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Theoretical Study of Isoindolines to Identify them as Cyclooxygenase-1 and –2 Inhibitors by Docking Simulations

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Abstract. This work describes a theoretical study of two series of isoindolines **1**(**a**-**h**) and **2**(**a**-**h**) as possible COX-1 and COX-2 inhibitors by Docking method. Whereas, the same study was carried out for isoindolilamides **3**-**5**, which have shown anti-inflammatory and analgesic effects, as well as ibuprofen **6** and dihydrodimethylbenzofuran **7,** which are well-known as excellent anti-inflammatory. Compounds **6** and **7** were used to identify the active sites on these two enzymes and compared with those obtained from isoindolines under docking studies. The analysis of Docking results show that compounds **1**(**a**-**h**) and **2**(**a**-**h**) could inhibit both cyclooxygenases (COXs), due to the fact that they act in the same region as those taken as reference (**3**-**7**) make several interactions with the amino acid residues that conform the active sites of both COXs. ΔG values were obtained for all compounds, they are between –9.87 and –6.65 (Kcal/mol, COX-1) and -10.96 and –6.28 (Kcal/mol, COX-2), being COX-1-**1h** and COX-2-**1h** complexes more stable. Therefore, K_d (μM) values were obtained, they are in the range of 0.06 and 13.5 in COX-1 and finally the values between 0.01 and 24.7 in COX-2, where **1h** shows more affinity to both COX-1 and COX-2.
Keywords: Isoindolines, Docking, cyclooxygenase, anti-inflammatory, analgesic, amino acids.

Resumen. Este trabajo describe un estudio teórico de dos series de isoindolines **1**(**a**-**h**) y **2**(**a**-**h**) como posibles inhibidores de COX-1 y COX-2 por el método docking. Además, el mismo método se llevó a cabo para las isoindolilamidas **3**-**5**, las cuales han mostrado efectos anti inflamatorios y analgésicos, así como para el ibuprofeno **6** y dihirodimetilbenzofurano **7**, los cuales son bien conocidos como antiinflamatorios. Los compuestos **6** y **7** fueron usados para identificar los sitios activos de estas dos enzimas y comparados con aquellos obtenidos en las isoindolinas por simulación docking. El análisis de los resultados por docking mostraron que los compuestos **1**(**a**-**h**) y **2**(**a**-**h**) pudieran inhibir ambas ciclooxigenasas (COXs), debido al hecho a que ellas actúan en la misma región como aquellos usados como referencia (**3**-**7**) mostrando varias interacciones con los residuos de los amino ácidos que conforman los sitios activos de ambas COXs. Los valores de ΔG fueron obtenidos para todos los compuestos, se encuentran entre –9.87 y –6.65 (Kcal/mol, COX-1), y –10.96 y –6.28 (Kcal/mol, COX-2), siendo los complejos COX-1-**1h** y COX-2-**1h** mas estables. Además, los valores de K_d (μM) fueron obtenidos, están en el intervalo de 0.06 y 13.5 en COX-1 y finalmente los valores entre 0.01 y 24.7 en COX-2, donde **1h** muestra más afinidad para ambas COX-1 y COX-2.
Palabras clave: Isoindolinas, Docking, ciclooxigenasa, anti-inflamatorios, analgésicos, amino ácidos.

Introduction

Most non-steroidal anti-inflammatory drugs (NSAIDs) currently used in clinic are known to inhibit both isoforms of Prostaglandins H synthase (PGHS) with little selectivity, and during extended therapy many NSAIDs cause ulcerogenic side effects most likely due to PGHS-1 inhibition in the stomach. There are two isoforms which have been shown to differ pharmacologically [1]. One of the research techniques to provide a evidence of the ligand binding sites on biological macromolecules is computational docking method [2-4], which has been used to obtain information about the structural basis of NSAID binding by PGHS-1 (COX-1) and PGHS-2 (COX-2), as well as evaluate drug as inhibitors of PGHS [5,6]. We have been interested in the biological activities of isoindolines due to various of them have been important as intermediates for the syntheses of novel multidrugs resistance reversal agents [7]; they have also shown diuretic activity [8,9]. They have been used for treating coronary vessel diseases and evaluated as alpha-adrenergic and adrenergic neuron blocking agents [10-12]. Therefore, several isoindolines have exhibited anti-inflammatory [13,14] and analgesic activity [14,15]. Our interest in the synthesis of organic compounds with biological applications [16-21], we have recently prepared some isoindolines derivatives of α-amino acids [18] with the aim to evaluate their biological properties. In this context, our current interest in the ligand-protein binding, which is important no only as an essential molecular recognition but also to discovery new drugs, we attempt a theoretical study of a various isoindolines **1**(**a**-**h**) and **2**(**a**-**h**) derived from glycine, leucine, isoleucine, methionine, phenylglycine, phenylalanine, tyrosine and tryptophane, respectively, to investigate their properties as possible inhibitors of cyclooxygenase-1 and -2 (COXs) by computational docking technique. While a similar docking study was carried out within the COX-1 and COX-2 for isoindolilamide **3**, isoindolil-*N*-methylamide **4** and isoindolil-*N*-dimethylamide **5**, which have exhibited anti-inflammatory and analgesic activity [14-15] and also for ibuprofen **6** [5] and dihydrodi-

methylbenzofuran **7** [6] known as excellent anti-inflammatory. Thus, is with the purpose of investigate from the analysis of docking results, if theoretically compounds **1**(**a**-**g**) and **2**(**a**-**g**) could show a similar biological behavior exhibited by compounds **3**-**7** used as reference.

Results and discussion

Molecular modeling (docking) study was carry out for two series of isoindolines **1**(**a**-**h**), **2**(**a**-**h**), where the different between them is the ester and carboxylic groups, respectively (Fig.1) and for isoindolilamides **3**-**5**, ibuprofen **6** and dihydrodimethylbenzofuran **7** showed in figure 2, within the COX-1 and COX-2 binding sites.

The interactions of compounds **1**(**a**-**h**), **2**(**a**-**h**), **6** and **7** within the COX-1 sites are depicted in Figure 3.

The following features are observed: a) COX-1-**1d**, **-1e**, **-1f**, **-1h**, **-2b**, **-2e**, **-2f**, **-2h** and **-6** complexes are in the chain A, where the compounds undergo interactions with the amino acid residues Cys36, Cys37, Tyr39, Pro40, Cys41, Gln42, His43, Gln44, Gly45, Ile46, Cys47 (compact domain) [22] Tyr130, Asp135, Ile151, Leu152, Pro153, Ser154, Val 155, Pro156, Arg459, Gln461, Glu465, Tyr466, Lys468 and Arg469 (catalytic domain) [22]; b) COX-1-**2a** and COX-1-**2c** complexes also are in the chain A but, the compounds interact with the amino acid residues Phe142, Ser143, His226, Gly227, Val228, Asp229, Tyr373, Arg374, Asn375, Arg376, Ile377, Gly533, Leu534, Gly536, Asn537 and Pro538 (catalytic domain); c) COX-1-**1a**, **-1b**, **-1c**, **-1g**, **-2d**, **-2g**, and **-7** complexes are in the chain B, where the compounds undergo interaction with the same type of amino acid residues of chain A observed for COX-1-**1d**, **-1e**, **-1f**, **-1h**, **-2b**, **-2e**, **-2f**, **-2h** and **-6** complexes and d) the interactions of compounds **3**-**5** within the COX-1 sites are shown in Figure 4, where enzyme-**3**, **-4**, **-5** complexes are between chain A and B. Thus compound **3** exhibits interaction with the amino acid residues Trp323, Glu326, Gln327, Gln330, Thr331, Leu334, Ser548, Thr549 and Gly551 of the chain A and Ile46, Cys47, Val48, Tyr136, Ile137, Ser138 of the chain B. Compound **4** exhibited interaction with the amino acids residues Phe367, Gly368, Ala369, Gln370, Phe371, Gln372, Tyr373, Pro542 and Glu543 in chain A (blue) and in the chain B (red) with Asn122, Phe367, Ala369, Phe371, Gln372. Compound **5** showed a similar type of interaction observed for **4**, except Gly368 (chain A), which is absent in **5** and the interaction with the amino acid residues Ile124 and Ser 126 (chain B) are observed only for **5**.

Docking results indicate that isoindolines **1**(**d**-**f**), **1h**, **2b**, **2e**-**f** and **2h** (Fig.5), independently of their functional group (ester or carboxylic acid) are bonded at chain A and they exhibit interactions with the amino acid residues observed for ibuprofen **6**, which is considered as inhibitor of the second type, due to its inhibition reversible for both COXs and also it compete with substrate for the COXs active site [5]. This suggest that isoindolines showed in figure 5 could inhibit to COX-1 by the same mechanism that **6**.

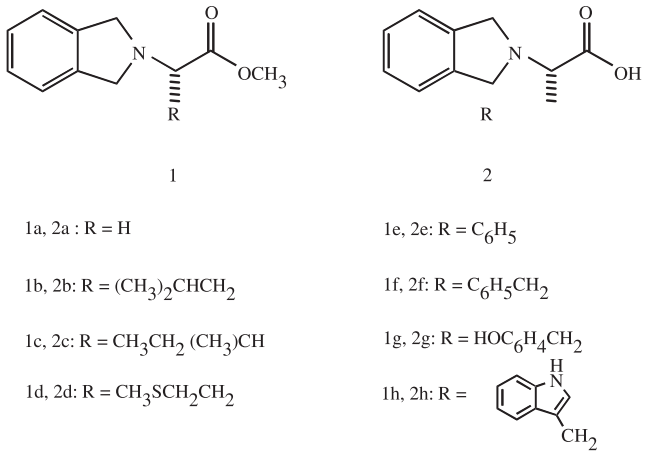

Fig. 1. Isoindolines derived from amino acids **1**(**a**-**h**) and 2(**a**-**h**).

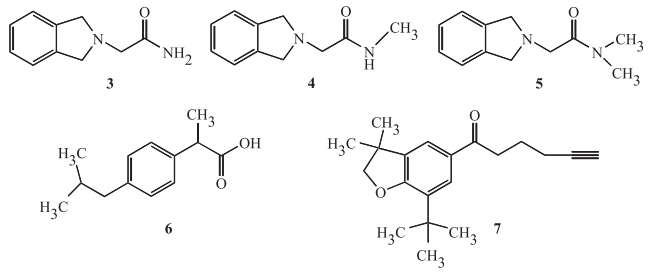

Fig. 2. Anti-inflammatory and analgesic compounds **3**-**7**.

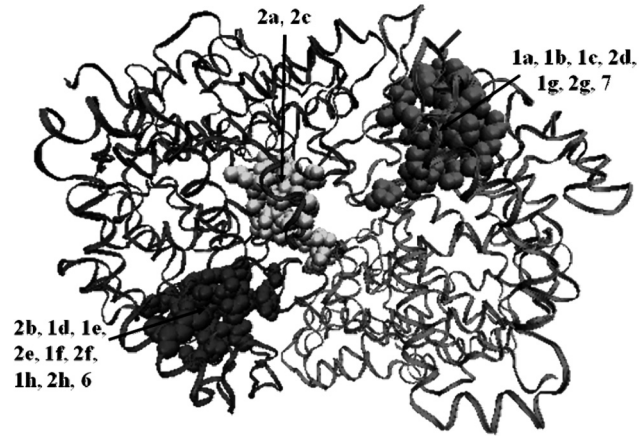

Fig. 3. Docking **1**(**a**-**h**), **2**(**a**-**h**), **6** and **7** (ball) in COX-1 site (ribbon)

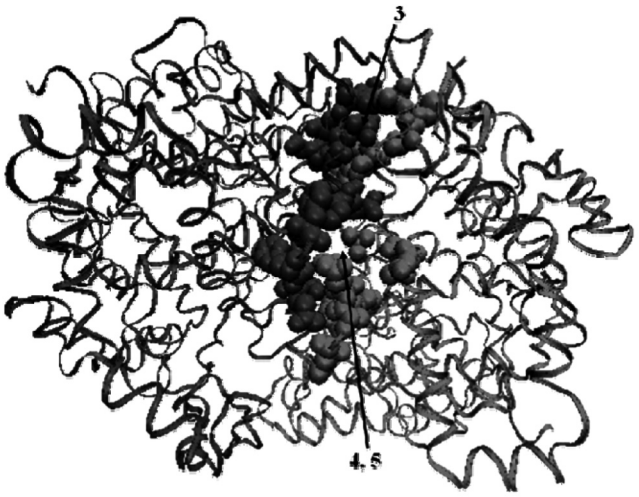

Fig. 4. Docking compounds **3-5** (ball) in COX-1 site (ribbon)

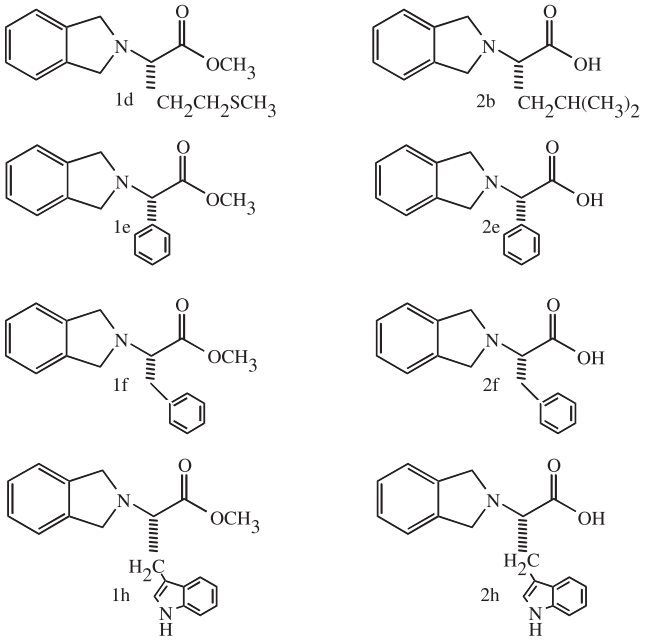

Fig. 5. Structure of isoindolines bonded at chain A within COX-1.

Therefore, docking results of isoindolines **1(a-c)**, **1g**, **2d**, **2g** and dihydrodimethylbenzofuran **7**. (Fig.6) also showed that independently of their functional group (ester or carboxylic acid), they are bonded at chain B and due to they exhibit interactions with the same amino acid residues observed for the compounds in chain A. In the case of isoindolines **2a** and **2c** (Fig.7), where both contain carboxylic group are binding into the hydrophobic channel nearby to catalytic pocket. These compounds could inhibit COX-1 as flurbiprofen, iodosuprofen and iodoindometacine, which are considered as blockers to the access of substrate to the active side cavity that lies at the end of the catalytic site [1].

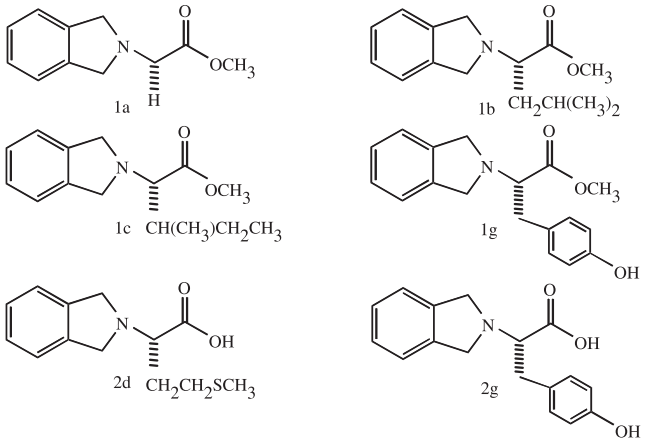

Fig. 6. Structure of isoindolines bonded at chain B within COX-1.

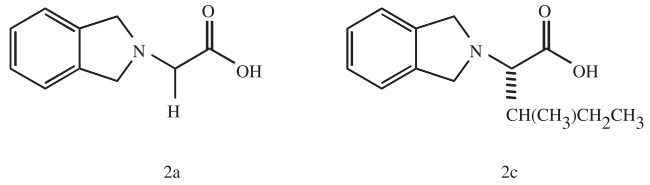

Fig. 7. Structures of isoindolines binding into the hydrophobic channel at chain A within COX-1

For isoindolines **3-5**, as mentioned before they are bending in COX-1 between chain A and B. Compound **3** is binding in the compact domain (chain B) and hydrophobic channel (A and B chains). Therefore, isoindolines **4** and **5** are binding at hydrophobic channel. It is important to notice that there is not docking study for these isoindolines and it is possible that their mechanism to inhibit COX-1 could be as blockers of the access to the active side cavity according their interaction with the amino acids to COX-1. These results could explain in part, their analgesic effect [14].

An analysis of the nearer distances between heteroatoms, carbons or protons of isoindolines **1(a-h)**, **2(a-h)**, as well as those of compounds **3** to **7** with atoms of amino acids residues of COX-1 was carried out with the aim to investigate if the compounds under study could exhibit analogous interactions within the COX-1. Thus, the values are between 1.88 and 4.98 Å, but only selected distances are giving in according to those with similar or shorter than the sum of van der Waals radius for atoms, as well as for hydrogen bond lengths for H bonds found in proteins [23]. Thus, compounds **1d**, **1e**, **1f** and **1h** having ester group and **6**, which are at chain A exhibit the following interactions within COX-1: oxygen of C=O group of **1d** shows a distance of 3.28 Å with an backbone oxygen of Cys36, for **1f**, **1h** and **6** show of 2.98, 3.54 and 3.23 Å, respectively with a carbon of the residue Pro40, for **6** of 2.85 Å with

backbone nitrogen atom of Cys41 and **1e** of 2.84 Å with a carbon of the residue Pro153. Oxygen atom of CH_3O group of **1f** exhibits a distance of 3.38 Å with a carbon of the residue Tyr39. Another interactions observed are both **1d** and **6** with Gly45 are of 3.14 Å between S atom with HO group and hydrogen bond of 2.14 Å for OH group with C=O group, respectively. Compound **1h** shows interaction of its HN with a carbon from residue Ile46 of 3.34 Å and hydrogen bond of 2.23 Å between nitrogen with HO group of Tyr130. Compounds **2b**, **2e**, **2f** and **2h**, where contain carboxylic group show the following interactions, oxygen of C=O group for compounds **2b**, **2e** and **2h** with backbone NH (3.14 Å) from Tyr39, with a carbon (3.20 Å) from Pro153 and with a carbon (2.87 Å) from Pro40, respectively. Hydroxyl group undergo interactions, for **2b** with backbone N (2.47 Å) and (2.86 Å) from Pro40 and Cys41, respectively; for **2b** with C=O group (1.91 Å) of Gly45; for **2f** with C=O (2.15 Å) group of Cys36, with two carbons (3.08 Å), (3.34) in Tyr39 and for **2h** with C=O (1.88 Å) of Cys36, and NH group of **2h** undertake interaction with OH (2.15 Å) of Tyr130 and a carbon (3.39 Å) of Pro153. Bond distance data for compounds **2a** and **2c** are: both **2a** and **2c** compounds exhibit hydrogen bond lengths between C=O and C=NH from Arg374 of 3.04 and 3.03 Å, respectively, therefore OH group of **2a** with NH_2 (2.19 Å) and for **2c** with C=O (2.05 Å) and backbone NH (2.60 Å) from Asn375. Bond distances between atoms of isoindoline **1d** and amino acid residues within COX-1 bending sites are illustrate in figure 8.

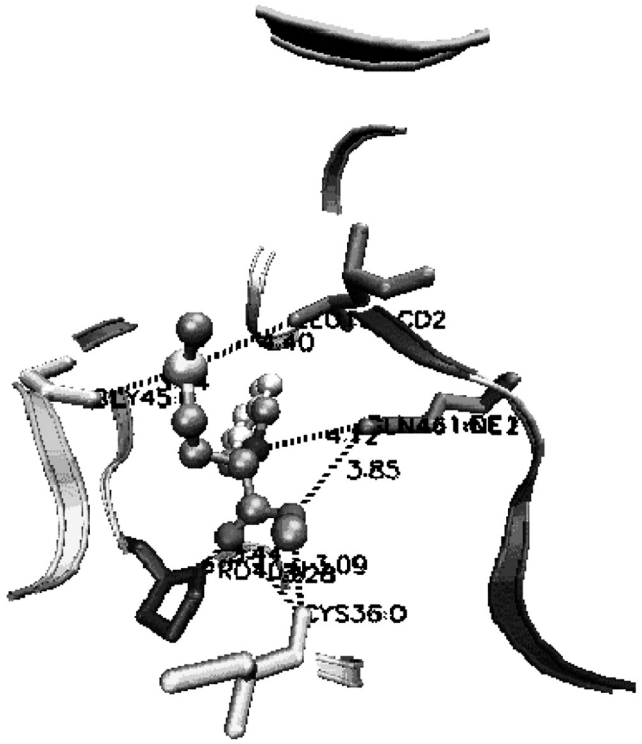

Fig. 8. Docking **1d** in the active site of COX-1, bond distances are indicated by broken lines.

In spite of compounds **1**(**a**-**c**), **1g**, **2d**, **2g** and **7,** which are fitted at chain B and among a similar amino acid residues observed for the compounds at chain A, in general they reveal superior values than sum of van der Waals radii for the atoms considered, as well as for hydrogen bond lengths for H bonds found in proteins [23]. The more relevant interaction are showing for some of them as follow: N atom of **1b** undertake interaction with backbone C=O (2.80 Å) and N (3.02 Å) of Cys41, sulfur atom of compound **2d** undergo contact with a carbon (3.62) of Cys36 and OH with backbone N (2.21 Å) of Cys41, compound **2g** exhibits interaction between HO with a carbon (3.12 Å) of Tyr39, C=O group with a carbon (2.86 Å) of Pro40 and OH belong to R group with a carbon (2.49 Å) of Ile151, and compound **7** shows interactions between C=C and backbone C=O (3.47 Å) of Ile151 and with a carbon (3.61 Å) of Pro151. Bond distances between atoms of isoindoline **2d** and amino acids residues within COX-1 binding sites are illustrate in figure 9. It is important to notice that isoindolines **1**(**e**-**h**) and **2**(**e**-**h**), which the R group hold aromatic group are overlapped within COX-1.

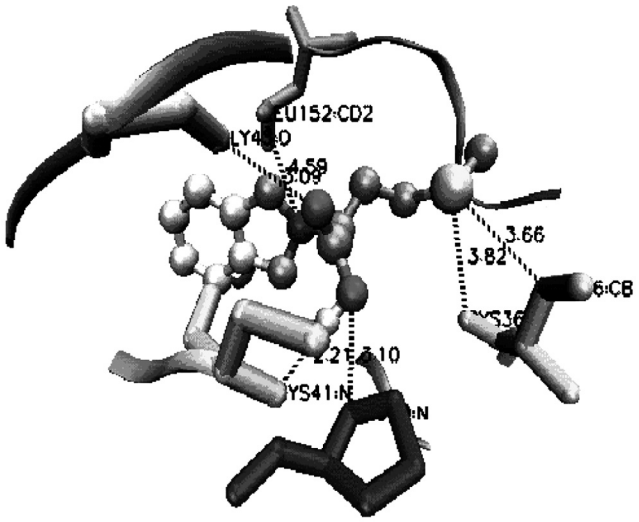

Fig. 9. Docking **2d** in the active site of COX-1, bond distances are indicated by broken lines.

As mentioned before, compounds **3** to **5** binding to COX-1 in both chains **A** and **B**, compound **3** exhibits more interactions within the sum of van der Waals radii for atoms and hydrogen bond lengths for H bonds found in proteins than **4** and **5**. Thus, **3** shows C=O interaction with a carbon (3.23 Å) of Ser138 (chain B) and a carbon (3.00 Å) of Glu330 (chain A), N atom with a carbon (3.38 Å) of Tyr136 (chain B), proton of NH undergo interactions with the following residues: carbon (2.32 Å) of Ser138 (chain B), oxygen (2.10 Å) of Gln327 (chain A), and with N atom (1.98 Å) and oxygen atoms (2.06 Å) of Thr331 (chain A). Compound **4** shows hydrogen bonds of NH with oxygen (1.96 Å) and N atom (2.12 Å) of Phe371 (chain B), and **5** reveals interaction of C=O group with oxygen (2.53 Å) of Ala369 (chain B), N atom with a carbon (3.32 Å) of Gln370 (chain B) and NCH_3 with backbone NH (3.40 Å) of Gln372 (chain A).

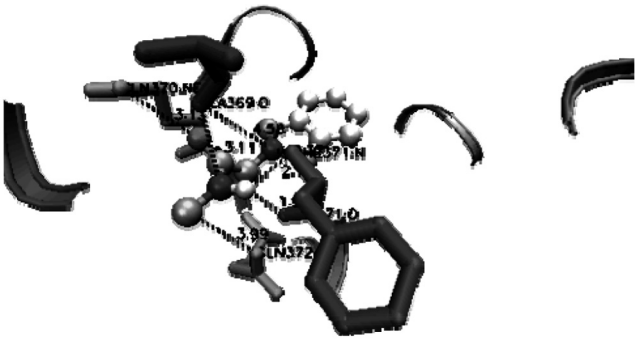

Fig. 10. Docking **4** in the active site of COX-1, bond distances are indicated by broken lines.

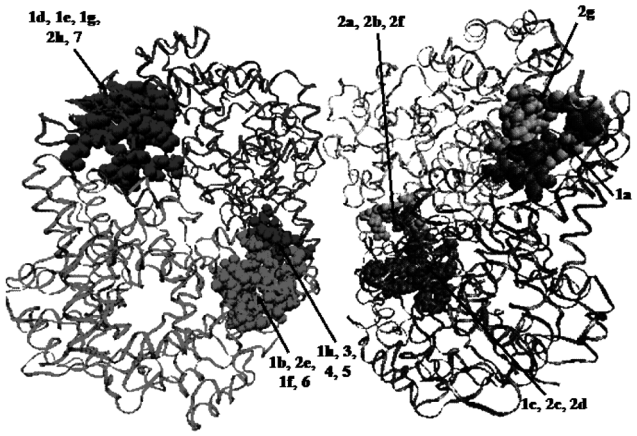

Fig. 11. Docking **1**(**a**-**h**), **2**(**a**-**h**), **3**-**7** (ball) in COX-2 sites (ribbon).

By Docking, the compound **4** in the COX-1 active sites is depicted in Figure 10.

Docking compounds **1**(**a**-**h**), **2**(**a**-**h**), **6** and **7** in the COX-2 active sites are depicted in Figure 11.

Docking results show that COX-2-**1d**, -**1e**, -**1g**, -**2h**, -**7**; COX-2-**1b**, -**2e**, -**1f,** -**6** ; COX-2-**1c**, -**2c**, -**2d** and COX-2-**2g** complexes are in the chain A, B, C and D, respectively, where the compounds undergo interactions with the amino acid residues Cys36, Asn39, Pro40, Cys41, Gln42, Asn43, Arg44, Gly45, Glu46, Cys47, Met48 (compact domain) [22] Asp125, Thr129, Tyr130, Tyr134, Gly135, Tyr136, Lys137, Arg150, Ala151, Leu152, Pro153, Pro154, Val155, Ala156, Cys159, Gln461, Glu465, Tyr466, Lys468, Arg469 (catalytic domain) [22]. Therefore, COX-2-**1h**, -**3**,-**4** and -**5** complexes are between A and B chains interacting with the following amino acid residues His320, Trp323, Glu326, Gln327, Gln330, Thr331, Leu334, Ser548, Thr549 and Gly551 in chain A and Cys36, Asn39, Glu46, Cys47, Met48, Ser49 Tyr130, Gly135, Tyr136, Lys137, Ser138, Pro153, Pro154, Ala156 in chain B, the last three residues interact only with **1h**. Compounds **1a**, **2a**, **2b** and **2f** interact within COX-2 between C and D chains, thus enzyme-**1a** complex shows interaction with Glu322, Trp323, Gln327, Gln330 and Thr331 in chain C and Glu46, Cys47, Met48, Ser49, Tyr130, Gly135, Tyr136, Lys137 in chain D. Compounds **2a**, **2b** and **2f** exhibit a similar interaction within COX-2 with some differences, in chain C: for compounds **2b** and **2f**, Asn34, Cys36, Cys37, Ser39, Cys41, Arg44, the next interaction are observed for **2a**, **2b** and **2f**, Glu46, Cys47, Met48, Ser49, Asp129, Tyr130, Gly135, Tyr136, Lys137, Ser138 and the follow are only observed for compound **2b**, Leu152, Pro153, Pro154, Val155, Ala156. In chain D: Gln327, Leu328, Gln330, Thr331, Leu334 amino acid residue interactions are observed for **2a** and **2f**, while the amino acid residue interactions Ser548, Thr549 and Gly551 are observed for **2a**, **2b** and **2f**. Analysis of the nearer distances between the atoms of isoindolines **1**(**a**-**h**), **2**(**a**-**h**), as well as compounds **3** to **7** with those atom amino acids residues of COX-2 was also carry out. Thus, the length values are between 1.93 and 5.53 Å, but only a selected distance are giving agreement to those with similar or shorter than the sum of van der Waals radii for atoms considered, as well as for hydrogen bond lengths for H bonds found in proteins [23]. Compound **1d** shows interaction of sulfur atom with oxygen (3.85 Å) of Pro153 (chain A). Compounds **7**, **2d** and **2g** undergo interactions with Asn39 as follow: =C= with N (3.28 Å, chain A), HO with a carbon (3.13 Å, chain C) and PhOH with a carbon (2.95 Å, chain D), respectively. Compounds **7**, **1f**, **1c**, **2d** and **2g** show the following contact with Cys41: C=O with N atom (3.28 Å, chain B), OCH_3 with oxygen (2.90 Å, chain B), OCH_3 with oxygen (3.30 Å, chain C), OH with N atom (2.18 Å, chain C) and PhOH with N atom (2.57 Å, chain D), respectively. Therefore, **1g** exhibits contact of C=O with a carbon (3.21 Å, chain A) of Pro153. Compound **1g** undergo interaction between PhOH with nitrogen atom (2.36 Å, chain A) of Asn43. Whereas **1b** (chain B) undergoes interaction between nitrogen and oxygen (3.01 Å) from Cys41. Compounds **2h**, **2e**, **2c** and **2g** exhibit hydrogen bond with Arg44, thus OH with NH of 2.20 Å (chain A), OH with NH of 2.29 Å (chain B), OH with N of 2.55 Å (chain C) and OH with NH of 1.96 Å (chain D), respectively, the same interaction is observed for **6** and **2c**, OH with oxygen (2.13 Å, chain B) of Glu465 and OH with NH (1.93 Å, chain C) of Arg469, respectively. While, **7** exhibits another interaction between =C and oxygen (3.87 Å, chain A) of Val155. Compound **2h** shows the contact of HN with a carbon (3.57 Å) of Glu46, NH with nitrogen (2.41 Å, chain A) of Cys47. But the time **2c** shows contact between nitrogen and a carbon (3.49 Å, chain C) of Arg44. Carbonyl group of **1c** (chain C) and **2g** (chain D) undergoes interaction with a carbon of Leu152, which values are 3.06 and 2.99 Å, respectively. Compounds **1h**, **3**, **4**, **5** at A and B chains and **1a**, **2a**, **2b**, **2f** at C and D chains show the following contacts with residues of COX-2: first are giving the interactions more frequently observed: nitrogen atom of compounds **1h**, **3**, **4**, **5**, **1a** and **2a** show contact with a carbon of Tyr136 as follow, 3.40, 3.25, 3.19, 2.44 Å (chain B), 3.09 Å (chain D) and 3.15 Å (chain C), respectively. Also **2f** exhibits interaction of nitrogen with oxygen (2.52 Å, chain C) of Cys47. Hydrogen from NH group of compounds **1h**, **3** and **4**

shows the following contact: for **1h** with a carbon (2.57 Å, chain A) of Leu334 and with backbone nitrogen (2.32 Å, chain B) of Ser138; for **3** with an oxygen (2.00 Å) and a carbon (2.66 Å) of Gln327, as well as with backbone nitrogen (2.16 Å) and oxygen (2.06 Å) of Thr331 both of them in chain A and for **4** with backbone nitrogen (2.44 Å, chain B) of Ser138. Nitrogen from NH group of compounds **3** shows interaction with a carbon (3.27 Å, chain A) of Gln330. Methyl from NCH_3 group of compounds **4** and **5** exhibits the following contacts: for **4** with oxygen (2.84 Å, chain A) of Gln330 and for **5** with a carbon (3.64 Å, chain B) of Met 48. Oxygen from C=O group of compounds **3**, **4**, **5**, **1a**, **2b** and **2f** shows the following contacts: for **3** with backbone N (2.87 Å, chain B) of Ser138; for **4** with oxygen (2.79 Å, chain A) of Thr331; for **5** with a carbon (3.36 Å, chain B) of Met48; for **1a** with backbone N (2.63 Å, chain C) of Gln327; for **2b** with a carbon (3.20 Å, chain D) of Thr549 and for **2f** with backbone N (2.66 Å, chain C) of Ser49. Hydrogen bond is exhibits from OH group of compounds **2a**, **2b** and **2f** with oxygen (2.41 Å, chain D) of Gln327, backbone N (2.41 Å, chain C) of Lys137 and with nitrogen (2.29 Å, chain D) of Gln327, respectively. These data suggest that isoindolines **1d**, **1e**, **1g**, **2h** (chain A); **1b**, **2e**, **1f** (chain B); **1c**, **2c**, **2d** (chain C) and **2g** (chain D) independently in which chain are joined, they could inhibit to COX-2 by the same mode that compounds **6** and **7** due to they have similar interaction within COX-2. Therefore, is expected that isoindolines **1h** (A and B chains); **1a**, **2a**, **2b**, and **2f** (C and D chains) could inhibit COX-2 in the same way that compounds **3**, **4** and **5** due to they have similar interactions with the amino acids residues in COX-2. It was founded that Tyr130 exhibits interaction with all compounds, except with **1d**, **1e**, **1b**, **2e**, **6** and **1h**.

ΔG (Kcal/mol) values were obtained for all compounds: -9.87 (**1h**), -9.75 (**2h**), -9.69 (**7**), -8.96 (**1f**), -8.91 (**2g**), -8.87 (**2f**), -8.83 (**1g**), -8.2 (**3**), -8.08 (**2c**), -8.06 (**2e**), -7.95 (**2b**), -7.91 (**1b**), -7.90 (**1e**), -7.74 (**1c**), -7.68 (**2d**), -7.64 (**4**), -7.56 (**5**), -7.51 (**1d**), -7.05 (**6**), -6.74 (**2a**), -6.65 (**1a**) in COX-1; -10.96 (**1h**), -9.01 (**7**), -8.74 (**3**), -8.15 (**5**), -8.02 (**4**), -7.85 (**1f**), -7.83 (**2g**), -7.77 (**1g**), -7.71 (**2f**), -7.49 (**1e**), -7.49 (**2e**), -7.36 (**2a**), -7.27 (**1b**), -7.22 (**2b**), -7.16 (**2c**), -7.13 (**2h**), -6.71 (**1c**), -6.65 (**2d**), -6.55 (**1d**), -6.48 (**1a**), -6.28 (**6**) in COX-2. Enzyme-**1h** complex shows to be more stable in both cases. Therefore, K_d (μM) values were obtained, 0.06 (**1h**), 0.07 (**2h**), 0.08 (**7**), 0.27 (**1f**), 0.29 (**2g**), 0.31 (**2f**), 0.33 (**1g**), 0.98 (**3**), 1.18 (**2c**), 1.18 (**2e**), 1.47 (**2b**), 1.53 (**1b**), 1.61 (**1e**), 2.10 (**1c**), 2.35 (**2d**), 2.53 (**4**), 2.91 (**5**), 3.12 (**1d**), 6.76 (**6**), 11.5 (**2a**), 13.5 (**1a**) in COX-1; 0.01 (**1h**), 0.25 (**7**), 0.39 (**3**), 1.07 (**5**), 1.32 (**4**), 1.76 (**1f**), 1.8 (**2g**), 2.0 (**1g**) 2.23 (**2f**), 3.23 (**1e**), 3.23 (**2e**), 4.03 (**2a**), 4.69 (**1b**), 5.02 (**2b**), 5.60 (**2c**), 5.85 (**2h**), 12 (**1c**), 13.2 (**2d**), 15.8 (**1d**), 17.9 (**1a**), 24.7 (**6**) in COX-2, where **1h** shows more affinity to both COX-1 and COX-2.

In conclusion, we describe a theoretical study of two series of isoindolines **1**(**a**-**h**) and **2**(**a**-**h**) as possible COX-1 and COX-2 inhibitors, which results were compared with those done for compounds **3** to **7** used as reference to find the active sites within COX-1 and -2. Analysis of the data showed that the isoindolines investigated exhibit interactions at the same or nearby of amino acid residues within both cyclooxygenases sites that reference compounds, consequently they could inhibit equally the enzymes. Whereas, Enzyme-**1h** complex shows to be more stable in both cases and **1h** shows more affinity to both COX-1 and COX-2 demonstrated from its K_d value. The biological activity of isoindolines will be carried out in the near future.

Molecular modeling (Docking) methodology

The ligands were drawn using Isis/draw program [24] and converted to three-dimensional format (i.e. pdb) using the WebLab Viewer and Molekel Visualization Package [25,26]. The geometry pre-optimization 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 [27] located in a Pentium IV computer. To understand the recognition mechanism between COX enzymes and the ligands, Docking simulations were done on the 3-D structure of COX 1 and COX 2 (PDB codes: 1PRH and 5COX respectively) [28,29]. Before starting the docking evaluations, the partial atomic charges (Gasteiger-Marsili formalism), as well as all possible rotable bonds of the ligands and the Kollman charges for all atoms in enzymes were assigned by using the AutoDock Tools [30]. 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 latest version of AutoDock (3.0.5) was chosen because its algorithm allows full flexibility of small ligands [30]. 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 [31]. First, a rectangular grid box were constructed over all protein (127 X 127 X 127 $Å^3$) 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.

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 (1.0×10^7). 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 [32].

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