Molecular Modeling Study of 2-Substituted Isoindoline Derivatives of α-Amino Acids as Inhibitors of Lipoxygenase by Docking Simulations

Teresa Mancilla Percino,*1 José Correa Basurto,2,3,4 Karla S. Alavés Carbajal,2 Nagchielli Valle-Sandoval,2 and José Trujillo Ferrara2,3,4

1Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Departamento de Química. Apartado Postal 14-740, CP 07000, México, D. F. México. tmancill@cinvestav.mx
2Instituto Politécnico Nacional, Escuela Superior de Medicina, Sección de Estudios de Posgrado e Investigación
3Departamento de Bioquímica, 4Departamento de Farmacología. Plan de San Luis y Díaz Mirón s/n Col. Casco de Santo Tomás, Delegación Miguel Hidalgo, C. P. 11340, México, D. F.

Received October 24, 2008; accepted January 23, 2009

Abstract. In this work two series of isoindolines 1(a-g) and 2(a-g) were evaluated as possible inhibitors of lipoxygenase (LOX) by docking studies, as well as for the antiinflammatory isoindolilamides 3-5 and ibuprofen 6, as part of a theoretical study to found dual LOX/COX inhibitory activities. Therefore, dihydrodimethylbenzofuran 7, licofelone 8 and darbufelone 9 were also evaluated, which are well-known as dual LOX/COX inhibitors and consequently, in this work they were used to identify their binding sites on the LOX and compared with those obtained from 1(a-g), 2(a-g) and 3 to 6 under study. Analysis of the results showed that all compounds under study could inhibit to the LOX, since they are binding in the same or close to the region as the compounds 7-9 taken as references. Several interactions of heteroatom of all compounds with the amino acid residues of binding sites of LOX were determined. The $\Delta G$ values were obtained for all the complexes (LOX-compound), among all the complexes, LOX-8 (-12.76 kcal/mol) resulted to be the most stable; and from the compounds under study, LOX-1f (-8.97 kcal/mol) resulted to be more stable than the other compounds tested. Whereas, theoretical dissociation constant values $K_d$ ($\mu$M) were obtained. Among all compounds, 8 (0.000433 $\mu$M) showed more affinity to LOX; while from compounds under study, 1f (0.266 $\mu$M) exhibited more affinity to LOX. These results also show that compounds 1(a-g) and 2(a-g), and 3-6 could have a dual LOX/COX inhibition, as have been shown for 7-9 and from their similar docking study within the COX-1 and COX-2 previously reported.

Key words: Lipoxygenase, Docking, Isoindolines, Antiinflammatory, Analgesic, α-Amino acids.

Introduction

The pain is the main cause of medical attention and also, it is a symptom of some diseases defined as “unlikable sensitive and emotional experience, related with real or potential tissue damage” [1]. It is known that cyclooxygenases (COX-1 and -2) and lipoxygenase (LOX) are involved in the metabolism of arachidonic acid generating eicosanoids, which have been implicated in the pathogenesis of a variety of human diseases [2]. It is established that conventional nonsteroidal antiinflammatory drugs (NSAIDs) and selective COX-2 inhibitors decrease the bioconversion of arachidonic acid to pro inflammatory prosta-glandins (PGs) by inhibiting the COX catalytic activity [3]. In spite of COX-2 inhibitors have showed to have more effective anti-inflammatory properties than classical NSAIDs, still they are not totally safe, due to the implication of COX-2 in several physiological functions; for instance, renal homeostasis, in different stage of pregnancy, in the protection of the gastric mucosa, as well as in the cardiovascular system [4-7]. On the other hand, it is known that leukotrienes and lipoxins produced via the LOX pathway, play role in inflammation and promote the development of gastrointestinal tract [4, 8, 9]. Moreover, expression of LOX and the presence of their products are associated with immune, proliferative diseases, inflammatory bowel diseases, psoriasis, asthma, allergic, inflammatory disorders, cochlear acoustic injury, atherosclerosis and osteoporosis [2,10-19]. Thus, it has been considered that the inflammation is a multifactorial process and biochemical pathways...
should be taken into account, including the LOX pathway [8]. In consequence, drugs able to block COX and LOX pathway (drugs inhibitors) have been developed and pharmacologically investigated, which, should not only present a superior anti-inflammatory profile but also fewer side effects than NSAIDs and selective COX-2 inhibitors [2, 4, 8, 9, 11, 13, 20-28]. On the other hand, we have been interested in the biological activities of isoindolines, various of them have been important as intermediates for the syntheses of novel multidrugs resistance reversal agents [29]. They have also shown diuretic activity [30, 31], as well as used for treating coronary vessel diseases and evaluated as alpha-adrenergic and adrenergic neuron blocking agents [32-34]. Therefore, several isoindolines have exhibited anti-inflammatory [35,36] and analgesic activity [36, 37]. Our interest in the synthesis [38] and biological application of isoindolene derivatives of α-amino acids, we have recently reported the theoretical study as COX-1 and COX-2 inhibition as well as inhibitors in vitro [39,40]. Thus, we attempt a theoretical study of isoindolines 1(a-g) and 2(a-g) by docking, as well as for isoindolilamides 3-5 and ibuprofen 6 as part of a theoretical study to found dual LOX/COX inhibitors. Therefore, dihydrodimethylbenzofuran 7 [26], licofelone 8 [14, 15, 27] and darbufelone 9 [9] were also evaluated, which have showed dual 5-LOX/COX inhibition and in this work they were used to identify the active sites on the 15-LOX and compared with those obtained from 1(a-g), 2(a-g) and 3-6.

**Result and discussion**

Molecular modeling (docking) study was carried out for two series of isoindolines 1(a-g), 2(a-g), (Fig.1), as well as for isoindolilamides 3-5, ibuprofen 6 (Fig. 2) and also for dihydrodimethylbenzofuran 7, licofelone 8, and darbufelone 9 (Fig. 3). The results show that both isoindolines 1(a-g) and 2(a-g) series, as well as compounds 3-9 bind within LOX.

Compounds 1c, 1d, 1e, 1g, 2c, 2d, 2f, 2g, 5, and 6, as well as compounds 7-9 taken as references, binding within LOX in the vicinity of the amino acid residues 96-168 (β-barrel domain) and amino acid residues 377-396, (Fig. 4, Tables 1 and 2), which are part of catalytic domain (360-366 and 537-543 residues); whereas this domain is characterized for containing the residues His 361, 366, 541, and 545, and they are coordinated to the non-haem catalytic iron [10]. Due to compounds 1c, 1d, 1e, 1g, 2c, 2d, 2f, 2g, 5, and 6 are binding to the LOX at the same region that compounds taken as reference; this indicates that these compounds could be LOX inhibitors.

The compounds 1a, 1b, 1f, 2a, 2b and 4 are binding to LOX in the region of the core of the catalytic domain (360-366 and 537-543 residues), (Table 3). Although these compounds are not binding within to LOX exactly at the same region as compounds 7 to 9, they can be considered as feasible LOX inhibitors due they are within the catalytic domain.
While compounds 2e and 3 are binding to the LOX among amino acids residues near to catalytic iron atom, 3 also binds in the vicinity of catalytic domain (Table 4). These results also reveal that these compounds could also inhibit to LOX.

An analysis of the near distances between atoms of amino acids residues of LOX with heteroatoms, carbons or protons of isoindolines 1(a-g) and 2(a-g), as well as those of the compounds 3 to 9 was carried out with the aim to investigate the binding mode for LOX. Thus, the selected distances are in agreement to those with similar or shorter than the sum of van der Waals radius for atoms and hydrogen bond lengths found in proteins [41]. Thus compounds 5, 7, 8 and 9 exhibit the following distances: 4.21 Å between a methyl group of N(CH$_3$)$_2$ of 5 with backbone O of Pro96; 2.88 Å between C=O group of 7 with backbone oxygen of C=O of Pro 96; 3.08 Å between HO group of 8 and side chain CB of Pro96 and 2.82 Å involving N1 of 9 with backbone O Pro96. The distance from backbone O of Cys97 to N atom of 1d, 2c and 2d is of 2.90 Å, 3.23 Å and 3.24 Å, respectively. The OH and N of 2f interact with side chain NH$_2$ and N of Arg99 showing a distance of 2.27 Å and 3.04 Å respectively. Compound 2g shows interaction of The C=O group of 2g exhibits a distance of 3.32 Å with side chain N of Arg99. The distance between C=O group of 1d with side chain C of Leu109 is of 3.21 Å. The HO group of 2f shows a distance of 2.83Å with backbone O of Leu109. The OH group of 6 exhibits hydrogen interaction of with backbone O of Val111 with a distance of 2.21 Å. The OH group of 2g interacts with side chain NH$_2$ of Arg136 (1.88 Å). The O atom (furan ring) of 7 interacts with backbone with side chain N of Arg136 (3.58 Å). Compound 1g undergoes the following interactions, HN with backbone O of Asp164 (3.08 Å) and NH with backbone O of Glu165 (2.67 Å). The distance between CH$_3$ group of compound 1e and backbone C=O of Arg166 is 3.21 Å. The atoms of compounds 1a, 2a and 4 display the following distances: 3.12 Å between N atom of 1a and side chain C=O of Glu357; N atom of compound 2a shows distance of 3.10 Å and 2.06 Å with side chain OE and OH groups and with backbone O of Glu357, respectively. Nitrogen atom of 4 shows a distance of 3.42 Å with side lateral OE2 of Glu357. Oxygen and carbon atoms of C=O and OCH$_3$ groups of 1a, exhibit distances of 3.57 and 2.79 Å with side chain CD1 and CD2 of Leu362, respectively. Methyl group of 1g shows a distance of 3.06 Å with side chain C of Thr373. Carbonyl group of 2c exhibits a distance
values obtained for isoindolines in inhibitory compound as compound).

These results show that compounds more affinity to LOX and while from compounds under study, LOX
compound), among all the complexes, LOX-
with side chain C of Leu624. Nitrogen atom of shows a distance of 2.94 Å with backbone N of Arg621. The N
atom of shows a distance of 2.70 Å with backbone C=O of Gly617. Carbonyl group of
shows a distance of 2.73 Å with backbone O of Val 391. Nitrogen atom of exhibits a distance of 3.53 Å with side chain CD1 of Leu408. Nitrogen atom of and exhibits a distance of 3.73 and 3.90 Å, respectively, with side chain CD1of Leu408 (Fig. 5).

Methyl group of exhibits a distance of 3.22 Å from side chain CE1 of Phe415. Carbonyl group of shows a distance of 2.61 Å with side chain N of Gln548 and NH group a distance of 2.51 Å with backbone N of Val594. The NH of 3 shows a distance of 1.71 Å with side chain C=O of Glu613. Carbonyl group of displays a distance of 2.50 Å with backbone O of Phe615. The NH of 3 shows a distance of 1.65 Å with backbone C=O of Phe615. The C=O group of 3 shows a distance of 2.70 Å with backbone C=O of Gly617. Carbonyl group of 2e shows a distance of 2.94 Å with backbone N of Arg621. The N atom of 3 display a distance of 2.81 Å with side chain C=NH of Arg621. Nitrogen atom of 2e displays a distance of 2.88 Å with side chain C of Leu624.

The ΔG values were obtained for all the complexes (LOX-compound), among all the complexes, LOX-8 (-12.76 kcal/mol) resulted to be more stable and from the compounds under study, LOX-1f (-8.97 kcal/mol) resulted to be more stable. Therefore, the ΔG values for 1b, 1g, 2b, 2e, 2f and 2g show to be also stables due to they are almost similar to the value obtained for 7, which is a dual inhibitor. Whereas, theoretical dissociation constant values $K_d$ (µM) were obtained, among all compounds 8 (0.000433 µM) showed more affinity to LOX, while from compounds under study 1f (0.266 µM) exhibited more affinity to LOX and 1g exhibits a similar affinity to 7. These results show that compounds 1f and 1g could be dual inhibitor as compound 7. Table 5 summarizes the ΔG and $K_d$ values obtained for isoindolines 1(a-g) and 2(a-g) and compounds 3-9.

### Molecular modeling (docking) methodology

The ligands were drawn using Isis/draw program [42] and converted to three-dimensional format (i.e. pdb) using the WebLab Viewer and Molekel Visualization Package [43,44]. The geometry pre-optimization (molecular mechanic, MM+) of the ligands was carried out by using HyperChem-6 software. The minimum energy structure of the ligands was obtained by means of Density Theory Functions (DFT) calculations at B3LYP/6-31G** level using Gaussian 98 software [45]. To understand the recognition mechanism between LOX enzyme and the ligands, docking simulations were done on the 3-D structure of LOX from rabbit (pdb cod: 1lox [10]). Before starting the docking evaluations, the partial atomic charges (Gasteiger-Marsili formalism), as well as all possible rotatable bonds of the ligands and the Kollman charges for all atoms in enzymes were assigned by using the AutoDock Tools 1.4.5 version [46]. Moreover, missing residues were also built and hydrogen atoms were added to the amino acids of the protein with the mentioned program. For docking studies, the AutoDock (3.0.5) was chosen because its algorithm allows full flexibility of small ligands [46]. It has been shown that it successfully reproduces many crystal structure complexes and includes an empirical evaluation of the binding free energy. The preparation of protein and ligand input structures and the definition of the binding sites were carried out under a GRID-based procedure [47]. First, a rectangular grid box were constructed over all protein (126 × 126 × 126 Å) with grid points separated by 0.375 Å under blind docking procedure. Previously, the enzyme structures were cleaned of its water molecules and co-crystallized ligands maintaining the haem group. All docking simulations were carried out by using the hybrid Lamarckian Genetic Algorithm, with an initial population of 100 randomly placed individuals and a maximum number of energy evaluations (1.0 × 10⁵). The resulting docked orientations within a root-mean square deviation of 0.5 Å were clustered together. The lowest energy cluster returned by AutoDock for each compound was used for further analysis. All other parameters were maintained at their default settings. All the docking result visualizations were achieved by using a Visual Molecular Dynamics (VMD) program [48].

In conclusion, we describe a theoretical study of two series of isoindolines 1(a-g) and 2(a-g) and compounds 3 to

Table 5. ΔG (kcal/mol) and $K_d$ (µM) values for compounds 1 to 9.

<table>
<thead>
<tr>
<th>Compound</th>
<th>1a</th>
<th>1b</th>
<th>1c</th>
<th>1d</th>
<th>1e</th>
<th>1f</th>
<th>1g</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_d$</td>
<td>9.678</td>
<td>0.696</td>
<td>16.908</td>
<td>5.740</td>
<td>2.230</td>
<td>0.266</td>
<td>0.360</td>
</tr>
</tbody>
</table>

### Molecular modeling (docking) methodology

The ligands were drawn using Isis/draw program [42] and converted to three-dimensional format (i.e. pdb) using the WebLab Viewer and Molekel Visualization Package [43,44]. The geometry pre-optimization (molecular mechanic, MM+) of the ligands was carried out by using HyperChem-6 software. The minimum energy structure of the ligands was obtained by means of Density Theory Functions (DFT) calculations at B3LYP/6-31G** level using Gaussian 98 software [45]. To understand the recognition mechanism between LOX enzyme and the ligands, docking simulations were done on the 3-D structure of LOX from rabbit (pdb cod: 1lox [10]). Before starting the docking evaluations, the partial atomic charges (Gasteiger-Marsili formalism), as well as all possible rotatable bonds of the ligands and the Kollman charges for all atoms in enzymes were assigned by using the AutoDock Tools 1.4.5 version [46]. Moreover, missing residues were also built and hydrogen atoms were added to the amino acids of the protein with the mentioned program. For docking studies, the AutoDock (3.0.5) was chosen because its algorithm allows full flexibility of small ligands [46]. It has been shown that it successfully reproduces many crystal structure complexes and includes an empirical evaluation of the binding free energy. The preparation of protein and ligand input structures and the definition of the binding sites were carried out under a GRID-based procedure [47]. First, a rectangular grid box were constructed over all protein (126 × 126 × 126 Å) with grid points separated by 0.375 Å under blind docking procedure. Previously, the enzyme structures were cleaned of its water molecules and co-crystallized ligands maintaining the haem group. All docking simulations were carried out by using the hybrid Lamarckian Genetic Algorithm, with an initial population of 100 randomly placed individuals and a maximum number of energy evaluations (1.0 × 10⁵). The resulting docked orientations within a root-mean square deviation of 0.5 Å were clustered together. The lowest energy cluster returned by AutoDock for each compound was used for further analysis. All other parameters were maintained at their default settings. All the docking result visualizations were achieved by using a Visual Molecular Dynamics (VMD) program [48].

In conclusion, we describe a theoretical study of two series of isoindolines 1(a-g) and 2(a-g) and compounds 3 to
6 as possible LOX inhibitor, which were compared with compounds 7 to 9 taken as reference to find the active sites within LOX. Analysis of the data showed that the compounds investigated exhibit interactions at the same or nearby of amino acid residues within lipoxynase sites. Due to compounds 1c, 1d, 1e, 1g, 2c, 2d, 2f, 2g, 5 and 6 are binding to the LOX at the same region that compounds taken as reference they could be considered as dual inhibitors. Although compounds 1a, 1b, 1f, 2a, 2b and 4 are not binding within to LOX exactly at the same region as compounds 7 to 9, they can be considered as feasible LOX inhibitors due they are in the region of the core of the catalytic domain. Compounds 2e and 3 showed bind to the LOX among amino acids residues near to catalytic iron atom and 3 shows also bind in the vicinity of catalytic domain, in consequence they could be inhibitor. On the other hand, distances between several atoms of all compounds with some atoms of amino acid residues were obtained.

Acknowledgments

Authors thank COFFA, SIP-IPN and for scholarship to K. S.A.C. and also Consejo Nacional de Ciencia y Tecnología (Conacyt- México) for financial support.

References

43. WebLab Viewer, available at http://www.liv.ac.uk/ctichem/
      16weblab.html
44. Flükiger, P. F., Development of the Molecular Graphics Package
      MOLÈKEL and its Application to Selected Problems in Organic
      and Organometallic Chemistry, Thèse No 2561, Département de
45. Frisch, M. J.; Trucks, G. W.; Schlegel, H.B.; Scuseria, G. E.;
      Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery,
      Jr. J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam,
      J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.;
      Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.;
      Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Peterson, G.
      A.; Ayala, P.Y.; Cui Q.; Morokuma, K.; Malick, D. K.; Rabuck,
      A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J.
      V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz,
      P.; Komaromi, I.; Gomperts, R.; Martin, R.L., Fox, D. J., Keith,
      T.; Al-Laham, M. A.; Peng, C.Y.; Nanayakkara, A.; Challacombe,
      M., Gill P. M. W., Johnson, B., Chen, W., Wong, M. W., Andres,
      J.L.; Gonzalez, C.; Head-Gordon, M.; Replogle E. S.; Pople, J. A.;
46. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart,
      W.E.; Belew, R.K.; Olson, A. J. J. Comp. Chem. 1998, 19, 1639-
      1662.
48. Humphrey, W.; Dalke, A.; Schulten K. J. Mol. Graph. 1996, 14,
      33-38.