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S-3-hydroxyphenyl (N-hydroxycarbamamido) (diphenyl) ethanethioate derivatives as potential inhibitors of 5 -Lipoxygenase (5-LOX)

Danilo L Ariza-Rua, MSc[1], Juan Rebollo-Pérez, Ph.D[2], Edisson Chavarro-Mesa, Ph.D[3], Yamil Ballestas-Casallas[4]
[1-4] Universidad Tecnológica de Bolívar, Colombia, dariza@utb.edu.co, jrebollo@utb.edu.co, echavarro@utb.edu.co, ballestasy@utb.edu.co

***Abstract* - S-3-hydroxyphenyl (N-hydroxycarbamamido) (diphenyl) ethanethioate derivatives were designed and docked into the crystal structure of 5-Lipoxygenase (5-LOX), a potential target for anti-inflammatory drugs. Four derivatives were found to have the same ability to inhibit 5-LOX activity as the anti-inflammatory drug Zileuton. This study demonstrates the potential of computational methods, specifically molecular docking, in finding 5-LOX inhibitors. The results suggest that these derivatives could be developed into new compounds for treating inflammation.**

***Keywords*—5-Lipoxygenase, molecular docking, anti-inflammatory drugs.**

I. INTRODUCTION

Lipoxygenases (LOXs) are a group of protein compounds containing a single non-heme iron cofactor involved in the hyperoxidation of polyunsaturated fatty acids such as linoleic acid (LA) and arachidonic acid (AA).[1]

There are different types of LOXs, including 5-LOX, 8-LOX, 9-LOX, 12-LOX, and 15-LOX, depending on the position of oxygen insertion within the substrate. Among these, 5-lipoxygenase (5-LOX) converts AA into pro-inflammatory metabolites, leukotrienes (LTs), 5-hydroxyeicosatetraenoic acid (5-HETE), 5-oxo-eicosatetraenoic acids (5-oxoETEs), as well as anti-inflammatory metabolites, lipoxins (LXs)[2].

LTs play an important role in inflammatory diseases such as asthma, allergic rhinitis, atherosclerosis, rheumatoid arthritis, psoriasis, autoimmune ulcerative colitis, inflammatory bowel disease, lupus, and cancer [3-8]. Therefore, 5-LOX is a potential target for the development of anti-inflammatory drugs, and the search for biologically active compounds for its inhibition is a promising direction in the prevention and treatment of inflammatory diseases of various etiologies[9-11]. However, Zileuton is the only 5-lipoxygenase inhibitor approved that targets rheumatoid arthritis, asthma, psoriasis, allergic rhinitis, and inflammatory bowel disease by inhibiting leukotriene (LT) biosynthesis.[12, 13]

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Computational methods are very useful tools to speed up the drug discovery process. In recent years, molecular docking has become one of the most effective methods for finding 5-LOX inhibitors. Molecular docking is a powerful computational tool for simulating molecular interactions and predicting binding and affinity modes between receptors and ligands. Using this strategy, we can test various non-existent or yet-to-be-synthesized molecules that can be designed using molecular fragments of biologically active compounds. The fragments can be a hydrophobic or hydrophilic binding site, a hydrogen bonding domain, or groups of Zileuton atoms.[14-17]

In this work, we aimed to design S-3-hydroxyphenyl (N-hydroxycarbamamido) (diphenyl) ethanethioate derivatives as potential inhibitors of 5 -Lipoxygenase derivates using different molecular fragments of known properties. A fragment of the selective 5-lipoxygenase inhibitor, Zileuton (A), two aryl hydrophilic binding sites (B and E), an aryl hydrophobic binding site (C), and a hydrogen bonding domain (D). This fragment B, C y D are present in representative structures of classical NSAIDs (nonselective COX inhibitors) and Structures of COX-2 selective inhibitors. The objective was to dock the designed compounds with the enzyme 5-LOX to predict the binding affinity in the active site of the enzyme that is composed of amino acid residues Trp147, Phe177, Tyr181, His 362, Glu363, Thr364, His 367, Leu368, His372, Leu373, His 367, Ile406, Asn407, Glu412, Arg411, Leu414, Ile415, Glu417, Phe421, His550, Trp599, His600, Arg603, Arg60, Leu607, and Ile673.[18, 19]

II. MATERIAL AND METHODS

A. *Ligand preparation and optimization*

A 167-member library of S-3-hydroxyphenyl (N-hydroxycarbamide) (diphenyl) ethanethioate derivatives with electron-donating and electron-withdrawing groups at the R^1, R^2, and R^3 positions of the phenyl ring was designed. See figure 1.

Three-dimensional (3D) ligand structures were drawn in ACD/ChemSketch (https://www.acdlabs.com/resources/free-chemistry-software-apps/chemsketch-freeware) and saved in

SDF file format. SDF files were converted to PDB format using PyMOL. Gasteiger charges and non-polar hydrogens were assigned to the compounds using Autodock Vina, and the compounds were saved in PDBQT format and optimized with the 500-step MMFF94 force field of the steepest descent algorithm set in the Avogadro 1.2.0 package software.

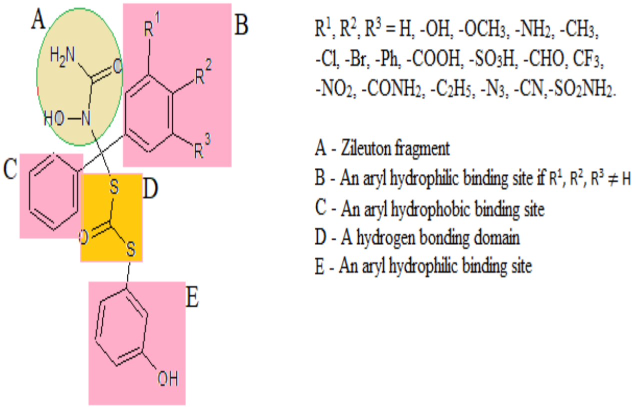

Fig 1: R^1, R^2 and R^3 substituted S-3-hydroxyphenyl (N-hydroxycarbamamido) (diphenyl) ethanethioate derivatives

To compare the interactions and possible inhibitory effects of S-3-hydroxyphenyl (N-hydroxycarbamamido) (diphenyl) ethanethioate derivatives, the selective 5-lipoxygenase inhibitor Zileuton was used as a reference.

B. PASS-biological activity assess of ligands

The online software PASS (Prediction of Activity Spectra for Substances) [20] was used to predict the biological activity of the ligands. The program is designed to predict over 4000 biological activities by relating probabilities of activity (Pa) and inactivity (Pi). Activities, including drug and non-drug effects, can be used to identify the most likely targets with 90 % accuracy.

C. Protein preparation

The crystal structure of LOX-5 with a resolution of 2.39 Å was retrieved from the Protein Data Bank (PDB:3O8Y): https://www.rcsb.org/structure/3O8Y and edited with AutoDockTools (ADT) to remove all the co-crystalline water molecules, chains, and heteroatoms not required, add polar hydrogen atoms to amino acid residues, and assign partial charges to the receptor. The resulting protein structure was optimized using a molecular mechanics method with a Kollman force field and Amber charge with an energy gradient of 0.001 kcal/mol per 1000 interactions. Finally, the 3D structure of 5-LOX was saved in PDBQT format.

D. Molecular docking

Molecular docking of S-3-hydroxyphenyl (N-hydroxycarbamamido) (diphenyl) ethanethioate derivatives into the active site of 5-LOX was performed by Autodock Vina[21]. The ligand docking sites for 5-LOX were defined by creating a 50 × 52 × 66 Å dot grid and setting the grid spacing to 1 Å. This includes active sites of proteins identified in literature reviews. The x, y, and z coordinates of 5-LOX were -0.104, 19.314, and 0.689, respectively. Ten runs were performed for each ligand and the best pose was saved for each run. Finally, the average binding affinity of the best poses was accepted as the binding affinity value for a particular complex.

III. RESULTS

A. Validation of docking protocol

To test the docking parameters of ligands, Zileuton was docked into the active site of 5-LOX, with an affinity of -7.77 kcal/mol. The reproducibility of the active site configuration and amino acid residue interaction types is shown in figure 2, and table 1.

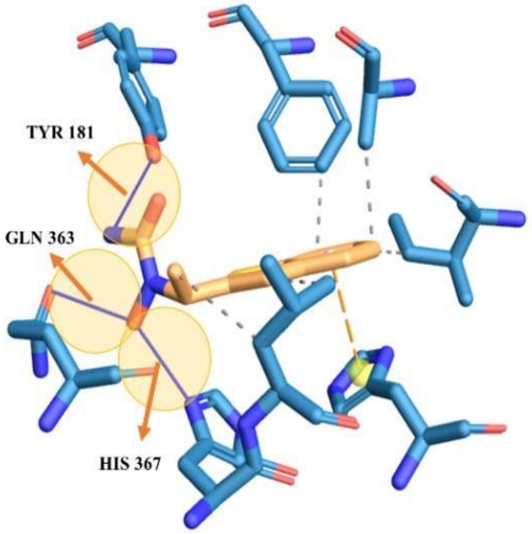

Fig 2: Interaction Protein-Ziluetón

TABLE I
LIPOXYGENASE-5 AMINO ACID RESIDUE INTERACTION TYPES WITH ZILEUTON

Residue	H-A (Å)	D-A (Å)	Donor Angle
GLN 363	2.01	2.79	135.88
HIS 367	2.21	3.00	151.47
TYR 181	3.52	4.08	116.92

B. Molecular docking of S-[(N-hydroxycarbamamido) (diphenyl)methyl] S-3-hydroxyphenyl (N-hydroxycarbamamido) (diphenyl) ethanethioate derivatives into the active site of 5-LOX .

The molecular docking protocol was performed by ten runs of the 5-LOX (PDB: 3O8Y) at its binding site. The conformations with the best affinity value show that AutoDock Vina successfully reproduces the binding of this ligand in the binding site of the protein. The estimated free energy of binding (kcal/mol) S-3-hydroxyphenyl (N-hydroxycarbamamido) (diphenyl) ethanethioate derivatives on 5-LOX structure are presented in table 2. Of the 167 compounds tested, only 4 ligands had negative value between -3,5 and -6,49 kcal/mol-

TABLE II
MOLECULAR DOCKING RESULTS OF LIGANDS TO LOX-5

Ranking	**No.**	**Compound**	**Estimated FreeEnergy of Binding (kcal/mol)**
1	1	R1=R2=R3= H	-6,46
2	22	R2= CH_3, R1=R3=H	-6,06
3	2	R1= OH, R2=R3=H	-4,99
4	5	R1= CH_3, R2=R3=H	-3,5
		Zilueton	-7.77

These S-3-hydroxyphenyl (N-hydroxycarbamamido) (diphenyl) ethanethioate derivatives with scores ranging from -3.5 to -6.46 kcal/mol could be potential 5-LOX ligands.

*C. Interactions of 5-*LOX *amino acid residues and* S-3-*hydroxyphenyl (N-hydroxycarbamamido) (diphenyl) ethanethioate derivatives*

The compounds satisfactorily fit into the binding pocket, considering the number of interactions and their theoretical value of affinity for the binding site. hydrophobic-type bonds and hydrogen bonds predominated in the coupling. Hydrogen bonds give greater stability to the binding of the ligand with the enzyme.to the binding site.

The interactions of S-[(N-hydroxycarbamamido) (diphenyl)methyl] S-3-hydroxyphenyl carbonodithioate derivatives (R1=R2=R3= H) with 5-LOX amino acid residues at the protein binding site are shown in figure 3and table 3.

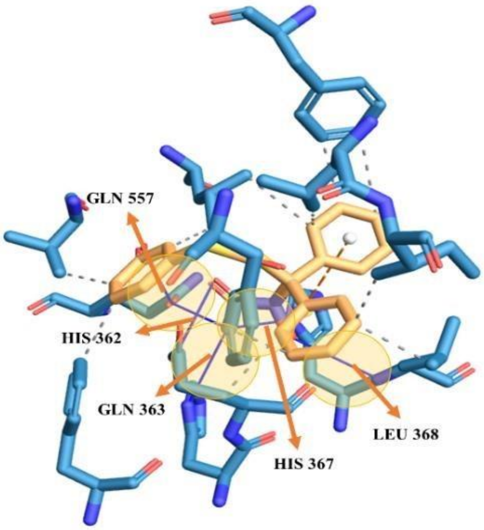

Fig 3. Amino acid residues in the interaction of 5-LOX with compound No.1

TABLE III
LIPOXYGENASE-5 AMINO ACID RESIDUE INTERACTION TYPES WITH COMPOUND NO. 1

Residue	H-A (Å)	D-A (Å)	Donor Angle
GLN 557	1.74	2.67	150.60
HIS 367	1.91	2.67	147.53
GLN 363	2.93	3.41	116.84
LEU 368	3.45	3.92	117.26
HIS 362	3.46	3.89	107.27

The interactions of S-[(N-hydroxycarbamamido) (diphenyl)methyl] S-3-hydroxyphenyl carbonodithioate derivatives (R2= CH3, R1=R3=H) with 5-LOX amino acid residues at the protein binding site are shown in figure 4 and table 4.

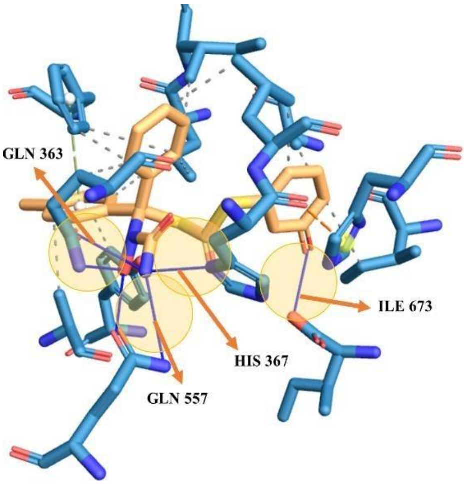

Fig 4. Amino acid residues in the interaction of 5-LOX with compound No.22

TABLE IV
LIPOXYGENASE-5 AMINO ACID RESIDUE INTERACTION TYPES WITH COMPOUND NO. 22

Residue	H-A (Å)	D-A (Å)	Donor Angle
HIS 367	1.92	2.65	141.58
ILE 673	2.19	2.97	135.53
GLN 363	2.53	3.09	114.02
GLN 557	3.32	3.64	105.43

S-[(N-hydroxycarbamamido) (diphenyl)methyl] S-3-hydroxyphenyl carbonodithioate derivatives (R1= OH, R2=R3=H) interact with 5-LOX amino acid residues at the protein binding site are shown in figure 5 and table 5.

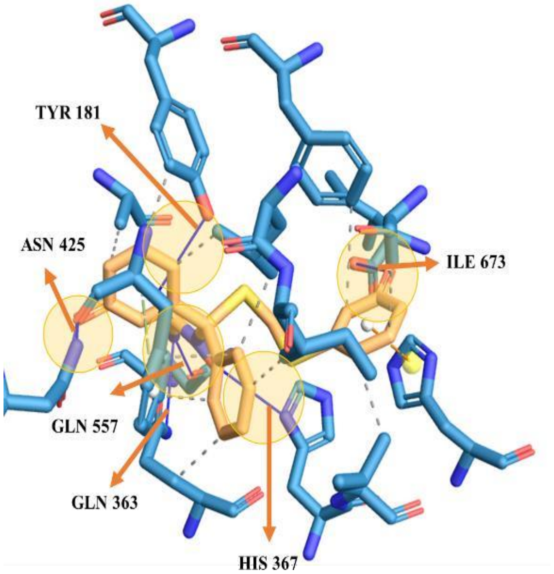

Fig 5. Amino acid residues in the interaction of 5-LOX with compound No.2

TABLE V
LIPOXYGENASE-5 AMINO ACID RESIDUE INTERACTION TYPES WITH COMPOUND NO. 2

Residue	H-A (Å)	D-A (Å)	Donor Angle
GLN 557	1.66	2.28	115.52
ASN 425	1.83	2.59	146.27
ILE 673	2.25	3.06	141.15
GLN 363	2.81	3.47	135.06
HIS 367	2.86	3.58	142.35
TYR 181	2.84	3.60	155.64

Figure 6 and table 6 show he interactions of S-[(N-hydroxycarbamamido) (diphenyl)methyl] S-3-hydroxyphenyl carbonodithioate dertivatives (R2= CH3, R1=R3=H) with 5-LOX amino acid residues at the protein binding site.

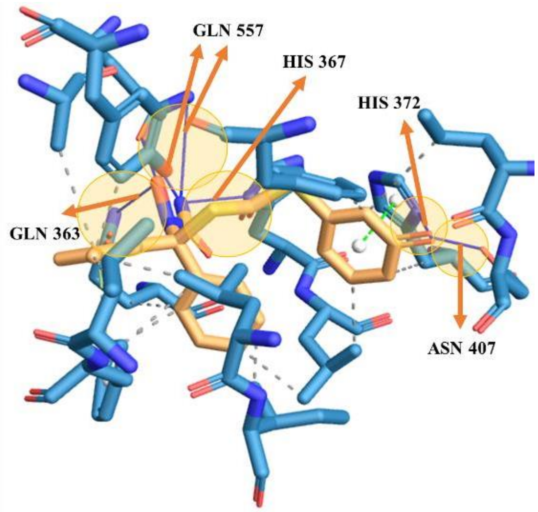

Fig 6. Amino acid residues in the interaction of 5-LOX with compound No.5

TABLE VI
LIPOXYGENASE-5 AMINO ACID RESIDUE INTERACTION TYPES WITH COMPOUND NO. 5

Residue	H-A (Å)	D-A (Å)	Donor Angle
HIS 367	1.82	2.60	149.64
ASN 407	1.95	2.70	131.91
GLN 557	2.08	2.74	120.25
HIS 372	2.83	3.45	130.46
GLN 363	3.21	3.74	121.65

IV. DISCUSSION

The protein-ligand complex structure reveals that His 367, Gln 363 and Gln 557 formed a hydrogen bond with the four complexes, while Ile 673 appeared in two complexes with a hydrogen bond (Table 7). This suggests that these amino acid residues are essential for protein binding. [22]

The four S-[(N-hydroxy carbamamido) (diphenyl)methyl] -S-3-hydroxy phenyl carbonodithioate derivatives interact with two of the amino acids (Gln-363, His-367) similar to those in the pocket where Zileuton binds the protein, implying that all three derivatives act at the same site as Zileuton. The findings also show that compound 3 binds three amino acids (Tyr-181, Gln-363, His-367) to the active site of 5-LOX like zileuton, but its binding energy is lower than that of zileuton.

TABLE VII
LIPOXYGENASE-5 RESIDUES INTERACTING S-[(N-HYDROXYCARBAMAMIDO) (DIPHENYL)METHYL] -S-3-HYDROXY PHENYL CARBONODITHIOATE DERIVATIVES

Compounds	Protein amino acid residues interacting with mono-substituted 4-nitrochalcone derivatives
1	His-362, Gln-363, His-367, Leu-368, Gln-557
2	Gln-363, His-367, Gln-557, Ile 673
3	Tyr-181, Gln-363, His-367, Asn-425, Gln-557, Ile 673
4	Gln-363, His-367, His-372, Gln-557, Asn-407
Zileuton	Tyr-181, Gln-363, His-367

Better free energy of binding was obtained for compound 1, which has no electron-donating or electron-withdrawing groups at the R1, R2, or R3 positions. However, compound 3 with an electron donating substituent in R1 such as OH has a good affinity value and interacts with amino acid residues similar to those of Zileuton.

V. CONCLUSIONS

Lipoxygenase-5 protein was successfully docked to four S-[(N-hydroxycarbamamido) (diphenyl)methyl]-S-3-hydroxyphenylcarbonodithioate derivatives. These derivatives interact at the active site of 5-LOX in the same way as Zileuton. Although the values obtained by molecular docking analysis should only be considered as theoretical approximations, this is useful for studying possible anti-inflammatory mechanisms of these derivatives.

Molecular docking studies confirmed the biological activity predicted by PASS online, which demonstrated that the proposed ligands have action against 5-lipoxygenase.

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