JOURNAL OF CHEMICAL INFORMATION AND MODELING

Article

pubs.acs.org/jcim

Comparative Binding Effects of Aspirin and Anti-Inflammatory Cu Complex in the Active Site of LOX-1

E. Vrontaki,[†] G. Leonis,[‡] M. G. Papadopoulos,[‡] M. Simcic,[§] S. Golic Grdadolnik,[§,∥] A. Afantitis,[⊥] G. Melagraki,[⊥] S. K. Hadjikakou,*[,#] and T. Mavromoustakos*[,†]

[†]Organic Chemistry Laboratory, Department of Chemistry, University of Athens, Panepistimiopolis-Zografou, 15771 Athens, Greece

[‡]Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, Vas. Constantinou 48, Athens 11635, Greece

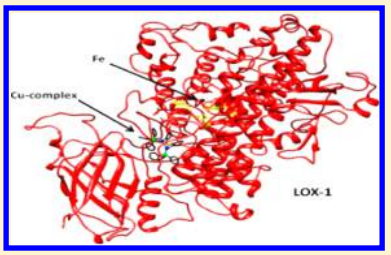
[§]EN-FIST Centre of Excellence, Dunajska 156, SI-1000 Ljubljana, Slovenia

[∥]Laboratory of Biomolecular Structure, National Institute of Chemistry, Hajdrihova 19, SI-1001 Ljubljana, Slovenia

[⊥]Department of Chemoinformatics, NovaMechanics Ltd, Nicosia, Cyprus

[#]Section of Inorganic and Analytical Chemistry, Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece

ABSTRACT: ^{1}H NMR Saturation Transfer Difference (STD) experiments were applied to study the binding of aspirin and of an anti-inflammatory complex of Cu(I), namely $[Cu(tpp)(pmt)]_2$ [pmt = 2-mercaptopyrimidine), synthesized in an attempt to develop novel metallotherapeutic molecules. While aspirin showed only very weak binding, the complex $[Cu(tpp)(pmt)]_2$ clearly favored binding to LOX-1. *In silico* docking experiments in LOX-1 showed that aspirin does only weakly bind to LOX-1, while the complex binds with high affinity. In addition, docking experiments and molecular dynamics (MD) simulations showed that the complex binds via hydrogen bonding (HB), to an allosteric site of LOX-1, revealing that this enzyme has more than one accessible site for complex metallotherapeutic molecules. When aspirin was added in the solution containing LOX and the complex $[Cu(tpp)(pmt)]_2$, the former was shown to hinder the binding of the Cu complex significantly. This may be interpreted as the copper complex aiding the transfer of aspirin through an acid−base reaction at the LOX enzyme which subsequently blocks its binding.



INTRODUCTION

The phospholipase A_2 (PLA_2) superfamily of enzymes consists of a broad range of enzymes that are capable of catalyzing the hydrolysis of the ester bond at the *sn*-2 position of phospholipids, yielding free fatty acids, including arachidonic acid (AA), and lysophospholipids.[1−4] Polyunsaturated fatty acid metabolism is governed by two enzymes, cyclooxygenase (COX) and lipoxygenase (LOX), and lead to the synthesis of eicosanoids.

The activity of COX is important in biological systems as compounds produced by the action of a lipid intermediate in many physiological and pathological conditions such as pain, fever, inflammation, control of renal function, and maintenance of mucous tissue in the stomach. There are two isoforms of the enzyme COX (COX-1 and COX-2), which are encoded by two different genes.[5−7]

Lipoxygenases constitute a large gene family of nonheme, iron-containing fatty acid dioxygenases, which are ubiquitous in plants and animals (isoforms from soybeans and rabbits). LOXs catalyze the regio- and stereospecific dioxygenation of polyunsaturated fatty acids (PUFAs), containing a (1Z,4Z)-pentadiene system. In plants, LOXs are classified with respect to their positional specificity of lipid acid (LA) oxygenation. LA is oxygenated either at carbon atom 9 (9-LOX) or at C-13 (13-LOX) of the hydrocarbon backbone of the fatty acid, leading to two groups of compounds, the (9S)-hydroperoxy and the (13S)-hydroperoxy derivatives of LA.[8−11] LOX inhibition is found to induce apoptosis, while the lipid peroxides derived from fatty acids metabolism by LOX can regulate cellular proliferation. Thus, LOX inhibition provides a potential, novel target for the treatment and chemoprevention of a number of different cancers.

Aspirin is a well-known nonsteroidal, anti-inflammatory drug (NSAID), which belongs to a group called salicylates and differs from the others in the mechanism of action.[12,13] Aspirin has similar effects (antipyretic, anti-inflammatory, analgesic) to the other NSAIDs by inhibiting the same enzyme cyclooxygenase but does so in an irreversible manner and affects more the COX-1 variant than the COX-2 of the enzyme as well as animal LOX 11- and 15-lipoxygenases.[14,15]

Most current drugs, including aspirin, are purely organic compounds. With the success of inorganic complexes, e.g., cisplatin in the treatment of many tumor types, the interest in using metal complexes for therapeutic purposes has been increased. Within cells, metal complexes can participate in reactions that cannot be achieved with conventional organic substances.

A number of epidemiological studies have indicated the beneficial long-term effects of aspirin/NSAID use as it is associated with 30−50% reduction in risk of colorectal cancer or adenomatous polyps or death from colorectal cancer.[16] In other

Received: June 15, 2012
Published: November 3, 2012

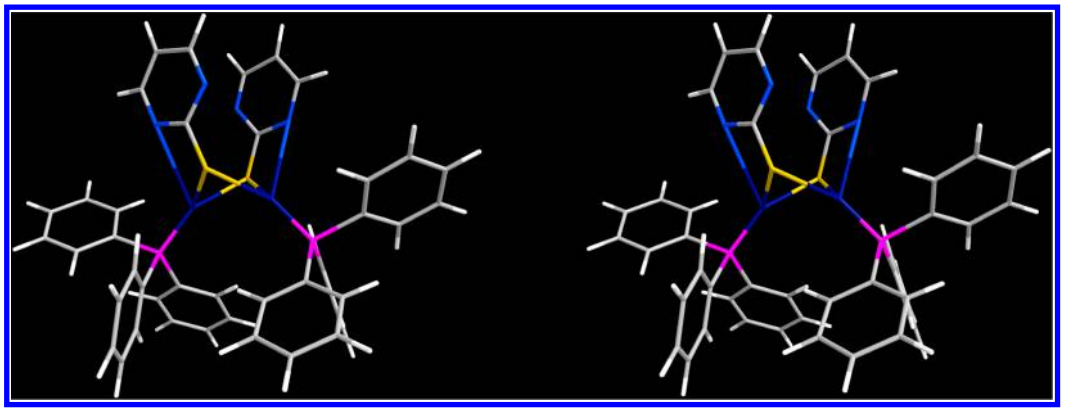


Figure 1. Stereo view of the complex $[Cu(tpp)(pmt)]_2$.

epidemiologic studies it has been found associations between aspirin use and a lower death rate from cancers of the esophagus, stomach, breast, lung, prostate, urinary bladder, and ovary.[17,18] On the other hand, it is well-known that many drugs which inhibit the growth of tumor cells act either by interfering with the bases and/or nucleotides of the double helix of DNA or with the metalloenzymes that are necessary for the rapid growth of malignant cells.[19,20] Aiming at the development of new antitumor agents, we are studying here the biological activity of new copper(I) complexes in the presence of aspirin.

The above findings suggest that it may be possible to improve antitumor activity in the case of aspirin by binding it to an organometallic fragment. An example constitutes the hexacarbonyldicobalt−aspirin complex (Co-Aspirin). The effect of aspirin stems from the acetylation of a serine residue in the active center of COX; while Co-Aspirin does not attack this side chain, instead it acetylates several other sites. This may block access to the active center of the enzyme, resulting in a different activity spectrum for the drug.[12] Also, silver complexes have been found to have biomedical uses in antibacterial action and antitumor activity.[21−25] However, despite the well-known pharmaceutical importance of aspirin as a drug, only few structures of its metal complexes are currently available.[26−33]

As mentioned above, the NSAID aspirin exerts its pharmacologic effect by inhibiting the enzymes of the COX family. In particular, the mechanism of action of acetylsalicylic acid is based on the inhibition of prostaglandin synthesis from arachidonic acid through COX. Arachidonic acid is also metabolized to hydroperoxides and hydroxy-fatty acids through LOX. These products of LOX may also have an important role in anti-inflammatory activity.

Since the relationship between inflammation and carcinogenesis has been examined in numerous biochemical studies,[34,35] the synthesis of new metal complexes is one of the strategies employed for the development of new metallotherapeutics. Thus, a new Cu(I) complex of formula $[Cu(tpp)(pmt)]_2$ [pmt = 2-mercaptopyrimidine] was synthesized (Figure 1). The complex was characterized by elemental analyses, spectroscopic techniques, and X-ray crystallography under ambient conditions.[36]

In this study, the binding mode of the complex and aspirin in LOX was studied *in silico* using docking and MD calculations. The binding of the complex and aspirin toward LOX was also investigated using STD ^{1}H NMR experiments (Saturation Transfer Difference ^{1}H NMR). In a recent study, a STD ^{1}H NMR experiment was used to characterize the binding of anti-inflammatory drugs to COX-1 and COX-2 enzymes. The authors used competition experiments and *in silico* docking to extract valuable information on the molecular basis of action of these anti-inflammatory drugs.[37]

Competitive studies using a complex of copper[36] and aspirin were also performed to investigate whether aspirin could be transferred to the active site of LOX-1.

MATERIALS AND METHODS

NMR Spectroscopy. NMR samples for STD experiments were prepared in a 99.9% D_2O buffer containing 20 mM TRIS (98% D_{11}), 7 mM $(ND_4)_2SO_4$ (98% D_8), 3.5 mM $MgCl_2$, and 0.3 mM DTT (98% D_{10}), pD 7.2 (lower pH was used to avoid acetylsalicylic acid hydrolysis). Ligands' concentration was 0.4 mM, and the protein concentration was 0.004 mM, resulting in a protein−ligand ratio of 1:100 (actual concentration of complex $[Cu(tpp)pmt]_2$ was lower due to poor solubility). Samples were subjected to STD experiments directly or after bath sonication for 30 min at 37 °C.

STD NMR experiments[38] were recorded on Varian 800 MHz spectrometer with spectral width of 8223 Hz and 8192 complex data points. Pulse sequences provided in Varian libraries of pulse programs were used. Relaxation delay was set to 10 s. Selective on-resonance irradiation frequency was set to 0.32 ppm with a saturation time of 0.4 s. Selective saturation was achieved by a train of 50 ms Gauss-shaped pulses separated by a 1 ms delay. Off-resonance irradiation frequency for the reference spectrum was applied at 30 ppm. Water suppression was achieved with excitation sculpting.[39,40] Spectra were zero filled twice, and a line broadening function of 1 Hz was applied.

Docking Experiments. The computational studies were performed using Molegro Virtual Docker software (MVD; version-5) provided by Molegro ApS.[41]

Molegro Virtual Docker 5.0. All structures were prepared using Molegro's Molecules and Protein Preparation Wizard. Proper bond assignments, bond orders, hybridization, and charges were calculated by Molegro Virtual Docker software.[41] Explicit hydrogen atoms were added, and their hydrogen bonding patterns were also determined by MVD.

In order to predict the potentially active regions of LOX-1 (PDB code: 1F8N), in which an inhibitor may bind, the grid-based cavity detection algorithm[42] was employed using the molecular surface feature. Two potential cavities were detected, and the corresponding volume for each was 473.60 Å^3 for cavity 1 and 1617.92 Å^3 for cavity 2. Docking calculations were performed using the heuristic search algorithm MolDock SE (simplex evolution) in combination with the grid-based version of the MolDock Score [GRID].[43]

dx.doi.org/10.1021/ci3002759 | J. Chem. Inf. Model. 2012, 52, 3293−3301

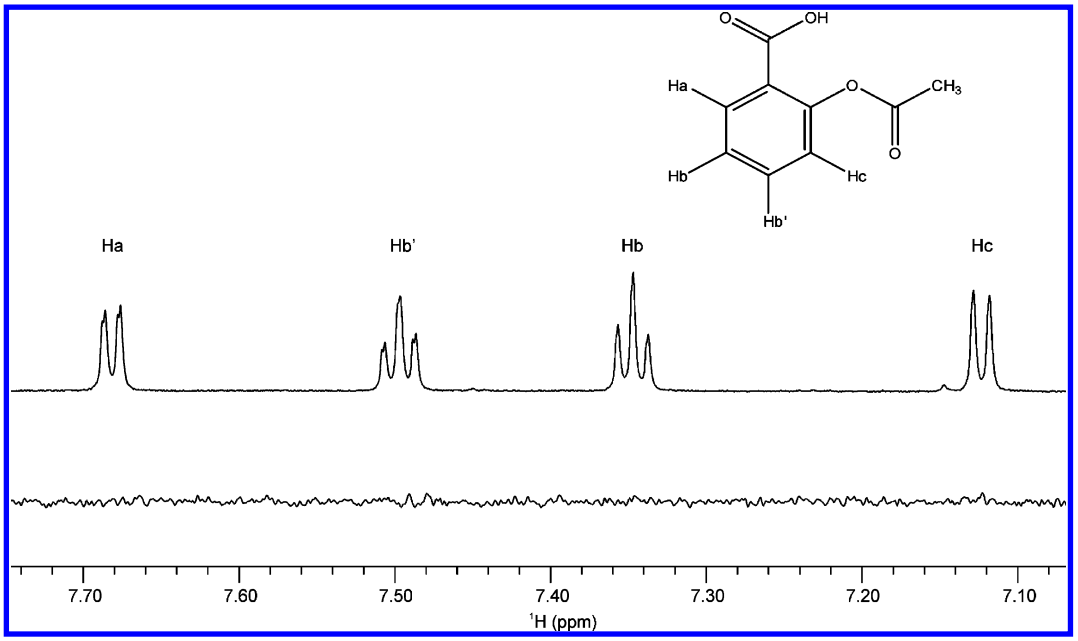


Figure 2. (top) 1D ^{1}H NMR reference spectrum and (bottom) STD NMR spectrum for complex aspirin−protein (LOX-1).

For each ligand, five poses were generated. The MolDock Score [Grid] is a grid-based scoring function, which precalculates potential-energy values on an evenly spaced cubic grid in order to speed up calculations.[43] A grid resolution of 0.30 Å was set to initiate the docking process, and the binding site on the protein was defined as extending in *x*, *y*, and *z* directions around the selected cavity with a radius of 15 Å. For the pose generation, the default setting was applied (MolDock SE), namely a maximum of 1500 iterations combined with a population size of 50. These poses were built incrementally from their rigid root point. The pose generator tests a number of different torsion angles, rotations, and translations; evaluates the affected part of the molecule; and chooses the value which results in the lowest energy contribution.[41] If the generated pose has an energy below the predefined "energy threshold" (100.0 expressed in arbitrary units in our study), it is accepted into the initial population for the "simplex evolution" algorithm. The "simplex evolution" algorithm performs a combined local/global search on the poses generated by the pose generator. The number of the maximum iterations of the simplex evolution algorithm (Nelder−Mead simplex minimization) was set to 300, while the neighbor distance factor, the factor which determines how close the point of the initial simplex will be to the other randomly selected individuals in the population, was set to 1.0 (causes the initial simplex to span the neighbor points exactly).[41]

Molecular Dynamics Calculations. The structure of Cu complex−LOX-1 as obtained by the docking procedure (with the Cu complex inside cavity 2) was considered for further MD calculations. An all-atom, unrestrained MD simulation in explicit solvent has been carried out for the protein complex using the SANDER program under the AMBER 11 software package.[44] Missing residues 1−5, 19−30, and 117−120 have been included in the crystal structure with the Maestro module of Schrödinger.[45] Crystal water molecules were removed from the structure before adding missing hydrogen atoms with the tLEaP module of AMBER. Atomic partial charges, bond lengths, bond angles, dihedral angles, force constants, and van der Waals parameters for LOX-1 were represented by the AMBER ff99SB force field.[46] Force field parameters and partial charges for the Cu complex were assigned as follows: missing hydrogen atoms were added with the program reduce.[47] Next, the geometry of the Cu complex was optimized with Gaussian 09,[48] using the HF/6-31G* basis set for C, P, N, S, and H atoms and the Stuttgart RSC 1997 effective core potential (ECP)[49] for Cu(I) [EMSL Basis Set Exchange web].[50] Nonbonded parameters for Cu(I) (r_{vdw}= 1.70, ε_{vdw} = 0.050) have been taken from Op't Holt and Merz.[51] Finally, the ANTECHAMBER module was used to derive the RESP atomic partial charges[52] for the Cu complex, and the general AMBER GAFF force field was employed to obtain the force field parameters.[53] Equilibrium distances, angles, and dihedrals in the Cu complex have been defined according to crystal structure parameters given by Batsala et al.[36] The atom of iron was modeled in a +II oxidation state (r_{vdw}= 1.200, ε_{vdw} = 0.050), with no bond restraints among Fe and its ligand residues. The system was neutralized with tLEaP by adding 12 Na^+ counterions, and it was solvated with 26 000 water molecules. Explicit solvation has been represented by the TIP3P water model[54] in truncated octahedral periodic boundary conditions, with a cutoff distance of 8 Å. Long range electrostatic interactions have been calculated using the particle mesh Ewald (PME) method.[55] A multiple-step, extensive energy minimization process with the steepest descent method (followed by the conjugate gradient algorithm) was used to relieve unwanted steric interactions that may appear after adding the missing residues and to direct the system toward an energetically favorable conformation. The first step kept the solute (Cu complex−COX-1 system) practically fixed with a harmonic force constant of 5 × 10^{24} kcal mol^{-1} $Å^{-2}$, while the water molecules were allowed to relax. Next, the strength of the restraint was gradually reduced in 14 steps to 2 kcal mol^{-1} $Å^{-2}$. Finally, the restraint was removed, to allow all atoms to move freely. Each of the 15 steps was realized in 1000 cycles with a cutoff of 20 Å. For the unrestrained, last-step minimization, 5000 steps were used. The next procedure involved the gentle heating of the system under constant volume, over 100 ps with a gradual increase of the temperature from 0 to 300 K. The SHAKE algorithm[56] was applied to constrain all bond lengths involving hydrogen to their equilibrium distance, and a 2 fs time step was used. The Langevin

dx.doi.org/10.1021/ci3002759 | J. Chem. Inf. Model. 2012, 52, 3293−3301

thermostat with a collision frequency of 2.0 ps^{-1} was used to keep the temperature constant.[57] A restraint of 10 kcal mol^{-1} $Å^{-2}$ was also applied to the solute. The same restraint was kept for the next 100 ps of equilibration in the NPT ensemble. A final equilibration stage of 100 ps was performed with all atoms of the system unrestrained. The subsequent MD calculations lasted for 15 ns. The SHAKE algorithm and Langevin thermostat, along with a 10 Å nonbonded cutoff were applied during the heating, equilibration, and production MD periods. Further analysis (RMSD and HB calculations) was performed on the resulting trajectory with the ptraj module under AMBER. Cutoffs of 3.5 Å for the donor−acceptor distance and of 120° for the donor−hydrogen−acceptor angle have been used to define HB interactions.

RESULTS AND DISCUSSION

STD ^{1}H NMR Spectroscopy Studies. *Aspirin Binding to LOX-1.* As can be seen in the bottom spectrum of Figure 2, no signal has been observed in the aromatic region of aspirin. Thus, the STD experiment indicates that aspirin does not bind in the regime $\mu M < K_D <$ mM. It should be mentioned that a small signal due to the methyl group appeared, but this we believe is an artifact for the following reasons: (a) a signal can be sometimes observed when no protein is added; (b) some other impurity as well as DMSO and buffer signals are visible; (c) it is unlikely that only the methyl group is in contact with the protein.

Aspirin Binding in the Presence of the Complex [Cu(tpp)(pmt)]$_2$. The STD experiment showed that complex [Cu(tpp)(pmt)]$_2$ binds to LOX-1 (Figure 3).

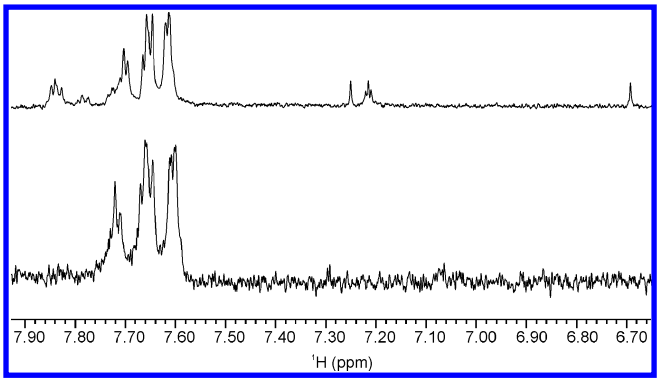


Figure 3. The complex of [Cu(tpp)(pmt)]$_2$ [22]. (top) 1D ^{1}H NMR reference spectrum and (bottom) STD NMR spectrum for complex [Cu(tpp)(pmt)]$_2$−LOX-1.

As shown in Figure 4, when aspirin is added to the protein−[Cu(tpp)(pmt)]$_2$ complex, the intensities of the aromatic protons of the [Cu(tpp)(pmt)]$_2$ complex in the STD spectrum are reduced. More specifically, in accordance with peak integrals or peak heights, the STD amplification factor of the [Cu(tpp)(pmt)]$_2$ complex is reduced approximately 3-fold when it is compared with the experiment where no aspirin was added.

Two explanations appear as the most possible:

(a) Aspirin, as we mentioned above, alone, either binds strongly or does not bind at LOX-1. Let us suppose that aspirin does not bind at the active site. The results show that the [Cu(tpp)(pmt)]$_2$ complex may aid its weak binding. Since the copper complex carries the basic segment of 2-mercaptopyrimidine, it is possible that the carboxylate group of aspirin may be attracted and transferred to the active site of LOX. The incorporation of the more flexible molecule of aspirin hinders the full incorporation of the Cu complex.

(b) If we assume that aspirin binds strongly at the active site, then the Cu complex appears to compete with the active site. As a consequence, aspirin appears to have a weak binding and Cu complex to lower its binding effect.

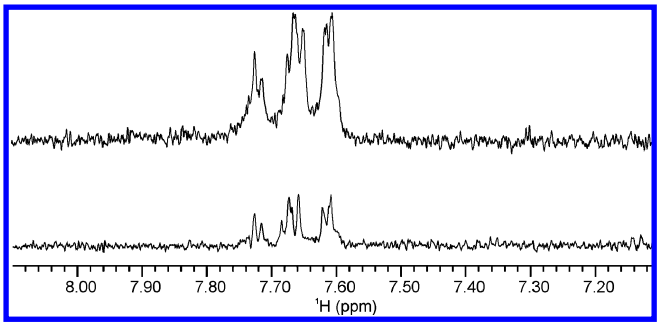


Figure 4. STD NMR spectrum of the complex [Cu(tpp)(pmt)]$_2$−protein without (top) and with (bottom) acetylsalicylic acid.

This binding of aspirin is shown in the STD ^{1}H NMR experiment (Figure 5). In the STD ^{1}H NMR experiment, the aliphatic protons of aspirin are eminent, and some aromatic peaks (Figure 5 bottom at *ca* 7.1 ppm) are discernible.

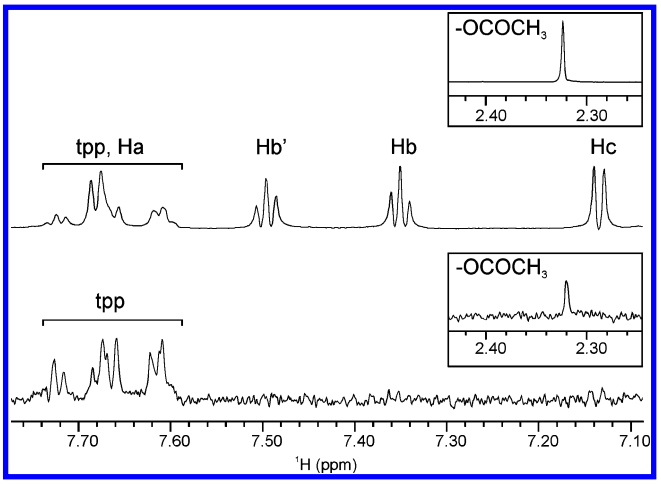


Figure 5. STD ^{1}H NMR of soybean LOX-1 with complex [Cu(tpp)(pmt)]$_2$ and aspirin in TRIS/D_2O (after sonication): (top) The off-resonance NMR spectrum of aliphatic and aromatic regions and (bottom) STD NMR spectrum of aliphatic and aromatic regions.

Docking Studies. In order to investigate further the binding of Cu−complex to the active site of LOX, *in silico* docking studies were performed. Docking experiments at the LOX-1 enzyme were depicted using Molegro Virtual Docker software according to the protocol described in the Materials and Methods. The obtained results confirmed the STD ^{1}H NMR experiments.

In this study, the crystallographic structure of the soybean lipoxygenase-1 in the apo-form is used, which was published by Tomchick et al.[58] The crystallographic study is listed in the Brookhaven Protein Database with entry code 1F8N. The high-resolution of the X-ray structure was 1.40 Å.

In the literature, it was reported that the active center of lipoxygenase-1 was the atom of iron with its four or six ligands.[59,60] Boyington et al. published the crystallographic data of lipoxygenase-1 with a high resolution of 2.60 Å and suggested

dx.doi.org/10.1021/ci3002759 | J. Chem. Inf. Model. 2012, 52, 3293−3301

that in the active center, the iron is attached to four ligands, namely three histidines (His499, His504, and His690), and the carboxyl end of Ile839.[59] Minor et al., three years later, concluded after obtaining the crystal structure of plant lipoxygonase-1 with a higher resolution of 1.40 Å (1YGE) that the center of the iron has six ligands, including a water molecule and Asn694, in addition to the four previously reported.[60]

The amide group oxygen of Asn694 is properly oriented toward the iron atom, but at a relatively long distance ($Fe-O_{694}$ = 3.05 Å); therefore it was concluded that the amino acid of asparagine serves as a rather weak ligand. In the recent crystallographic structure 1F8N, the $Fe-O_{694}$ distance was estimated more accurately (2.87 Å).[58]

Therefore, the recent crystallographic studies of LOX-1 converge on the view that the center of iron is associated with six ligands, and with the amino acid of Asn694 not tightly attached. It is noted that the atom of iron is in the oxidation state +2, contrary to the other LOXs, which are in oxidation state +3.

Then, the copper complex was docked in the two observed cavities of LOX-1. The copper complex is well accommodated in the cavity 2 (V = 1617 Å^3), as is depicted by its MolDock Score of −234.416 (expressed in arbitrary units). The complex is characterized by hydrogen bonding with Tyr525 and numerous steric interactions with amino acids Arg533, Asp768, Lys526, Thr529, and Trp772 (Figure 6a). In the case of the smaller cavity 1 (V = 473.6 Å^3), the MolDock Score was −179.641, and only two hydrogen bonds with Tyr493 and Lys587 were eminent (Figure 6b). The docking results suggest that the copper complex prefers cavity 2 for exerting its binding activity.

Then, the aspirin was docked in the two cavities of LOX-1. The MolDock score was −79.918 in cavity 2 (V = 473.6 Å^3). Aspirin is characterized by hydrogen bonding with Arg767 and numerous steric interactions with amino acids Arg767, Asn769, Pro530, and Trp772 (Figure 7a). In the case of the smaller cavity 1, the MolDock Score was −74.710, and only two hydrogen bonds and one steric interaction with Lys587, Arg360, and Asp 578, respectively, were eminent (Figure 7b). The docking results suggest that aspirin also prefers cavity 2 for exerting its binding activity.

Molecular Dynamics Analysis. The MD simulation of Cu complex−LOX-1 (Figure 8) was initiated from the conformation obtained by the docking calculations on 1F8N. Conformational changes of the protein were observed during the first 5 ns of the simulation, which eventually resulted in a converged trajectory. The highly stable structure of the system is indicated by the stabilization of structural deviations during the last 2/3 of the simulation (Figure 9). A Ca-based RMSD calculation with respect to the initial structure of LOX-1 yielded an average value of 2.0 Å, thus suggesting that the simulated protein equilibrates toward conformations that resemble the crystal structure. The Cu complex also appeared very stable inside cavity 2 of LOX-1 throughout the simulation, with an average RMSD of 1.1 Å (Figure 9, red).

Hydrogen bonding analysis on the MD trajectory of the complex attributed the high stability of the system to an HB network, which involves the Cu complex and the backbone of residues Asp768, Arg533, and Tyr532 in LOX-1. Five HB interactions that appear during the simulation stabilize the Cu complex inside LOX-1 in a tight structure. It was additionally suggested that the minor structural change of the protein during the first 5 ns may have been originated by certain HB rearrangements: the MD simulation indicated that the HB between the Cu complex and Tyr525 (observed in the docking scheme) is potentially unstable, as a result of the reorientation of the ring in the complex with respect to Tyr525. HB interactions as a percent occurrence during the simulation are summarized in Table 1 and are highlighted in Figure 10.

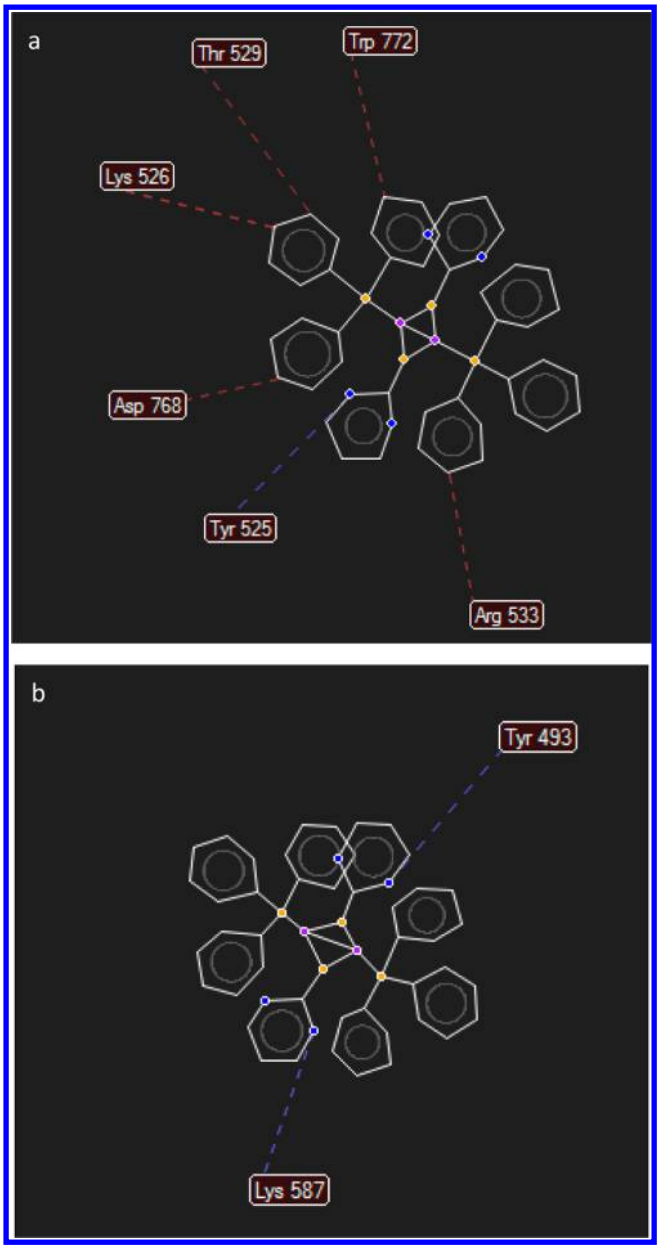


Figure 6. Ligand map (blue, hydrogen bonds; red, steric interactions) of the complex (a) in cavity 2 and (b) in cavity 1 of LOX-1 (1F8N).

CONCLUSION

Aspirin showed no binding to LOX-1 in the regime $\mu M < K_D <$ mM. In contrast to aspirin, the Cu complex $[Cu(tpp)(pmt)]_2$ binds in this regime, as signals of all protons of the molecule are observable by a ^{1}H NMR STD experiment. A weak binding effect to LOX-1 is depicted by aspirin in a competitive ^{1}H NMR STD experiment in the presence of the Cu complex. Thus, the Cu complex appears to aid in the transferring of aspirin at the active site probably through an acid−base transfer mechanism or to ameliorate its effects if aspirin has strong binding. However, molecular docking calculations showed that aspirin has an inferior binding relative to its Cu complex. Additionally, *in silico*

dx.doi.org/10.1021/ci3002759 | J. Chem. Inf. Model. 2012, 52, 3293−3301

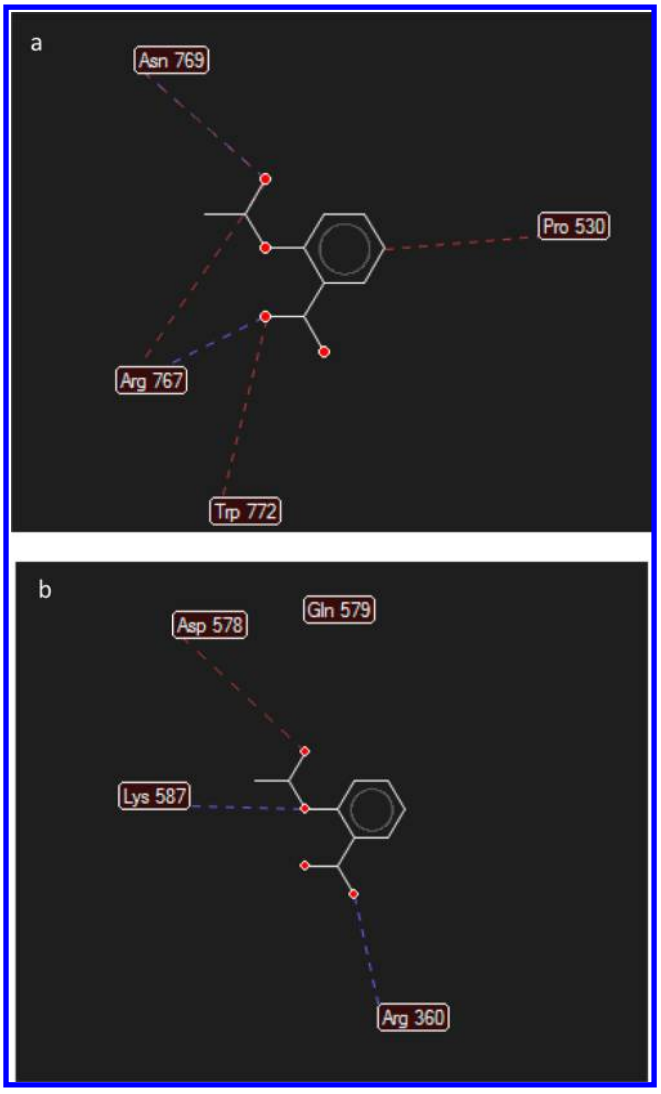


Figure 7. Ligand map (blue, hydrogen bonds; red, steric interactions) of aspirin (a) in cavity 2 and (b) in cavity 1 of LOX-1 (1F8N).

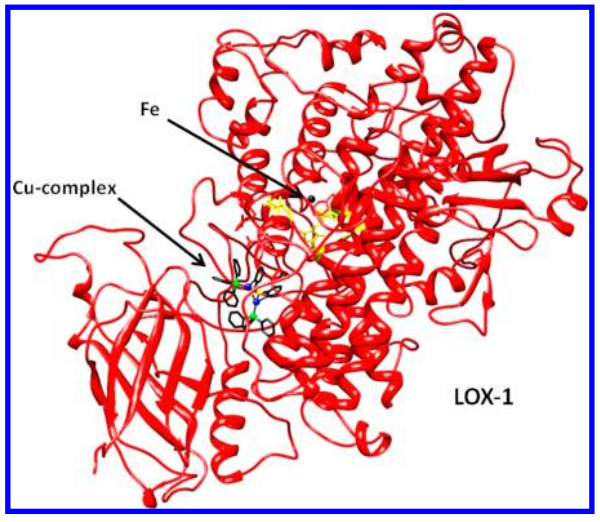


Figure 8. The anti-inflammatory Cu complex inside the binding cavity 2 of LOX-1. The atom of Fe(II) in the active site of cavity 1, along with its coordination sites (His499/504/690, Ile839, and Asn694, in yellow) are also displayed.

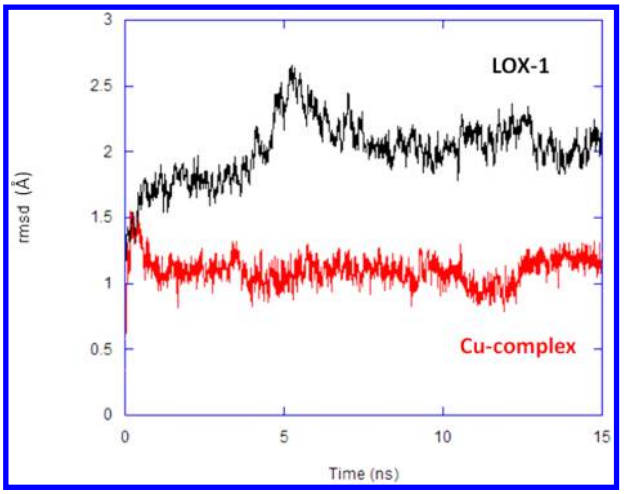
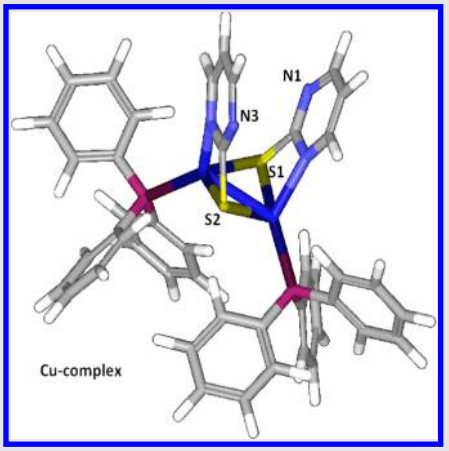


Figure 9. RMSD of LOX-1 and of the Cu complex in the Cu complex–LOX-1 simulated system. Calculations were initiated from the structure obtained after docking and overlapped on the same structure. For LOX-1 and Cu complex, superposition involved Cα atoms and all heavy atoms, respectively.

Table 1. Main Hydrogen Bonding Interactions between Cu Complex and LOX-1



interaction[a] Cu complex–LOX-1	occurrence[b]	comment
N1–Asp768 N-H	58.1%	appears mostly during the first 3/4 of the simulation
N3–Arg533 N-H	55.7%	appears during the last 3/4 of the simulation
S2–Arg533 N-H	31.8%	evenly distributed throughout the simulation
S1–Asp768 N-H	14.3%	appears mostly during the last 1/3 of the simulation
N3–Tyr532 N-H	7.4%	evenly distributed throughout the simulation

[a]N and H refer to LOX-1 backbone nitrogen and hydrogen atoms, respectively. [b]Occurrence is defined as the percentage of simulation time that a specific interaction exists; interactions occurring less than 5% of the simulation time are not shown.

studies revealed the existence of two possible binding cavities in LOX-1: the iron-containing cavity 1 and a larger pocket, cavity 2. Interestingly, cavity 2 appears the most probable binding site for both aspirin and the Cu complex. Since the Cu complex appears to have a more favorable binding to the protein than aspirin, the most plausible explanation for the data is that the Cu complex

dx.doi.org/10.1021/ci3002759 | J. Chem. Inf. Model. 2012, 52, 3293–3301

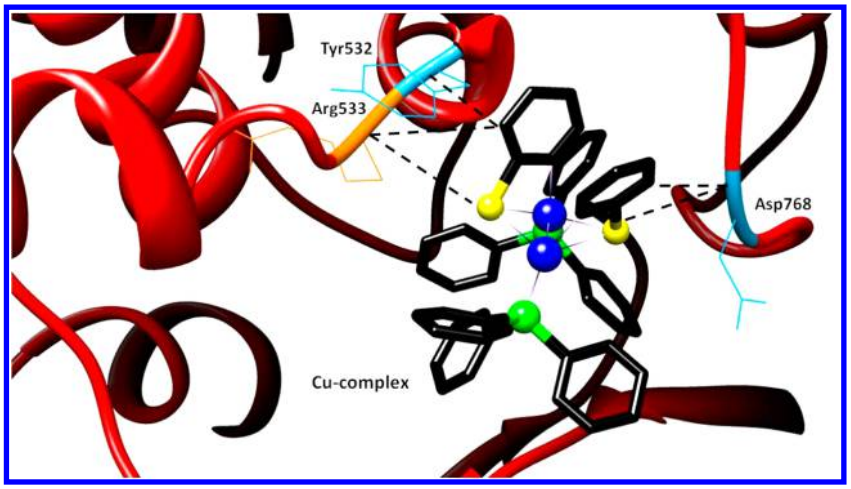


Figure 10. Hydrogen bonds between Cu complex and residues in binding cavity 2 of LOX-1. Interactions are represented by dotted lines and involve backbone N−H groups of LOX residues and sulfur (yellow) and nitrogen (black) atoms in the Cu complex; Cu(I) and P atoms are shown in blue and green, respectively.

aids the transfer of aspirin in the active site and aspirin by itself has no significant binding to LOX-1. This explanation is in accordance with our previous published data, in which it was shown that aspirin has a higher IC_{50} value than 300 μM.[61]

AUTHOR INFORMATION

Corresponding Author

*Tel.: +30 210 7274774 (T.M.), +30 26510 08374 (S.K.H.). Fax: +30 210 7274761 (T.M.), +30 26510 08786 (S.K.H). E-mail: tmavrom@chem.uoa.gr, shadjika@uoi.gr.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The NMR studies were supported by EN-FIST Center of Excellence (Dunajska 156, SI-1000 Ljubljana, Slovenia) and Ministry of Higher Education, Science and Technology of Slovenia. Funding provided by the European Commission for the FP7-REGPOT-2009-1 Project "ARCADE" (Grant Agreement No. 245866) is also acknowledged. The authors would like to thank Thomsen, R. for providing Molegro software.

REFERENCES

(1) Schaloske, R. H.; Dennis, E. A. The phospholipase A(2) superfamily and its group numbering system. *Biochim. Biophys. Acta* **2006**, *1761*, 1246−1259.

(2) Kudo, I; Murakami, M. Phospholipase A2 enzymes. *Prostaglandins Other Lipid Mediators* **2002**, *68−69*, 3−58.

(3) Balsinde, J.; Winstead, M. V.; Dennis, E. A. Phospholipase A2 regulation of arachidonic acid mobilization. *FEBS Lett.* **2002**, *531*, 2−6.

(4) Six, D. A.; Dennis, E. A. The expanding superfamily of phospholipase A(2) enzymes: classification and characterization. *Biochim. Biophys. Acta* **2000**, *1488*, 1−19.

(5) Picot, D.; Loll, P. J.; Garavito, R. M. The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1. *Nature* **1994**, *367*, 243−249.

(6) Kurumbail, R. G.; Stevens, A. M.; Gierse, J. K.; McDonald, J. J.; Stegeman, R. A.; Park, J. Y.; Glidehaus, D.; Miyashiro, J. M.; Penning, T. D.; Seibert, K. Structural basis of selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature* **1996**, *384*, 644−648.

(7) Luong, C.; Mille, A.; Barnett, J.; Chow, J.; Ramesha, C.; Browner, M. F. Flexibility of the NSAID binding site in the structure of human cyclooxygenase-2. *Nat. Struct. Biol.* **1996**, *3*, 927−933.

(8) Liavonchanka, A.; Feussner, I. Lipoxygenases: occurrence, functions and catalysis. *J. Plant Physiol.* **2006**, *163*, 348−357.

(9) Oliw, E. H. Plant and fungal lipoxygenases. *Prostaglandins Other Lipid Mediators* **2002**, *68−69*, 313−323.

(10) Andreou, A.; Feussner, I. Lipoxygenases-Structure and reaction mechanism. *Phytochemistry* **2009**, *70*, 1504−1510.

(11) Liavonchanka, A.; Feussner, I. Lipoxygenases: occurrence, functions and catalysis. *J. Plant Physiol.* **2006**, *163*, 348−357.

(12) Siegel, M. I.; McConnell, R. T.; Porter, N. A.; Selph, J. L.; Truax, J. F. Vinegar, R.; Cuatrecasas, P. Aspirin-like drugs inhibit arachidonic acid metabolism via lipoxygenase and cyclo-oxygenase in rat neutrophils from carrageenan pleural exudates. *Biochem. Biophys. Res. Commun.* **1980**, *92*, 688−695.

(13) Paajanen, H.; Männistö, J.; Uotila, P. Aspirin inhibits arachidonic acid metabolism via lipoxygenase and cyclo-oxygenase in hamster isolated lungs. *Prostaglandins* **1982**, *23*, 731−741.

(14) Ingo, O. Modulation of the Biological Properties of Aspirin by Formation of Bioorganometallic Derivative. *Angew. Chem., Int. Ed.* **2009**, *48*, 1160−1163.

(15) Zha, S.; Yegnasubramanian, V.; Nelsona, W. G.; Isaacs, W. B.; De Marzo, A. M. Cyclooxygenases in cancer: progress and perspective. *Cancer Lett.* **2004**, *215*, 1−20.

(16) Garcia Rodriguez, L. A.; Huerta-Alvarez, C. Reduced Incidence of Colorectal Adenoma among Long-Term Users of Nonsteroidal Antiinflammatory Drugs: A Pooled Analysis of Published Studies and a New Population-Based Study. *Epidemiology* **2000**, *11*, 376−381.

(17) Moran, E. M. Epidemiological and clinical aspects of nonsteroidal anti-inflammatory drugs and cancer risks. *J. Environ. Pathol. Toxicol. Oncol.* **2002**, *21*, 193−201.

(18) Thun, M. J.; Henley, S. J.; Patrono, C. Nonsteroidal Anti-inflammatory Drugs as Anticancer Agents: Mechanistic, Pharmacologic, and Clinical Issues. *J. Natl. Cancer Inst.* **2002**, *94*, 252−266.

(19) Roberts, J. J.; Pascoe, J. M. Cross-linking of complementary strands of DNA in mammalian cells by antitumor platinum compounds. *Nature* **1972**, *235*, 282−284.

(20) Williams, D. R. Bioinorganic Drugs. Part 1. *Educ. Chem.* **1974**, *11*, 124−127.

(21) Rosenkranz, H. S.; Rosenkranz, S. Silver sulfadiazine: inter- action with isolated deoxyribonucleic acid. *Antimicrob. Agents Chemother.* **1972**, *2*, 373−379.

(22) Kyros, L.; Kourkoumelis, N.; Kubicki, M.; Male, L.; Hursthouse, M.; Verginadis, I. I.; Gouma, E.; Karkabounas, S.; Charalabopoulos, K.; Hadjikakou, S. K. Structural Properties, Cytotoxicity, and Anti-Inflammatory Activity of Silver(I) Complexes with tris(p-tolyl)-Phosphine and 5-Chloro-2-Mercaptobenzothiazole. *Bioinorg. Chem. Appl.* **2010**, Article ID 386860.

(23) Zartilas, S.; Hadjikakou, S. K.; Hadjiliadis, N.; Kourkoumelis, N.; Kyros, L.; Kubicki, M.; Baril, M.; Butler, I. S.; Karkabounas, S.; Balzarini, J. Tetrameric 1:1 and monomeric 1:3 complexes of silver(I) halides with tri(p-tolyl)-phosphine: A structural and biological study. *Inorg. Chim. Acta* **2009**, *362*, 1003−1010.
(24) Zachariadis, P. C.; Hadjikakou, S. K.; Hadjiliadis, N.; Michaelides, A.; Skoulika, S.; Balzarini, J.; De Clercq, E. Synthesis, characterization and in vitro study of the cytostatic and antiviral activity of new polymeric silver (I) complexes with ribbon structures derived from the conjugated heterocyclic thioamide 2-mercapto-3,4,5,6-tetrahydropyrimidine. *Eur. J. Inorg. Chem.* **2004**, 1420−1426.
(25) Hadjikakou, S. K.; Ozturk, I. I.; Xanthopoulou, M. N.; Zachariadis, P. C.; Zartilas, S.; Karkabounas, S.; Hadjiliadis, N. Synthesis, structural characterization and biological study of new organotin(IV), silver(I) and antimony(III) complexes with thioamides. *J. Inorg. Biochem.* **2008**, *102*, 1007−1015.
(26) Ochsenbein, P.; Bonin, M.; Masson, O.; Loyaux, D.; Chapuis, G.; Schenk, K. J. Direct Evidence for a Furtive State in the Degradation of Carbasalatum Calcicum. *Angew. Chem., Int. Ed.* **2004**, *43*, 2694−2697.
(27) Fujimori, T.; Yamada, S.; Yasui, H.; Sakurai, H.; In, Y.; Ishida, T. Orally active antioxidative copper(II) aspirinate: synthesis, structure characterization, superoxide scavenging activity, and in vitro and in vivo antioxidative evaluations. *J. Biol. Inorg. Chem.* **2005**, *10*, 831−841.
(28) Viossat, B.; Daran, J.-C.; Savouret, G.; Morgant, G.; Greenaway, F. T.; Dung, N.-H.; Pham-Tran, V. A.; Sorenson, J. R. J. Low-temperature (180 K) crystal structure, electron paramagnetic resonance spectroscopy, and propitious anticonvulsant activities of $Cu^{II}_2(aspirinate)_4(DMF)_2$ and other $Cu^{II}_2(aspirinate)_4$ chelates. *J. Inorg. Biochem.* **2003**, *96*, 375−385.
(29) Ma, Z.; Moulton, B. Mixed-ligand coordination species: A promising approach for "second-generation" drug development. *Cryst. Growth Des.* **2007**, *7*, 196−198.
(30) Santana, M. D.; Lozano, A. A.; Garcia, G.; Lopez, G.; Perez, J. Five-coordinate nickel(II) complexes with carboxylate anions and derivatives of 1,5,9-triazacyclododec-1-ene: structural and 1H NMR spectroscopic studies. *Dalton Trans.* **2005**, 104−109.
(31) James, B. D.; Kivlighon, L. M.; Skelton, B. W.; White, A. H. Triphenyltin(IV) compounds with biologically active anionic groups: Crystal and molecular structures of the *p*-ethoxybenzoic acid, acetylsalicylic acid, phthalic acid and salicylaldehyde derivatives. *Appl. Organomet. Chem.* **1998**, *12*, 13−23.
(32) Vasquez-Arciga, H.; Perez-Benitez, A.; Alvarez-Hernandez, A.; Bernes, S.; Mendez-Rojas, M. A. Bis(acetylsalicylato-x^2O,O')-diaquacadmium(II). *Acta Crystallogr., Sect. E* **2004**, *60*, 1621−1623.
(33) Lemoine, P.; Viossat, B.; Dung, N.-H.; Tomas, A.; Morgant, G.; Greenaway, F. T.; Sorenson, J. R. J. Synthesis, crystal structures, and anti-convulsant activities of ternary $[ZnII(3,5\text{-diisopropylsalicylate})_2]$, $[ZnII(salicylate)_2]$ and $[ZnII(aspirinate)_2]$ complexes. *J. Inorg. Biochem.* **2004**, *98*, 1734−1949.
(34) Pidgeon, G. P.; Lysaght, J.; Krishnamoorthy, S.; Reynolds, J. V.; O′ Byrne, K.; Nie, D.; Honn, K. V. Lipoxygenase metabolism: roles in tumor progression and survival. *Cancer Metastasis Rev.* **2007**, *26*, 503−524.
(35) Cuendet, M.; Pezzuto, J. M. The role of cyclooxygenase and lipoxygenase in cancer chemoprevention. *Drug Metab. Drug Interact.* **2000**, *17*, 109−157.
(36) Batsala, G. K.; Dokoru, V.; Kourkoumelis, N.; Manos, M. J.; Tasiopoulos, A. J.; Mavromoustakos, T.; Simcic, M.; Golic-Grdadolnik, S.; Hadjikakou, S. K. Copper(I)/(II) or silver(I) ions towards 2-mercaptopyrimidine: An exploration of a chemical variability with possible biological implication. *Inorg. Chim. Acta* **2012**, *382*, 146−157.
(37) Viegas, A.; Manso, J.; Corvo, M. C.; Marques, M. M. B.; Cabrita, E. J. Binding of Ibuprofen, Ketorolac, and Diclofenac to COX-1 and COX-2. Studied by Saturation Transfer Difference NMR. *J. Med. Chem.* **2011**, *54*, 8555−8562.
(38) Mayer, M.; Meyer, B. Group Epitope Mapping by Saturation Transfer Difference NMR To Identify Segments of a Ligand in Direct Contact with a Protein Receptor. *J. Am. Chem. Soc.* **2001**, *123*, 6108−6117.
(39) Hwang, T. L.; Shaka, A. J. Water suppression that works. Excitation sculpting using arbitrary wave-forms and pulsed-field gradients. *J. Magn. Reson. Ser. A* **1995**, *112*, 275−279.
(40) Dalvit, C. Efficient multiple-solvent suppression for the study of the interactions of organic solvents with biomolecules. *J. Biomol. NMR* **1998**, *11*, 437−444.
(41) *MVD 2011.5.0 Molegro Virtual Docker User Manual*; CLC bio: Aarhus, Denmark, 2012.
(42) Thomsen, R.; Christensen, M. H. MolDock: A new technique for high-accuracy molecular docking. *J. Med. Chem.* **2006**, *49*, 3315−3321.
(43) Ul-Haq, Z.; Khan, W. Molecular and structural determinants of adamantly susceptibility to HLA-DRs allelic variants: an in *silico* approach to understand the mechanism of MLEs. *J. Comput.-Aided Mol. Des.* **2011**, *25*, 81−101.
(44) (a) Case, D. A.; Cheatham, T.; Darden, T.; Gohlke, H.; Luo, R.; Merz, K. M., Jr.; Onufriev, A.; Simmerling, C.; Wang, B.; Woods, R. The amber biomolecular simulation programs. *J. Comput. Chem.* **2005**, *26*, 1668−1688. (b) Case, D. A.; Darden, T. A.; Cheatham, III, T. E.; Simmerling, C. L.; Wang, J.; Duke, R. E.; Luo, R.; Walker, R. C.; Zhang, W.; Merz, K. M.; Roberts, B. P.; Wang, B.; Hayik, S.; Roitberg, A.; Seabra, G.; Kolossváry, I.; Wong, K. F.; Paesani, F.; Vanicek, J.; Liu, J.; Wu, X; Brozell, S. R.; Steinbrecher, T.; Gohlke, H.; Cai, Q.; Ye, X.; Wang, J.; Hsieh, M. J.; Cui, G.; Roe, D. R.; Mathews, D. H.; Seetin, M. G.; Sagui, C.; Babin, V.; Luchko, T.; Gusarov, S.; Kovalenko, A.; Kollman, P. A. *AMBER 11*; University of California: San Francisco, CA, 2010.
(45) *Maestro*, version 9.1, User Manual; Schrödinger, LLC: New York, NY, 2008.
(46) Hornak, V.; Abel, R.; Okur, A.; Strockbine, B.; Roitberg, A.; Simmerling, C. Comparison of multiple Amber force fields and development of improved protein backbone parameters. *Proteins* **2006**, *65*, 712−725.
(47) Word, J. M.; Lovell, S. C.; Richardson, J. S.; Richardson, D. C. Asparagine and glutamine: using hydrogen atom contacts in the choice of side-chain amide orientation. *J. Mol. Biol.* **1999**, *285*, 1735.
(48) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo,, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma,, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09*, Revision A.02; Gaussian, Inc.: Wallingford CT, 2009.
(49) (a) Bergner, A.; Dolg, M.; Küchle, W.; Stoll, H.; Preuss, H. Ab-initio Energy-Adjusted Pseudopotentials for Elements of Groups 13 - 17. *Mol. Phys.* **1993**, *80*, 143. (b) Kaupp, M.; Schleyer, P.v.R.; Stoll, H.; Preuss, H. Pseudopotential Approaches to Ca, Sr, and Ba Hydrides. Why are Some Alkaline Earth MX_2 Compounds Bent? *J. Chem. Phys.* **1991**, *94*, 1360. (c) Dolg, M.; Stoll, H.; Preuss, H.; Pitzer, R. M. Relativistic and correlation effects for element 105 (hahnium, Ha): a comparative study of M and MO (M = Nb, Ta, Ha) using energy-adjusted *ab initio* pseudopotentials. *J. Phys. Chem.* **1993**, *97*, 5852.
(50) https://bse.pnl.gov/bse/portal (accessed November 2012).
(51) Op't Holt, B.; Merz, K. M., Jr. Insights into Cu(I) Exchange in HAH1 Using Quantum Mechanical and Molecular Simulations. *Biochemistry* **2007**, *46*, 8816−8826.
(52) Woods, R. J.; Khalil, M.; Pell, W.; Moffat, S. H.; Smith, V. H. Derivation of Net Atomic Charges from Molecular Electrostatic Potentials. *J. Comput. Chem.* **1990**, *11*, 297−310.

(53) Wang, J.; Wolf, R. M.; Caldwell, J. W.; Kollman, P. A.; Case, D. A. Development and testing of a general amber force field. *J. Comput. Chem.* **2004**, *25*, 1157−1174.
(54) Jorgensen, W. L.; Madura, J. D.; Impey, R. W.; Klein, M. L. Comparison of simple potential functions for simulationg liquid water. *J. Chem. Phys.* **1983**, *79*, 926−935.
(55) Darden, T.; York, D.; Pedersen, L. Particle mesh Ewald: an N.Log(N) method for Ewald sums in large systems. *J. Chem. Phys.* **1993**, *98*, 10089−10092.
(56) Ryckaert, J.-P.; Ciccotti, G.; Berendsen, H. J. C. Numerical integration of the cartesian equations of motion of a system with constraints: Molecular dynamics of n-alkanes. *J. Comput. Phys.* **1977**, *23*, 327−341.
(57) Izaguirre, J. A.; Catarello, D. P.; Wozniak, J. M.; Skeel, R. D. Langevin stabilization of molecular dynamics. *J. Chem. Phys.* **2001**, *114*, 2090−2098.
(58) Tomchick, D. R.; Phan, P.; Cymborowski, M.; Minor, W.; Holman, T. R. Structural and Functional Characterization of Second-Coordination Sphere Mutants of Soybean Lipoxygenase-1. *Biochemistry* **2001**, *40*, 7509−7517.
(59) Boyington, J. C.; Gaffney, B. J.; Amzel, L. M. The Three-Dimensional Structure of an Arachidonic Acid 15-Lipoxygenase. *Science* **1993**, *260*, 1482−1486.
(60) Minor, W.; Steczko, J.; Stec, B.; Otwinowski, Z.; Bolin, J. T.; Walter, R.; Axelrod, B. Crystal Structure of Soybean Lipoxygenase L-1 at 1.4 Å Resolution. *Biochemistry* **1996**, *35*, 10687−10701.
(61) Banti, C. N.; Giannoulis, A. D.; Kourkoumelis, N.; Owczarzak, A. M.; Poyraz, M.; Kubicki, M.; Charalabopoulos, K.; Hadjikakou, S. K. Mixed ligand−silver(I) complexes with anti-inflammatory agents which can bind to lipoxygenase and calf-thymus DNA, modulating their function and inducing apoptosis. *Metallomics* **2012**, *4*, 545−560.