODEL TO UNDERSTAND TRADITIONAL CHINESE MEDICINE

We demonstrated by MTOI analysis that optimal solutions for a disease network often involve mild but simultaneous interventions of multiple targets. Our findings remind us of traditional Chinese medicine (TCM), which uses a mixture of several herbs containing a cocktail of many natural compounds. Thus, we used the AA metabolic network model in combination with molecular docking to understand the anti-inflammatory functions of TCM herbs and formulations.[49]

DOI: 10.1021/acs.accounts.5b00226
Acc. Chem. Res. 2015, 48, 2242−2250

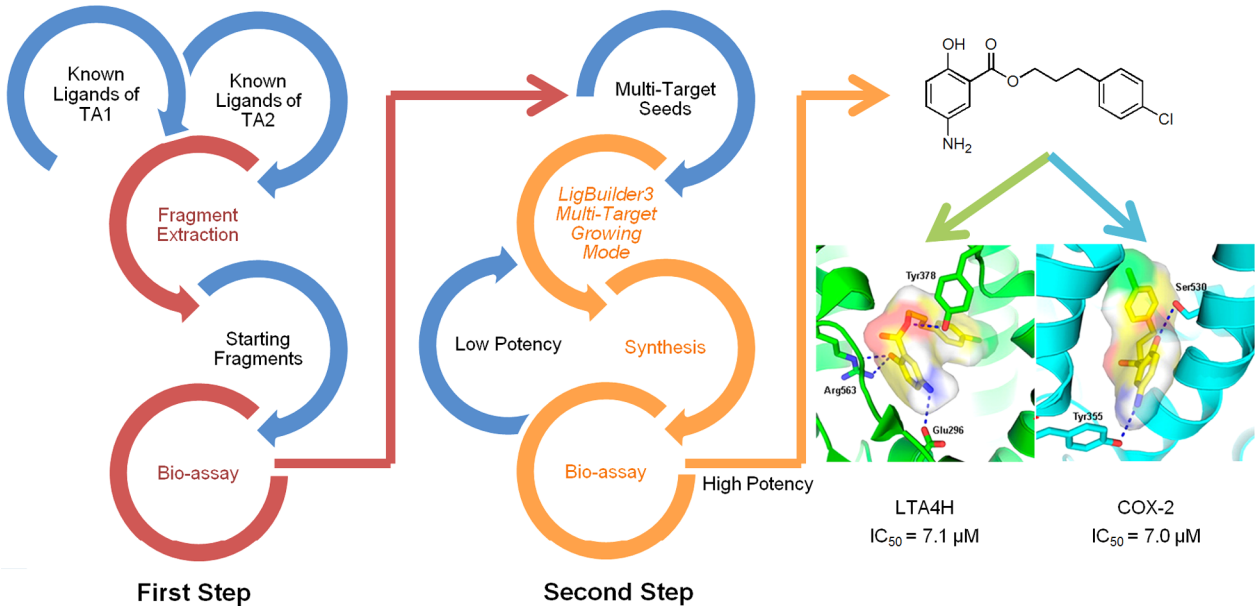


Figure 5. Flowchart of *de novo* multitarget ligand design method.

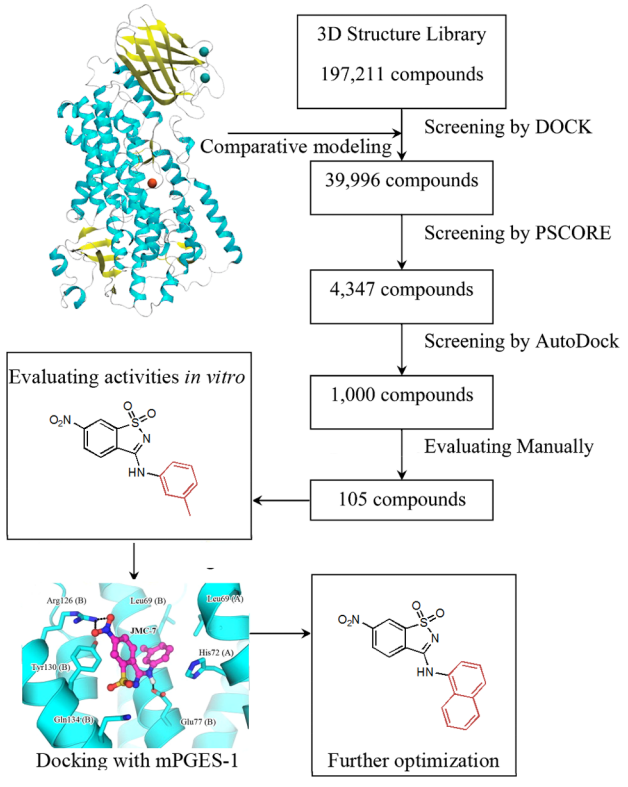


Figure 6. Flowchart of the sequential docking of compounds to 5-LOX and mPGES-1.

Twenty-eight commonly used TCM herbs related to "heat-clearing" or "syndrome-relieving", which are often associated with inflammation, were selected for analysis. The chemical components of the selected TCM plants were from The Traditional Chinese Medicine Database.[50] First, an overall docking of these molecules to drug targets in the AA metabolic network, including COX-1, COX-2, LTA4H, 5-LOX, and mPGES-1, was conducted to identify the targets of each herb. The inhibitory effect of each herb was modeled as the sum of its inclusive molecules. All of the inhibition constants could be deduced from docking scores. Using these estimated parameters, we then conducted dynamic network simulations to calculate the efficacy and side effects of the herbs and three herbal formulations. Most of the herbs significantly reduced LTB_4 production but not PGE_2 production in our computational model, which differed from the reductions in PGE_2 production observed with most NSAIDs. A likely explanation is that the herbs have historically been chosen to treat syndromes such as a cough or asthma, which are often related to elevated LTB_4 levels. Further, the predicted ratio of $[PGI_2]/[TXA_2]$ ranged from 0.44 to 1.49, which was near or slightly higher than the normal value (0.68). Consequently, the side effects of these herbs are predicted to be mild.

The docking results demonstrated that distinct ingredients of a formula tend to inhibit different targets. Therefore, the combination of ingredients could cover virtually the whole network and collectively achieve a superior therapeutic effect. The other advantage of the formulations is that a much lower dosage can be used to achieve the goal of simultaneous inhibition of both the PGE_2 and LTB_4 pathways compared with the use of a single herb. This method provides a new technique to elucidate the regulatory functions of TCM and offers a computational approach to evaluate TCM from a systems biology perspective. Moreover, new TCM combinations or formulas can also be predicted using this method. To this end, we have used a similar approach to understand anti-influenza TCM formulations.[51]

5. UP-REGULATING ACTIVITIES OF LIPOXIN-PRODUCING ENZYMES

It was reported several years ago that an alternative means of inflammation control is to promote the endogenous resolution of inflammation. In the 1980s, Serhan et al. discovered a series of anti-inflammatory mediators formed from AA in human leukocytes called lipoxins (LXs).[52] These endogenous molecules demonstrate the capacity to promote the resolution of inflammation as well as the return to tissue homeostasis. Hence, introducing these proresolving molecules may activate the

DOI: 10.1021/acs.accounts.5b00226
Acc. Chem. Res. 2015, 48, 2242−2250

body's natural pathways for curbing inflammation and might be safer than current anti-inflammatory therapeutics.[53] However, because LXs are unstable and difficult to synthesize chemically, direct application of LXs for inflammation control requires further development. An alternative is to increase the activity of the key enzymes involved in the generation of anti-inflammatory mediators to achieve therapeutic benefits. In order to accomplish this, however, methodologies for enzyme activator discovery must be developed.

The design of activators requires information regarding allosteric sites and knowledge of the mechanisms of activation. To achieve reasonable modulation of protein activity through allosteric sites, predictive methods are needed. Accordingly, we have developed an approach to identify allosteric sites in proteins based on the coarse-grained two-state Gō model and successfully discovered novel allosteric inhibitors of *Escherichia coli* phosphoglycerate dehydrogenase.[54]

We recently used a protein dynamics-based allosteric-site prediction approach[55] together with a pocket-finding method[56] to predict potential allosteric sites in 15-LOX, which is the key enzyme that produces endogenous anti-inflammatory effectors, including LXs[57] and 15-hydroxyeicosatetraenoic acid (15-HETE).[58] Novel allosteric sites in 15-LOX were predicted and successfully used in virtual screening to identify both activators and inhibitors. The most potent activator increased 15-LOX product levels and reduced production of pro-inflammatory mediators in human PMNs, human whole blood, and a mouse model of peritonitis. In addition, combined treatment with the 15-LOX activator and inhibitors of 5-LOX or COX balanced different pathways in the AA network, which provided a novel strategy for blocking inflammation with fewer adverse effects (Figure 2). This work is in submission.[59]

6. CONCLUSION AND PERSPECTIVE

Traditional, selective single-target drugs cannot effectively control complex diseases including cancer, diabetes, and inflammation. Hence, efforts to discover drugs to treat these diseases should involve multitarget interventions from a systems biology perspective.[60] We utilized the inflammation-related AA network as a model system to demonstrate how to understand the molecular mechanisms of a complex disease at the network level and how the network model can be used to identify key drug design targets and optimal intervention solutions. Much has been learned from our AA metabolic network study, including the realization that a network-wide analysis provides useful information for understanding related diseases and interventions and that controlling multiple targets is typically more efficient than controlling a single target. We also learned from TCM that mild and balanced interventions can aid in avoiding potential side effects and that more attention should be paid to protein activation, because activation is equal in importance to inhibition. Finally, we concluded that structure-based drug design and experimental strategies should be updated to reflect the concept of multiple-target mild control.

In this Account, we primarily introduced different strategies that we used to analyze the AA network and to design effective inhibitors or activators to treat inflammation. Many other groups have made important contributions to multitarget inhibitor development and other strategies for superior anti-inflammatory effects with reduced health risks.[25,27,61−66] Some dual-target inhibitors have already entered clinical trials, including azelastine,[66] tepoxaline,[67] and licofelone.[27] Consequently, we believe following systems biology guidelines will enable the discovery of drugs that are safer and more effective than currently available therapies.

AUTHOR INFORMATION

Corresponding Authors

*Luhua Lai: e-mail, lhlai@pku.edu.cn; phone, +86-010-62757486.

*Ying Liu: e-mail, liuying@pku.edu.cn; phone, +86-010-62751490.

Author Contributions

All authors contributed to writing this article.

Funding

This work was supported by the National Natural Science Foundation of China (Grants 91313302 and 21173013) and the Ministry of Science and Technology of China (Grants 2015CB910300 and 2012AA020308).

Notes

The authors declare no competing financial interest.

Biographies

Hu Meng received his B.S. (Chemistry) in 2009 and his Ph.D. (Physical Chemistry) in early 2015 from Peking University. His dissertation research focused on the discovery of allosteric regulators and drug design targeting key enzymes in the arachidonic acid network.

Ying Liu received her B.S. (Chemistry) and M.S. (Organic Chemistry) from Lanzhou University and her Ph.D. from Nankai University in 1999. Currently, she is an associate professor at the Peking University College of Chemistry and Molecular Engineering. Her research focuses on structure and biological network-based drug design and synthesis of novel organic molecules as probes to modulate biological systems.

Luhua Lai received her B.S. (Chemistry) in 1984 and her Ph.D. (Physical Chemistry) in 1989 from Peking University. She became a full professor at the Peking University College of Chemistry and Molecular Engineering in 1992 and a Changjiang Chair professor in 2001. Her research interests include structure and systems-based drug design, protein−protein interactions, and functional protein design, as well as molecular mechanisms involved in biological events.

ACKNOWLEDGMENTS

The authors thank all past and current members of the Lai group for their contributions to the research mentioned in this article.

REFERENCES

(1) Khanapure, S. P.; Garvey, D. S.; Janero, D. R.; Letts, L. G. Eicosanoids in inflammation: Biosynthesis, pharmacology, and therapeutic frontiers. *Curr. Top. Med. Chem.* **2007**, *7*, 311−340.

(2) Davies, P.; Bailey, P. J.; Goldenberg, M. M.; Fordhutchinson, A. W. The Role of Arachidonic-Acid Oxygenation Products in Pain and Inflammation. *Annu. Rev. Immunol.* **1984**, *2*, 335−357.

(3) Hyde, C. A. C.; Missailidis, S. Inhibition of arachidonic acid metabolism and its implication on cell proliferation and tumour-angiogenesis. *Int. Immunopharmacol.* **2009**, *9*, 701−715.

(4) Peters-Golden, M.; Gleason, M. M.; Togias, A. Cysteinyl leukotrienes: multi-functional mediators in allergic rhinitis. *Clin. Exp. Allergy* **2006**, *36*, 689−703.

(5) Copeland, R. A.; Williams, J. M.; Giannaras, J.; Nurnberg, S.; Covington, M.; Pinto, D.; Pick, S.; Trzaskos, J. M. . Mechanism of

DOI: 10.1021/acs.accounts.5b00226
Acc. Chem. Res. 2015, 48, 2242−2250

Accounts of Chemical Research Selective-Inhibition of the Inducible Isoform of Prostaglandin G/H Synthase. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 11202−11206.
(6) Penning, T. D.; Talley, J. J.; Bertenshaw, S. R.; Carter, J. S.; Collins, P. W.; Docter, S.; Graneto, M. J.; Lee, L. F.; Malecha, J. W.; Miyashiro, J. M.; Rogers, R. S.; Rogier, D. J.; Yu, S. S.; Anderson, G. D.; Burton, E. G.; Cogburn, J. N.; Gregory, S. A.; Koboldt, C. M.; Perkins, W. E.; Seibert, K.; Veenhuizen, A. W.; Zhang, Y. Y.; Isakson, P. C. Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: Identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide (SC-58635, Celecoxib). *J. Med. Chem.* **1997**, *40*, 1347−1365.
(7) Prasit, P.; Wang, Z.; Brideau, C.; Chan, C. C.; Charleson, S.; Cromlish, W.; Ethier, D.; Evans, J. F.; Ford-Hutchinson, A. W.; Gauthier, J. Y.; Gordon, R.; Guay, J.; Gresser, M.; Kargman, S.; Kennedy, B.; Leblanc, Y.; Leger, S.; Mancini, J.; O'Neill, G. P.; Ouellet, M.; Percival, M. D.; Perrier, H.; Riendeau, D.; Rodger, I.; Tagari, P.; Therien, M.; Vickers, P.; Wong, E.; Xu, L. J.; Young, R. N.; Zamboni, R.; Boyce, S.; Rupniak, N.; Forrest, N.; Visco, D.; Patrick, D. The discovery of rofecoxib, [MK 966, Vioxx (R), 4-(4 ′-methylsulfonylphenyl)-3-phenyl-2(5H)-furanone], an orally active cyclooxygenase-2 inhibitor. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1773−1778.
(8) Psaty, B. M.; Furberg, C. D. COX-2 inhibitors - Lessons in drug safety. *N. Engl. J. Med.* **2005**, *352*, 1133−1135.
(9) Singh, D. Merck withdraws arthritis drug worldwide. *BMJ.* **2004**, *329*, 816.
(10) Berger, W.; De Chandt, M. T. M.; Cairns, C. B. Zileuton: clinical implications of 5-Lipoxygenase inhibition in severe airway disease. *Int. J. Clin. Pract.* **2007**, *61*, 663−676.
(11) Penning, T. D. Inhibitors of leukotriene A(4) (LTA(4)) hydrolase as potential anti-inflammatory agents. *Curr. Pharm. Des.* **2001**, *7*, 163−179.
(12) Devi, N. S.; Doble, M. Leukotriene C4 Synthase: Upcoming Drug Target For Inflammation. *Curr. Drug Targets* **2012**, *13*, 1114−1125.
(13) Bauer, J.; Waltenberger, B.; Noha, S. M.; Schuster, D.; Rollinger, J. M.; Boustie, J.; Chollet, M.; Stuppner, H.; Werz, O. Discovery of Depsides and Depsidones from Lichen as Potent Inhibitors of Microsomal Prostaglandin E2 Synthase-1 Using Pharmacophore Models. *ChemMedChem* **2012**, *7*, 2077−2081.
(14) Csermely, P.; Agoston, V.; Pongor, S. The efficiency of multi-target drugs: the network approach might help drug design. *Trends Pharmacol. Sci.* **2005**, *26*, 178−182.
(15) Csermely, P.; Korcsmaros, T.; Kiss, H. J.; London, G.; Nussinov, R. Structure and dynamics of molecular networks: a novel paradigm of drug discovery: a comprehensive review. *Pharmacol. Ther.* **2013**, *138*, 333−408.
(16) Barabasi, A. L.; Gulbahce, N.; Loscalzo, J. Network medicine: a network-based approach to human disease. *Nat. Rev. Genet.* **2011**, *12*, 56−68.
(17) Yang, K.; Ma, W.; Liang, H.; Qi, O.; Tang, C.; Lai, L. Dynamic simulations on the arachidonic acid metabolic network. *PLoS Comput. Biol.* **2007**, *3*, e55.
(18) Kanehisa, M.; Goto, S.; Hattori, M.; Aoki-Kinoshita, K. F.; Itoh, M.; Kawashima, S.; Katayama, T.; Araki, M.; Hirakawa, M. From genomics to chemical genomics: new developments in KEGG. *Nucleic Acids Res.* **2006**, *34*, D354−D357.
(19) Yang, K.; Bai, H.; Ouyang, Q.; Lai, L.; Tang, C. Finding multiple target optimal intervention in disease-related molecular network. *Mol. Syst. Biol.* **2008**, *4*, 228.
(20) Honn, K. V.; Cicone, B.; Skoff, A. Prostacyclin: a potent antimetastatic agent. *Science* **1981**, *212*, 1270−1272.
(21) Smith, J. B.; Araki, H.; Lefer, A. M. Thromboxane A2, prostacyclin and aspirin: effects on vascular tone and platelet aggregation. *Circulation* **1980**, *62*, V19−V25.
(22) Dai, Z.; Lai, L. Differential simulated annealing: a robust and efficient global optimization algorithm for parameter estimation of biological networks. *Mol. BioSyst.* **2014**, *10*, 1385−1392.
(23) He, C.; Wu, Y.; Lai, Y.; Cai, Z.; Liu, Y.; Lai, L. Dynamic eicosanoid responses upon different inhibitor and combination treatments on the arachidonic acid metabolic network. *Mol. BioSyst.* **2012**, *8*, 1585−1594.
(24) Liedtke, A. J.; Keck, P. R. W. E. F.; Lehmann, F.; Koeberle, A.; Werz, O.; Laufer, S. A. Arylpyrrolizines as Inhibitors of Microsomal Prostaglandin E-2 Synthase-1 (mPGES-1) or as Dual Inhibitors of mPGES-1 and 5-Lipoxygenase (5-LOX). *J. Med. Chem.* **2009**, *52*, 4968−4972.
(25) Koeberle, A.; Zettl, H.; Greiner, C.; Wurglics, M.; Schubert-Zsilavecz, M.; Werz, O. Pirinixic Acid Derivatives as Novel Dual Inhibitors of Microsomal Prostaglandin E-2 Synthase-1 and 5-Lipoxygenase. *J. Med. Chem.* **2008**, *51*, 8068−8076.
(26) Chen, Z.; Wu, Y.; Liu, Y.; Yang, S.; Chen, Y.; Lai, L. Discovery of Dual Target Inhibitors against Cyclooxygenases and Leukotriene A(4) Hydrolyase. *J. Med. Chem.* **2011**, *54*, 3650−3660.
(27) Laufer, S. A.; Augustin, J.; Dannhardt, G.; Kiefer, W. (6,7-Diaryldihydropyrrolizin-5-Yl)Acetic Acids, a Novel Class of Potent Dual Inhibitors of Both Cyclooxygenase and 5-Lipoxygenase. *J. Med. Chem.* **1994**, *37*, 1894−1897.
(28) Meirer, K.; Steinhilber, D.; Proschak, E. Inhibitors of the Arachidonic Acid Cascade: Interfering with Multiple Pathways. *Basic Clin. Pharmacol. Toxicol.* **2014**, *114*, 83−91.
(29) Snelgrove, R. J.; Jackson, P. L.; Hardison, M. T.; Noerager, B. D.; Kinloch, A.; Gaggar, A.; Shastry, S.; Rowe, S. M.; Shim, Y. M.; Hussell, T.; Blalock, J. E. A critical role for LTA4H in limiting chronic pulmonary neutrophilic inflammation. *Science* **2010**, *330*, 90−94.
(30) Jiang, X.; Zhou, L.; Wei, D.; Meng, H.; Liu, Y.; Lai, L. Activation and inhibition of leukotriene A(4) hydrolase aminopeptidase activity by diphenyl ether and derivatives. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6549−6552.
(31) Jiang, X.; Zhou, L.; Wu, Y.; Wei, D.; Sun, C.; Jia, J.; Liu, Y.; Lai, L. Modulating the substrate specificity of LTA4H aminopeptidase by using chemical compounds and small-molecule-guided mutagenesis. *ChemBioChem* **2010**, *11*, 1120−1128.
(32) De Oliveira, E. O.; Wang, K.; Kong, H. S.; Kim, S.; Miessau, M.; Snelgrove, R. J.; Shim, Y. M.; Paige, M. Effect of the leukotriene A4 hydrolase aminopeptidase augmentor 4-methoxydiphenylmethane in a pre-clinical model of pulmonary emphysema. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6746−6750.
(33) Serhan, C. N. Resolution phase of inflammation: Novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. *Annu. Rev. Immunol.* **2007**, *25*, 101−137.
(34) Morphy, R.; Rankovic, Z. Designed multiple ligands. An emerging drug discovery paradigm. *J. Med. Chem.* **2005**, *48*, 6523−6543.
(35) Koeberle, A.; Werz, O. Multi-target approach for natural products in inflammation. *Drug Discovery Today* **2014**, *19*, 1871−1882.
(36) Hwang, S. H.; Wecksler, A. T.; Wagner, K.; Hammock, B. D. Rationally Designed Multitarget Agents Against Inflammation and Pain. *Curr. Med. Chem.* **2013**, *20*, 1783−1799.
(37) Wei, D.; Jiang, X.; Zhou, L.; Chen, J.; Chen, Z.; He, C.; Yang, K.; Liu, Y.; Pei, J.; Lai, L. Discovery of Multitarget Inhibitors by Combining Molecular Docking with Common Pharmacophore Matching. *J. Med. Chem.* **2008**, *51*, 7882−7888.
(38) Chen, J.; Lai, L. Pocket v.2: Further developments on receptor-based pharmacophore modeling. *J. Chem. Inf. Model.* **2006**, *46*, 2684−2691.
(39) Meng, H.; Liu, Y.; Zhai, Y.; Lai, L. Optimization of 5-hydroxytryptamines as dual function inhibitors targeting phospholipase A(2) and leukotriene A(4) hydrolase. *Eur. J. Med. Chem.* **2013**, *59*, 160−167.
(40) Penning, T. D.; Chandrakumar, N. S.; Chen, B. B.; Chen, H. Y.; Desai, B. N.; Djuric, S. W.; Docter, S. H.; Gasiecki, A. F.; Haack, R. A.; Miyashiro, J. M.; Russell, M. A.; Yu, S. S.; Corley, D. G.; Durley, R. C.; Kilpatrick, B. F.; Parnas, B. L.; Askonas, L. J.; Gierse, J. K.; Harding, E. I.; Highkin, M. K.; Kachur, J. F.; Kim, S. H.; Krivi, G. G.; Villani-Price, D.; Pyla, E. Y.; Smith, W. G.; Ghoreishi-Haack, N. S. Structure-activity relationship studies on 1-[2-(4-phenylphenoxy)ethyl]pyrrolidine (SC-

DOI: 10.1021/acs.accounts.5b00226
Acc. Chem. Res. 2015, 48, 2242−2250

22716), a potent inhibitor of leukotriene A(4) (LTA(4)) hydrolase. *J. Med. Chem.* **2000**, *43*, 721−735.
(41) Shang, E.; Yuan, Y.; Chen, X.; Liu, Y.; Pei, J.; Lai, L. De Novo Design of Multitarget Ligands with an Iterative Fragment-Growing Strategy. *J. Chem. Inf. Model.* **2014**, *54*, 1235−1241.
(42) Wang, R.; Gao, Y.; Lai, L. LigBuilder: A multi-purpose program for structure-based drug design. *J. Mol. Model.* **2000**, *6*, 498−516.
(43) Yuan, Y.; Pei, J.; Lai, L. LigBuilder 2: A Practical de Novo Drug Design Approach. *J. Chem. Inf. Model.* **2011**, *51*, 1083−1091.
(44) Ma, X. H.; Shi, Z.; Tan, C.; Jiang, Y.; Go, M. L.; Low, B. C.; Chen, Y. Z. In-silico approaches to multi-target drug discovery: computer aided multi-target drug design, multi-target virtual screening. *Pharm. Res.* **2010**, *27*, 739−749.
(45) Greiner, C.; Zettl, H.; Koeberle, A.; Pergola, C.; Northoff, H.; Schubert-Zsilavecz, M.; Werz, O. Identification of 2-mercaptohexanoic acids as dual inhibitors of 5-lipoxygenase and microsomal prostaglandin E-2 synthase-1. *Bioorg. Med. Chem.* **2011**, *19*, 3394−3401.
(46) Wu, Y.; He, C.; Gao, Y.; He, S.; Liu, Y.; Lai, L. Dynamic Modeling of Human 5-Lipoxygenase-Inhibitor Interactions Helps To Discover Novel Inhibitors. *J. Med. Chem.* **2012**, *55*, 2597−2605.
(47) He, S.; Li, C.; Liu, Y.; Lai, L. Discovery of Highly Potent Microsomal Prostaglandin E-2 Synthase 1 Inhibitors Using the Active Conformation Structural Model and Virtual Screen. *J. Med. Chem.* **2013**, *56*, 3296−3309.
(48) Shang, E.; Wu, Y.; Liu, P.; Liu, Y.; Zhu, W.; Deng, X.; He, C.; He, S.; Li, C.; Lai, L. Benzo[d] isothiazole 1,1-dioxide derivatives as dual functional inhibitors of 5-lipoxygenase and microsomal prostaglandin E-2 synthase-1. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 2764−2767.
(49) Gu, S.; Yin, N.; Pei, J.; Lai, L. Understanding traditional Chinese medicine anti-inflammatory herbal formulae by simulating their regulatory functions in the human arachidonic acid metabolic network. *Mol. BioSyst.* **2013**, *9*, 1931−1938.
(50) He, M.; Yan, X.; Zhou, J.; Xie, G. Traditional Chinese medicine database and application on the Web. *J. Chem. Inf. Model.* **2001**, *41*, 273−277.
(51) Gu, S.; Yin, N.; Pei, J.; Lai, L. Understanding molecular mechanisms of traditional Chinese medicine for the treatment of influenza viruses infection by computational approaches. *Mol. BioSyst.* **2013**, *9*, 2696−2700.
(52) Serhan, C. N.; Hamberg, M.; Samuelsson, B. Lipoxins - Novel Series of Biologically-Active Compounds Formed from Arachidonic-Acid in Human-Leukocytes. *Proc. Natl. Acad. Sci. U. S. A.* **1984**, *81*, 5335−5339.
(53) Leslie, M. Inflammation's stop signals. *Science* **2015**, *347*, 18−21.
(54) Qi, Y.; Wang, Q.; Tang, B.; Lai, L. Identifying Allosteric Binding Sites in Proteins with a Two-State G(o)over-bar Model for Novel Allosteric Effector Discovery. *J. Chem. Theory Comput.* **2012**, *8*, 2962−2971.
(55) McClendon, C. L.; Friedland, G.; Mobley, D. L.; Amirkhani, H.; Jacobson, M. P. Quantifying Correlations Between Allosteric Sites in Thermodynamic Ensembles. *J. Chem. Theory Comput.* **2009**, *5*, 2486−2502.
(56) Yuan, Y.; Pei, J.; Lai, L. Binding site detection and druggability prediction of protein targets for structure-based drug design. *Curr. Pharm. Des.* **2013**, *19*, 2326−2333.
(57) Levy, B. D.; Clish, C. B.; Schmidt, B.; Gronert, K.; Serhan, C. N. Lipid mediator class switching during acute inflammation: signals in resolution. *Nat. Immunol.* **2001**, *2*, 612−619.
(58) Huang, J. T.; Welch, J. S.; Ricote, M.; Binder, C. J.; Willson, T. M.; Kelly, C.; Witztum, J. L.; Funk, C. D.; Conrad, D.; Glass, C. K. Interleukin-4-dependent production of PPAR-gamma ligands in macrophages by 12/15-lipoxygenase. *Nature* **1999**, *400*, 378−382.
(59) Meng, H.; McClendon, C. L.; Dai, Z.; Li, K.; Zhang, X.; He, S.; Shang, E.; Liu, Y.; Lai, L.: Redistributing Flux of Metabolic Pathways in Arachidonic Acid Network using Allosteric Activators Identified by Computational and Structural Based Discovery, Manuscript in preparation.
(60) Pei, J.; Yin, N.; Ma, X.; Lai, L. Systems Biology Brings New Dimensions for Structure-Based Drug Design. *J. Am. Chem. Soc.* **2014**, *136*, 11556−11565.
(61) Jiang, B.; Huang, X. J.; Yao, H. Q.; Jiang, J. Y.; Wu, X. M.; Jiang, S. Y.; Wang, Q. J.; Lu, T.; Xu, J. Y. Discovery of potential anti-inflammatory drugs: diaryl-1,2,4-triazoles bearing N-hydroxyurea moiety as dual inhibitors of cyclooxygenase-2 and 5-lipoxygenase. *Org. Biomol. Chem.* **2014**, *12*, 2114−2127.
(62) Inagaki, M.; Tsuri, T.; Jyoyama, H.; Ono, T.; Yamada, K.; Kobayashi, M.; Hori, Y.; Arimura, A.; Yasui, K.; Ohno, K.; Kakudo, S.; Koizumi, K.; Suzuki, R.; Kato, M.; Kawai, S.; Matsumoto, S. Novel antiarthritic agents with 1,2-isothiazolidine-1,1-dioxide (gamma-sultam) skeleton: Cytokine suppressive dual inhibitors of cyclo-oxygenase-2 and 5-lipoxygenase. *J. Med. Chem.* **2000**, *43*, 2040−2048.
(63) Hanke, T.; Dehm, F.; Liening, S.; Popella, S. D.; Maczewsky, J.; Pillong, M.; Kunze, J.; Weinigel, C.; Barz, D.; Kaiser, A.; Wurglics, M.; Lammerhofer, M.; Schneider, G.; Sautebin, L.; Schubert-Zsilavecz, M.; Werz, O. Aminothiazole-Featured Pirinixic Acid Derivatives As Dual 5-Lipoxygenase and Microsomal Prostaglandin E-2 Synthase-1 Inhibitors with Improved Potency and Efficiency in Vivo. *J. Med. Chem.* **2013**, *56*, 9031−9044.
(64) Ohkawa, S.; Terao, S.; Terashita, Z.; Shibouta, Y.; Nishikawa, K. Dual Inhibitors of Thromboxane-A2 Synthase and 5-Lipoxygenase with Scavenging Activity of Active Oxygen Species - Synthesis of a Novel Series of (3-Pyridylmethyl)Benzoquinone Derivatives. *J. Med. Chem.* **1991**, *34*, 267−276.
(65) Lomas, A. L.; Lyon, S. D.; Sanderson, M. W.; Grauer, G. E. Acute and chronic effects of tepoxalin on kidney function in dogs with chronic kidney disease and osteoarthritis. *Am. J. Vet. Res.* **2013**, *74*, 939−944.
(66) Hamasaki, Y.; Shafigeh, M.; Yamamoto, S.; Sato, R.; Zaitu, M.; Muro, E.; Kobayashi, I.; Ichimaru, T.; Tasaki, H.; Miyazaki, S. Inhibition of leukotriene synthesis by azelastine. *Ann. Allergy, Asthma, Immunol.* **1996**, *76*, 469−475.
(67) Argentieri, D. C.; Ritchie, D. M.; Ferro, M. P.; Kirchner, T.; Wachter, M. P.; Anderson, D. W.; Rosenthale, M. E.; Capetola, R. J. Tepoxalin - a Dual Cyclooxygenase 5-Lipoxygenase Inhibitor of Arachidonic-Acid Metabolism with Potent Antiinflammatory Activity and a Favorable Gastrointestinal Profile. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 1399−1408.

DOI: 10.1021/acs.accounts.5b00226
Acc. Chem. Res. 2015, 48, 2242−2250