Simulation Study of Interactions Between Two Bioactive Components from *Zingiber cassumunar* and 5-Lipoxygenase

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Abstract

Introduction—Compound D and DMPBD are compounds extracted from Plai or *Zingiber cassumunar* Roxb., which have antiasthmatic properties. Thai herbal pharmacopoeia have indicated that approximate 50% of Thai prescriptions for asthma contain Plai. However, the inhibition mechanisms of these compounds are not clearly known.

Methods—In this study, molecular docking and molecular dynamics (MD) simulations have been used to simulate complex systems and analyze molecular interactions between these compounds and protein target, 5-lipoxygenase (5-LO) enzyme, which is an enzyme involved with asthma symptoms.

Results—From our MD simulations, Compound D and DMPBD molecules bind at the same binding site of its natural substrate (arachidonic acid) on 5-LO enzyme, which is similar to the binding of commercial asthma drug (Zileuton). Molecular mechanics generalized born surface area binding energy calculations of the 5-LO complex with Compound D and DMPBD are −26.83 and −29.15 kcal/mol, respectively.

Conclusions—This work indicated that Compound D and DMPBD are competitive inhibitors, which are able to bind at the same 5-LO substrate binding site. This reveals opportunities for using Compound D and DMPBD as novel antiasthmatic drugs.

Keywords—Molecular modeling, Molecular docking, Asthma, Ligand–protein interaction.

INTRODUCTION

Asthma is a type of chronic respiratory disease. The Global Asthma Network reported in 2014 that

approximately 334 million people have suffered from asthma.¹ Asthma symptoms can occur from three basic components, which are (1) narrowing of airways due to constriction of smooth muscle, (2) inflammation of airways, and (3) production of mucus to obstruct the airways. When asthma occurs, it can lead to a patient's death. In general, asthma drugs can be divided into two groups, which are (i) anti-obstruction drugs and (ii) anti-inflammation drugs. Anti-obstruction drugs can be used to relieve wheezing and breathlessness by reducing the constriction of airways. Anti-inflammation drugs, including tablet drugs and inhaled corticosteroids, can be used to control the symptoms until they reach resting-state. Thus, patients have to constantly use these medications in order to control the symptoms. By using inhaled corticosteroids, long-term side effects may occur such as osteoporosis, oral candidiasis, dysphonia, glaucoma, and adrenal insufficiency, *etc.* Moreover, there is a very high probability that patients will be permanently resistant to corticosteroids. In spite of severe corticosteroid side effects, it is still used in asthma treatment due to its low market price, compared to others with the same range of effectiveness. Hence, the development of alternative asthma drugs with a lower price and less side effects are necessary.^{16,23}

Leukotrienes (LTs) are potent lipid mediators which have been converted from arachidonic acid (AA) by 5-lipoxygenase enzyme (5-LO). They are secreted from a cell when the immune system has been stimulated. The stimulation of symptoms lead to the presence of asthma and involve various biological pathways. In general, they are initiated by the inhalation of an antigen which activates mast cells and Th2 cells in the airway. This induces the production of mediators responsible

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for inflammation, such as histamine, LTs, and cytokines, including interleukin-4 and interleukin-5, as shown in Fig. 1. Once mast cells are activated, they secrete histamine and LTs. Some interleukin-5 that has been secreted from an activated Th2 cell is transported to the bone marrow and causes terminal differentiation of eosinophils. After eosinophils enter the airway through cytokine activation, their survival is prolonged by interleukin-5. Once activation occurs, eosinophils release inflammatory mediators such as LTs and granule proteins.^{2,8} Human 5-LO is a monomeric enzyme with 673 amino acids residues, which consists of two major domains: N-terminal β -sandwich domain (residues 1–114), and C-terminal catalytic domain (residues 121–673).²⁸ The substrate binding site is located on the catalytic domain, composed of Phe177, Tyr181, His367, Leu368, His372, Leu414, Leu420, Phe421, Ala603, Leu607, and Ile673.¹⁰ It catalyzes the oxygenation reaction of AA to form 5-hydroperoxyeicosatetraenoic acid (5-HPETE) in the first step and then converts 5-HPETE to LTA₄ (LTA₄) in the second step by dehydration.^{26,27} After those enzymatic processes, LTA₄, because of its instability, is immediately converted to LTB₄ and cysteinyl LTs (CysLTs).^{18,21} CysLTs mediate a biological response through G-protein couple receptors called cysteinyl LT receptors,¹² which lead to airway injuries, including smooth muscle contraction⁵ (Fig. 1).

In the past decade, there have been many studies aimed to develop anti-LT substances in order to use them as alternative asthma drugs. Some have been used in clinical practice for curing asthma, such as Zileuton or ZYFLO CR[®] (Fig. 2), which is a commercial 5-LO inhibitor. However, those medications have lower efficacy than corticosteroids and have some severe major drawbacks, such as liver toxicity, low potency, and short half-life. In addition, the therapeutic efficacy of Zileuton currently does not reach expectations.^{3,14,22,30} Therefore, the development of new anti-LT with lower side effects and higher efficacy are necessary.

Plai or *Zingiber cassumunar* Roxb. is a traditional herb found in the Southeast Asia region. Thai herbal pharmacopoeia have indicated that approximate 50% of Thai prescriptions for asthma contain Plai.²⁹ Interestingly, Plai has antiasthmatic activity in children with no acute toxicology effects. A study on the phytochemical aspects of Plai showed that there are compounds that can be extracted from the Plai rhizome by using hexane. A study of the pharmacological properties of isolated compounds from Plai revealed that there are two compounds in the phenylbutanoid group which have antiasthmatic activities. These bioactive substances are Compound D ((E)-4-(3, 4'-dimethoxyphenyl)

but-3-en-1-ol)) and DMPBD ((E)-1-(3', 4'-dimethoxyphenyl)-butadiene), as shown in Fig. 2.²⁴ Jeenapongsa *et al.*¹³ and Pongprayoon *et al.*²⁵ reported that DMPBD and Compound D can be used to reduce ear edema in rats (ear edema induced by AA or tetradecanoylphorbol acetate). Therefore, they suggested that DMPBD and Compound D can inhibit 5-LO pathways.^{13,25} However, their molecular mechanisms are not fully understand. Thus, in order to study the molecular inhibition mechanisms of Compound D and DMPBD on 5-LO, molecular modeling approaches are used for ligand–protein complex investigation. Here we report the results of molecular dynamics (MD) simulations of 5-LO complexed with AA, Zileuton, Compound D, and DMPBD, with water molecules at 300 K. The binding energy, BE (ΔG) of each system is calculated by the molecular mechanics generalized born surface area (MM/GBSA) method. The dynamics of the enzyme and the motion of each amino acid residue at the active site, which may play a role in ligand binding, are examined. Our work indicated that Compound D and DMPBD are competitive inhibitors, which are able to bind at the same 5-LO substrate binding site. This reveals opportunities for using Compound D and DMPBD as novel antiasthmatic drugs.

MATERIALS AND METHODS

Molecular Preparation and Optimization

The structure of AA was extracted from X-ray structure downloaded from the RCSB Protein Data Bank (PDB ID 3V99).¹¹ Zileuton, Compound D, and DMPBD molecules were constructed by Gauss-View05 program.⁷ Geometrical optimization by density functional theory at B3LYP/6-31G (d,p) level was performed by using the Gaussian09 program⁹ for all four ligands, in order to identify the most stable structure. The X-ray structure of substrate-bound 5-LO (PDB ID 3V99), which is a mutated 5-LO with 12 mutated residues and 15-LOX activity,¹¹ is not complete. Most of these amino acid residues are residues which are involved in the substrate binding pocket. Therefore, the complete X-ray structure of substrate-free 5-LO was downloaded from the RCSB Protein Data Bank (PDB ID 3O8Y) which is a mutated variant of 5-LO with 14 mutated residues (stable 5-LOX),¹⁰ in order to use it as the host for molecular docking and MD simulations. Hydrogen atoms and water molecules were added to the prepared 5-LO structure. Structural minimization was performed by using AMBER force fields in the AMBER12 program package.⁴

FIGURE 1. Inflammation cascade in asthma and inhibition by anti-leukotrienes drugs.

Molecular Docking Calculations

Molecular docking of all ligands (small molecules) into the binding site of the 5-LO structure was carried out using the AutoDock 4.2 software package.¹⁷ All torsional angles within small-molecules (ligands) were set free to perform flexible ligand docking. Gasteiger charges were assigned for all molecules. The Lamarckian genetic algorithm was used at 100 dockings for each ligand. The grid sizes were set at a specified grid box with the size of $80 \times 80 \times 80$ Å with a grid point spacing of 0.375 Å. The grid center is located at the binding pocket of 5-LO, following the specified binding location of AA in the X-ray structure (PDB ID 3V99). All other parameters were run at the program's default settings.

Molecular Dynamics Simulations

The AMBER12 program with AMBER force fields was used for MD simulations. 5-LO complex systems

from molecular docking are solvated with a periodic truncated octahedral box of TIP3P water molecules. Energy minimization was performed with 1000 cycles of steepest descent, followed by 1000 cycles of conjugate gradient, to reduce steric conflicts between water molecules and the protein. Then, the solvent equilibration was performed. The system was gradually heated from 0 to 300 K over 20 ps with the volume held constant. After heating for 20 ps, the system had a temperature of 300 K and a density of 1 g/cm^3 , i.e., the experimental density of water. Then, the MD simulations were performed for 20 ns at 300 K and 1 atm (isothermal-isobaric ensemble, NPT) with 2 fs time steps. In addition, the 12-6-4 Lennard-Jones type model from Li and Merz¹⁵ for non-bonded divalent ions was used for Fe^{2+} ion in our system. The van der Waals constants for Fe^{2+} were 1.457 (van der Waals radius in Å) and 0.027 (energy representing the depth of the potential well in kcal/mol). The system properties that can be extracted from the data output files during MD simulations are energies, temperatures,

pressures, volumes, densities, and structural *RMSDs* (root mean square deviations). In addition, free *BE* estimation by the MM/GBSA method has been used to calculate the *BE* between each ligand and specific amino acid residues of 5-LO.

FIGURE 2. Chemical structures of arachidonic acid, Zileuton, Compound D ((E)-4-(3, 4'-dimethoxyphenyl) but-3-en-1-ol)), and DMPBD ((E)-1-(3', 4'-dimethoxyphenyl)-butadiene).

RESULTS AND DISCUSSION

Two available X-ray structures of 5-LO were downloaded from the RCSB Protein Data Bank. One is the substrate-free enzyme structure (PDB ID 3O8Y)¹⁰ and the other is the substrate-bound enzyme structure (PDB ID 3V99).¹¹ Both of them are reported by the same research group. Thirty-eight amino acid residues are missing in the substrate-bound structure (residue numbers 172–176, 190–197, 294–299, 414–429, and 611–613). The difference between these X-ray structures are investigated by their alpha-carbon atom (C_α) superimposition using Discovery Studio Visualizer 4.0 program.⁶

The difference between substrate-free and substrate-bound enzyme structures mostly occurs at the catalytic domain of 5-LO (*RMSD* = 2.43 Å). In the other

TABLE 1. The average binding energy of the selected system from molecular docking calculations and from MD simulations (during 15–20 ns) using MM/GBSA calculations.

Ligands	Molecular docking <i>BE</i> (kcal/mol)	MM/GBSA ΔG (kcal/mol)
AA	-6.68 ± 0.40	-48.21 ± 2.78
Zileuton	-7.22 ± 0.05	-29.40 ± 2.78
Compound D	-5.73 ± 0.12	-26.83 ± 2.58
DMPBD	-5.70 ± 0.06	-29.15 ± 1.87

FIGURE 3. Superimposition of 5-LO X-ray structures. Substrate-bound structure (PDB ID 3V99), presented as green ribbon with AA as the stick model. Substrate-free structure (PDB ID 3O8Y), presented as red ribbon. Fe^{2+} ions in both structure presented as a purple sphere. The missing amino acid residues are highlighted in yellow.

FIGURE 4. (a) Superimposition of 5-LO substrate complex from molecular docking (gray ribbon) and the X-ray structure (PDB ID 3V99, green ribbon). AA from docking and X-ray are shown as blue and orange stick models, respectively. The Fe^{2+} ion is shown as a sphere with the same color as AA in each complex structure. (b) 5-LO binding site and ligand conformations from molecular dockings. The 5-LO structure is shown as a ribbon. Thirteen interacting amino acid residues are shown with a green highlight. Arachidonic acid (AA), Zileuton (ZIL), Compound D (CD), and DMPBD (DMP) are shown as blue, magenta, yellow, and green stick models, respectively. Fe^{2+} ion is shown as purple spheres. All hydrogen atoms are omitted for clarity.

hand, difference in β -sandwich domain is much more less ($RMSD = 0.66 \text{ \AA}$). Therefore, overall C_{α} $RMSD$ of 5-LO is equals to $2.22 \text{ \AA}$. The position of the Fe^{2+} ion in these two structures is almost at the same position ($RMSD = 0.10 \text{ \AA}$), as shown in Fig. 3.

Molecular Docking Calculations

Molecular docking has been used to calculate the possibility of binding between each ligand complex

with 5-LO by fixing the enzyme structure and allowing the ligands to be flexible in the specified grid box. The criteria for selection of ligand conformations in each complex system depends on the average BE and the number of conformations in a cluster (frequency). Thus, if the frequency is equal or greater than 50 that cluster will be selected. If no frequency in any cluster is equal or greater than 50, the cluster with the lowest BE will be selected. The BE of selected clusters have been listed in Table 1.

FIGURE 5. 5-LO complex, solvated in a truncated octahedral water box. 5-LO structure is shown as a gray ribbon. Ligand is shown as a blue stick model. Fe^{2+} ion is shown as purple spheres. Water molecules are shown as a transparent stick model. Na^+ ions are shown as blue spheres.

FIGURE 6. *RMSD* plot of 5-LO complex systems during 20 ns of MD simulations. *C_α* *RMSD* of 5-LO shown as black line. All atoms *RMSD* of ligands shown as red line. Single atom distance of Fe^{2+} ion shown as blue line.

The substrate-free 5-LO enzyme with complete amino acid residues (PDB ID 3O8Y) was used in our molecular docking calculations. The validation of the docking simulation model was considered based on the position of the AA substrate, by superimposition with the substrate-bound X-ray structure (PDB ID 3V99). As a result, the carboxyl group of the AA structure, from X-ray and docking, are close to each other. However, the movement of long chain hydrocarbons can be observed within the binding site, as presented in Fig. 4.

All ligands can bind to 5-LO in the specified region composed of Tyr181, His367, His372, Ile406, Leu414, Leu420, Ala424, Asn425, Asn554, Gln557, Ala603,

Leu607, and Ile673, as shown in Fig. 4. However, the rigidity of the enzyme and the vacuum environment in the docking calculations are not similar to reality. Therefore, MD simulations are used to study the dynamics in aqueous solution of 5-LO with different four ligands.

Thermodynamics Energies of the Ligand–Protein Complexes in MD Simulations

The selected conformations of each ligand–protein complex from molecular docking calculations were used for further MD investigation. The β -sandwich domain of the 5-LO enzyme in each selected conformation was removed from the models in order to reduce the calculation time. Each ligand–protein complex was solvated into a truncated octahedral water box (Fig. 5), which is minimized and equilibrated for 20 ps. Then, the MD simulations were performed for 20 ns at 300 K and 1 atm (isothermal–isobaric ensemble, NPT).

The energies of the equilibrated systems were calculated in order to observe the behavior of systems. The energy of each 5-LO complex system (potential, kinetic, and total) is steady throughout the whole simulation (approximately -2 kcal/mol in total), which is considered as normal for a system in an isothermal water bath. Hence, in order to emphasize the equilibrium of systems, total energies have been averaged every 5 ps and plotted along with the original total energy. The average total energy shows stability with respect to time in every complex system after approximately 2 ns of MD simulations. The rapid increase of energy at the beginning of a simulation occurs due to the heating and minimization (at the first 20 ps of simulation time), and the decline after that is

FIGURE 7. Decomposition energy of each amino acid residue in each ligand-protein complex system during 15–20 ns of MD simulations.

an adjustment to reach equilibrium. Therefore, in the interval between 2 and 20 ns, the complex systems are in equilibrium.

Consequently, the *RMSD* of all ligands during the simulations have been observed in order to see the robustness of the method, as shown in Fig. 6. *RMSD* of AA was highly fluctuate, comparing to others, due to long chain of hydrocarbon in its structure. However, the fluctuation decreased after 3 ns and repeated around 2.5–3.5 Å. For Zileuton, Compound D, and DMPBD molecules, the fluctuations of their *RMSD* were repeated in pattern around 0.2–1.2 Å. For Fe²⁺ ion, single atom *RMSD* is the comparison of ion's

distance between reference frame and each frame during whole simulation by superimposition of the enzyme structures together. As the result, high fluctuation of ion's *RMSD* in each system have occurred. However, there are noticeable drop down peak at difference time as specified in Fig. 6 and then it started to present repeated behavior. Therefore, the high fluctuation of Fe²⁺ ion might not effect in the stability of ligand-protein complex system. Thus, all complex systems have been considered as adequate stable to perform further analysis.

The last 5 ns interval (15–20 ns) of each system were selected for the pairwise decomposition energy by the

MM/GBSA method. The 5000 frames during this interval are averaged, for calculation of BE between each ligand and the specified amino acid residues (5 Å around ligand). The average binding free energy (ΔG) is shown in Table 1, and the total decomposition energy is shown in Fig. 7.

The average binding free energy values are all negative which indicates that all ligands can bind with the 5-LO enzyme at the substrate binding site. All ligands are located in the substrate binding site of 5-LO and the negative BE represents a favorable ligand–protein

FIGURE 9. (a) 5-LO binding site and ligand conformations at 19 ns of MD simulations. The amino acid residues in the binding site are shown as green stick models. Arachidonic acid (AA), Zileuton (ZIL), Compound D (CD), and DMPBD (DMP) are shown as blue, magenta, yellow, and green stick models, respectively. All hydrogen atoms are omitted for clarity. (b) Ligand–protein interactions at 19 ns of MD simulations. Hydrogen bonds are shown as green dashed lines. Hydrophobic interactions are shown as pink curved lines. The π – π interactions are shown as purple dashed lines, and the π –sulfur interaction is shown as an orange dashed line. The distances are in Å units.

FIGURE 8. (a) Superimposition of 5-LO substrate complex from the MD snapshot (at 19 ns) and X-ray structure (3V99). 5-LO structure from X-ray and MD, shown as green and pink ribbons. AA from X-ray and MD, shown as orange and blue stick models. Fe^{2+} ion, shown as non-bonded spheres with the same color as AA in each complex structure. (b) Superimpositions of 5-LO conformations at 19 ns MD simulations. For 5-LO complexes with AA, Zileuton, Compound D, and DMPBD: the enzyme structures are shown as blue, magenta, yellow, and green ribbons, respectively. All ligands are presented as stick models with the same color as their 5-LO structures. All hydrogen atoms are omitted for clarity.

complex. This suggests that Zileuton, Compound D, and DMPDB are competitive inhibitors.

Decomposition energy of the 5-LO complex with AA, Zileuton, and DMPBD show a similar pattern in the arched helix (Ile406, Leu414, Leu420, and Phe421) and specific amino acid residues in the α helix (Trp599, His600, Ala603, Val604, Ala606 and Leu607), which indicate edges of active sites. However, the 5-LO complex with Compound D shows a different pattern of decomposition energy from the interaction with the C-terminus (Ile673) and helix $\alpha 2$ (Phe177, Asn180, and Tyr181), which define another edge of active sites. According to the average BE (ΔG), AA has a lower BE than other ligands because of van der Waals interactions with many amino acid residues.

Molecular Interactions Between 5-LO Enzyme and Each Ligand

Model validation has been performed by considering the AA position from the selected snapshot, at 19 ns of MD simulations, compared to the X-ray structure (PDB ID 3V99), as shown in Fig. 8. The C_{α} - $RMSD$ between them is 2.98 Å. The carboxyl group of AA from both structures is close to each other, but further movement of long chain hydrocarbon can be noticed. Moreover, the positions of Fe^{2+} ion in our MD simulations and in the X-ray structure are not far from each other. These results indicate that our MD simulations are able to mimic the behavior of the 5-LO enzyme when binding with AA substrate molecules and should be able to provide reliable prediction models of 5-LO, complexed with another ligands.

FIGURE 10. Grid water density based on occupancy by water oxygen in the binding pocket of each ligand. GIST contour level are at 5 and represent as pink wireframe. A light blue protein surface is shown in order to indicate the cavity inside 5-LO. The Fe^{2+} ion is shown as orange sphere.

The specific frame of each ligand–protein complex system at 19 ns of MD simulations was selected to investigate molecular interactions and dynamics properties. Superimpositions between systems have been performed in order to observe the difference between each 5-LO complex system (Fig. 8).

The overall structures of 5-LO, complexed with each ligand from our MD simulations, are similar, with *RMSD* 1.35–1.79 Å, which indicates that the 5-LO enzyme has the same dynamics motion even though it is binding with different four ligands. This also supports our assumption that Compound D and DMPDB are competitive substrate inhibitors, the same as the Zileuton commercial drug.

The ligand–protein complexed conformations at 19 ns of each MD simulations system are used to present the ligand's alignment and the molecular interactions with amino acid residues inside the binding pocket on 5-LO, as shown in Fig. 8. Detailed interactions of each ligand with amino acid residues in their binding pockets are presented in Fig. 9.

AA and Zileuton are located at the same binding site. There are two hydrogen bonds between hydroxyl group of AA and Zileuton with carbonyl group of Leu420 and the amino group of Ala424. Moreover, the aliphatic and aromatic hydrocarbon atoms of AA and Zileuton interact with the hydrophobic amino acid residues on 5-LO, which help to stabilize these protein–ligand complexes, indicated by their strong interaction energies (Table 1).

Water in the Binding Site

Water map analysis of the binding pocket of all ligand were explored by grid-based implementation of inhomogeneous solvation theory (GIST) approached.^{19,20} Five thousand conformations during the last 5 ns of each MD simulation were collected to investigate water occupancy in the binding pocket of each ligand by GIST. The GIST approach use to monitors the locations of water molecules by defined a grid box that divided into many small voxels and report the water occupancy in each voxel (a regular grid in three-dimensional space) ranging from low to high density regions. In each system, the grid center was located at center of ligand and the box was cover 5 Å around each ligand. The occupancy of water molecules within the binding pocket of each system, were illustrated in Fig. 10. Cavities of 5-LO in each system are represent as light blue surfaces and the high water density region at five times than of bulk appear as pink wireframe. In cavity of ligand binding site, AA and Compound D shown a few site of water occupied

(w1–w3) while no water found in binding site of DMPDB. The binding site of Zileuton shown several water molecules (w1–w5) are binding inside the pocket. The binding site of Fe^{2+} ion (orange sphere) shown the high density of water occupied in all energetically favor systems.

CONCLUSION

MD simulations reveal that Zileuton, Compound D, and DMPBD can bind at the AA substrate binding site on 5-LO. The AA substrate and Zileuton have a similar binding mode with different binding affinity. The hydroxyl groups of AA and Zileuton have two hydrogen bonds with Leu420 and Ala424. The *BE* of 5-LO with AA substrate (−48.21 kcal/mol) is much lower than the *BE* with Zileuton (−29.40 kcal/mol), Compound D (−26.83 kcal/mol), and DMPBD (−29.15 kcal/mol) due to van der Waals interactions of its hydrocarbon chain with many amino acid residues. Compound D and DMPBD bind at the same catalytic site of 5-LO, but the mode of molecular interactions with each amino acid residue is different. The overall structures of 5-LO, complexed with each ligand from our MD simulations, are similar, with *RMSD* 1.35–1.79 Å, which indicates that the 5-LO enzyme has the same dynamics motion even though it is binding with different ligands. This also supports our assumption that Compound D and DMPDB are competitive substrate inhibitors, the same as the Zileuton, a commercial drug. The binding affinities of Compound D and DMPBD for 5-LO enzyme reveal opportunities for using them as novel antiasthmatic drugs.

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CONFLICT OF INTEREST

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ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

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REFERENCES

- ¹Asher, I., and N. Pearce. Global burden of asthma among children. *Int. J. Tuberc. Lung Dis.* 18:1269–1278, 2014.
- ²Busse, W. W., and R. F. J. Lemanske. Asthma. *N. Engl. J. Med.* 344:350–362, 2001.
- ³Carter, G. W., P. R. Young, D. H. Albert, J. Bouska, R. Dyer, R. L. Bell, J. B. Summers, and D. W. Brooks. 5-Lipoxygenase inhibitory activity of Zileuton. *J. Pharmacol. Exp. Ther.* 256:929, 1991.
- ⁴Case, D. A., T. A. Darden, T. E. Cheatham, C. L. Simerling, J. Wang, R. E. Duke, R. Luo, R. C. Walker, W. Zhang, K. M. Merz, B. Roberts, S. Hayik, A. Roitberg, G. Seabra, J. Swails, A. W. Götz, I. Kolossváry, K. F. Wong, F. Paesani, J. Vanicek, R. M. Wolf, J. Liu, X. Wu, S. R. Brozell, T. Steinbrecher, H. Gohlke, Q. Cai, X. Ye, J. Wang, M.-J. Hsieh, G. Cui, D. R. Roe, D. H. Mathews, M. G. Seetin, R. Salomon-Ferrer, C. Sagui, V. Babin, T. Luchko, S. Gusarov, A. Kovalenko, and P. A. Kollman. AMBER 12. San Francisco: University of California, 2012.
- ⁵Dahlen, S. E. Treatment of asthma with antileukotrienes: first line or last resort therapy? *Eur. J. Pharmacol.* 533:40–56, 2006.
- ⁶Dassault Systèmes BIOVIA. Discovery Studio Modeling Environment. San Diego: Dassault Systèmes BIOVIA, 2016.
- ⁷Dennington, R., T. Keith, and J. Millam. GaussView. v. 5. Shawnee Mission, KS: Semichem, Inc., 2009.
- ⁸Drazen, J. M., E. Israel, and P. M. O'Byrne. Treatment of asthma with drugs modifying the leukotriene pathway. *N. Engl. J. Med.* 340:197–206, 1999.
- ⁹Frisch, M. J., G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, N. J. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox. Gaussian 09. Wallingford, CT: Gaussian, Inc., 2009.
- ¹⁰Gilbert, N. C., S. G. Bartlett, M. T. Waight, D. B. Neau, W. E. Boeglin, A. R. Brash, and M. E. Newcomer. The structure of human 5-lipoxygenase. *Science* 331:217–219, 2011.
- ¹¹Gilbert, N. C., Z. Rui, D. B. Neau, M. T. Waight, S. G. Bartlett, W. E. Boeglin, A. R. Brash, and M. E. Newcomer. Conversion of human 5-lipoxygenase to a 15-lipoxygenase by a point mutation to mimic phosphorylation at Serine-663. *FASEB J.* 26:3222–3229, 2012.
- ¹²Hedi, H., and G. Norbert. 5-Lipoxygenase pathway, dendritic cells, and adaptive immunity. *J. Biomed. Biotechnol.* 99–105:2004, 2004.
- ¹³Jeenapongsa, R., K. Yoovathaworn, K. M. Sriwatanakul, U. Pongprayoon, and K. Sriwatanakul. Anti-inflammatory activity of (E)-1-(3,4-dimethoxyphenyl) butadiene from *Zingiber cassumunar* Roxb. *J. Ethnopharmacol.* 87:143–148, 2003.
- ¹⁴Li, F., M. D. Chordia, K. A. Woodling, and T. L. Macdonald. Irreversible alkylation of human serum albumin by Zileuton metabolite 2-acetylbenzothiophene-S-oxide: a potential model for hepatotoxicity. *Chem. Res. Toxicol.* 20:1854–1861, 2007.
- ¹⁵Li, P., and K. M. Merz. Taking into account the ion-induced dipole interaction in the nonbonded model of ions. *J. Chem. Theory Comput.* 10:289–297, 2014.
- ¹⁶Luhadia, S. K. Steroid resistant asthma. *J. Assoc. Physicians India* 62:38–40, 2014.
- ¹⁷Morris, G. M., R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell, and A. J. Olson. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J. Comput. Chem.* 30:2785–2791, 2009.
- ¹⁸Mullane, K. The increasing challenge of discovering asthma drugs. *Biochem. Pharmacol.* 82:586–599, 2011.
- ¹⁹Nguyen, C. N., A. Cruz, M. K. Gilson, and T. Kurtzman. Thermodynamics of water in an enzyme active site: grid-based hydration analysis of coagulation factor Xa. *J. Chem. Theory Comput.* 10:2769–2780, 2014.
- ²⁰Nguyen, C. N., T. K. Young, and M. K. Gilson. Grid inhomogeneous solvation theory: hydration structure and thermodynamics of the miniature receptor Cucurbit [7] uril. *J. Chem. Phys.* 137:144101, 2012.
- ²¹O'Byrne, P. M. Leukotrienes in the pathogenesis of asthma. *Chest* 111:27s–34s, 1997.
- ²²O'Byrne, P. M. Asthma treatment: antileukotriene drugs. *Can. Respir. J.* 5(Suppl A):64a–70a, 1998.
- ²³Pandya, D., A. Puttanna, and V. Balagopal. Systemic effects of inhaled corticosteroids: an overview. *Open Respir. Med. J.* 8:59–65, 2014.
- ²⁴Panichyupakaranunt, P., and L. Latae. Plai: chemical compositions, pharmacological properties, toxicology, and quality control. *J. Thai Tradit. Altern. Med.* 11:123–137, 2013.
- ²⁵Pongprayoon, U., P. Tuchinda, P. Claeson, T. Sematong, V. Reutrakul, and P. Soontornsaratune. Topical antiin-

- flammatory activity of the major lipophilic constituents of the rhizome of *Zingiber cassumunar*. part II: hexane extractives. *Phytomedicine* 3:323–326, 1997.
- ²⁶Rådmark, O. Arachidonate 5-lipoxygenase. *Prostaglandins Other Lipid Mediat.* 68–69:211–234, 2002.
- ²⁷Rådmark, O., and B. Samuelsson. 5-Lipoxygenase: regulation and possible involvement in atherosclerosis. *Prostaglandins Other Lipid Mediat.* 83:162–174, 2007.
- ²⁸Rådmark, O., and B. Samuelsson. Regulation of the activity of 5-lipoxygenase, a key enzyme in leukotriene biosynthesis. *Biochem. Biophys. Res. Commun.* 396:105–110, 2010.
- ²⁹Thai Herbal Pharmacopoeia. Department of Medical Sciences, Ministry of Public Health, 2009.
- ³⁰Werz, O., and D. Steinhilber. Therapeutic options for 5-lipoxygenase inhibitors. *Pharmacol. Ther.* 112:701–718, 2006.