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IN-SILICO STUDY: COX2 (CYCLO-OXYGENASE 2), AN UNKNOWN TARGET OF HYDROXYCHLOROQUINE REPOSITIONED FOR THE TREATMENT OF COVID 19 INFECTION AND CARDIOVASCULAR EFFECTS

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Keywords:

Covid 19, Cardiovascular effects, Hydroxychloroquine, Cyclooxygenase 2, Molecular docking

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ABSTRACT: Hydroxychloroquine, widely used to treat certain autoimmune diseases and inflammatory conditions, has been repositioned as a new lead against the COVID-19 pandemic. Preliminary studies have suggested that hydroxychloroquine inhibits the entry of the SARS-CoV2 virus mediated by the ACE2 receptor and is a particularly interesting anti-inflammatory and immunomodulatory action in the cytokine storm during COVID-19 infection. Furthermore, cardiovascular effects have been reported in hypertensive patients. These last are thought to result from the inhibition of cyclooxygenase 2. Our results reveal for the first time in atomic detail how hydroxychloroquine, one of the most controversial drugs, has an affinity for a new molecular target (COX2) with a docking score: -7.6 (kcal/mol) and a root mean square deviation (RMSD = 2.542). Hydroxychloroquine binds to cyclo-oxygenase 2 via two hydrogen bonds (H-GLY136 and H-GLY45) and four hydrophobic bonds: Cl -CYS36, 0 -CYS36, 0 -CYS47 and 0 -PRO154). This mechanism has never been elucidated. Hydroxychloroquine proved to be promiscuous. Its structure, which differs from that of non-steroidal anti-inflammatory drugs , tends to show activity against a wide variety of biological targets : As an enzyme inhibitor (bioactivity = 0.15), GPCR receptor ligand (bioactivity = 0.35), ion channel modulator (bioactivity = 0.30), kinase inhibitor (bioactivity = 0.44) and protease inhibitor (bioactivity = 0.12). This approach improves the understanding of its mechanism of action and the side effects attributed to it.

INTRODUCTION: While the main scientific research has focused on chronic non-communicable diseases in recent decades. The year 2019-2020 was marked by the emergence of a new COVID-19 epidemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1, 2]. This pandemic has challenged health systems around the world [3].

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Hydroxychloroquine (4-amino-quinoline), widely used to treat some autoimmune diseases and inflammatory affections, has been repositioned as a new lead against COVID-19 infection [4, 5].

Preliminary studies have suggested that hydroxy-chloroquine inhibits ACE2 receptor-mediated entry of the SARS-CoV2 virus [6, 7] and has an anti-inflammatory and immunomodulating action particularly interesting in the cytokine storm during COVID-19 infection [8]. Thereafter, new research has highlighted the unfavourable evolution of this infection in patients treated with non-steroidal anti-inflammatory drugs (NSAIDs) [9]. Besides the gastro-digestive effects, serious cardiovascular complications have been previously reported when

using cyclo-oxygenase. In the light of scientific debates led by researchers from all over the world on the benefit/risk ratio of hydroxychloroquine, the objective is to check if, by sharing anti-inflammatory properties with NSAIDs by binding to cyclooxygenase, hydroxychloroquine, causes fatal cardiovascular effects in hypertensive patients with Covid 19.

MATERIALS AND METHODS:

Preparation of Receptor: In the literature, several crystalline structures of COX-1 and COX-2 in complex with several inhibitors and substrates have been identified. With arachidonic acid (their physiological substrate) [10], classic NSAIDs such as Diclofenac classic NSAIDs such as Diclofenac [11], Naproxen [12], as well as coxibs such as celecoxib [13]). COX-2 is induced by inflammation, while COX-1 is physiological. The X-ray crystallographic structure of ibuprofen bound to cyclooxygenase-2 (4PH9) (Mus musculus) [14], was obtained from the Protein Data Bank (PDB) (http://www.rcsb.org) with a resolution <3.00 Å (resolution: 1.81 Å, free R-value: 0.197, working R-value: 0.160, observed R-value: 0.162). This file is not directly used by the Autodock 1.5.6 tools. It was first visualized using Discovery Studio Visualizer version 2.5.5.

Discovery Studio is a software package of biological molecular design for chemists and computational biologists. (Discovery Studio 2.5 (CDOCKER Dock, Dassault Systemes BIOVIA, USA). Water molecules, ligands and other heteroatoms have been removed from the protein. This crystallographic structure has been preserved without any treatment for molecular docking. Co-crystallized inhibitors/substrates (ibuprofen) were used to define the corresponding active site, the flexible residues within this active site is a criterion for the validation of mooring calculations (re-docking). By a literature review, common residues involved in the binding site were identified [10, 15, 12, 16, 14]. The PDB file of the protein has been efficiently prepared using the AutoDock / Vina (Molecular Graphics Lab, Le plugin Scripps Research Institute, La Jolla, CA, USA) , and saved as pdbqt file. Polar hydrogens and Kollman chrages have been added.

Preparation of the Ligand: The necessary information about hydroxychloroquine was collected on the ChemSpider platform (http://www.chemspider.com/): Hydroxychloroquine (Molecular formula: $C_{18}H_{26}ClN_3O$, Average mass: 335.871 Da, Monoisotopic mass: 335.176453 Da, ChemSpider ID: 3526). - Chloroquine (Molecular formula $C_{18}H_{26}ClN_3$, Average mass 319,872 Da, Mono-isotopic mass 319.181519 Da, ChemSpider ID: 2618). Their chemical structures are almost similar to [17]. These two anti-malarial and anti-rheumatic drugs have antiviral activity *in-vitro*. Chloroquine interferes with different stages of the viral life cycle [18]. The 3D structure of the Ligand was downloaded from the Drugbak database (https://www.drugbank.ca/): Access number DB01611. Then the identification and verification were performed on Discovery Studio Visualizer version 2.5.5. The ligand was then exported to AutoDock tools 1.5.6. Torsional connections have been verified. The ligand was saved in the same docking folder in pdbqt format. Charges were then added.

Molecular Docking Protocol: Docking calculations were performed using the standard AutoDock Vina parameters. AutoDock Vina docking programme is generally in the top position among all the methods tested in CASF-2013. Vina is the best of all methods in terms of reception power. Ligand minimisation has a significant impact, reducing the performance difference between AutoDock and Vina [19]. The active site was placed in a 40 × 50 × 40 Å grid box, in the geometrical center of the set of selected flexible residues at 0.375 Å as the grid spacing. Values of the root mean square deviation (RMSD) between the docking and the initial poses have been calculated. Results of the docking were initially examined in Discovery Studio. (Discovery Studio 2.5 (CDOCKER Dock, Dassault Systemes BIOVIA, USA). The best poses were ranked according to Vina scores (kcal/mol), evaluated by the free bond energies (score S, kcal/mol) and the binding interactions between the ligand atom and the residues of the active site. To predict the bioactivity of hydroxychloroquine for cox2 receptors, we used Molinspiration (https://www.molinspiration.com/). This server predicts bioactivity score for the most important therapeutic targets such as GPCR receptors, kinase inhibitors, ion channel modulators, enzymes, and nuclear receptors [20]. SwissADME database (Swissadme.ch/index. php) was used to compare physiochemical properties, ADME parameters and pharmacokinetic properties of hydroxychloroquine with certain anti-

inflammatory drugs. Smiles (chemical structure) for each structure were downloaded from the Drug Bank database (Drugbank.ca/). Statistical analyses (mean and standard deviation) were performed using Minitab statistical software Version 16. Mann-Whitney test was used to comparison of median Docking values.

RESULTS AND DISCUSSION:

Identification of the COX2 Interaction Site: Each COX isoform is considered as heterodimer with a catalytic site and an allosteric site [21]. Inhibitors may act at one or both sites, depending on the structure and concentration of the inhibitor [22]. Whatever the site, binding requires that a small molecule must first enter through the binding domain to the four-helix membrane in an open area called the "lobby" [23]. As shown in **Fig. 1a** and **1b**, Ibuprofen was first identified in the pocket of the COX2 enzyme binding site.

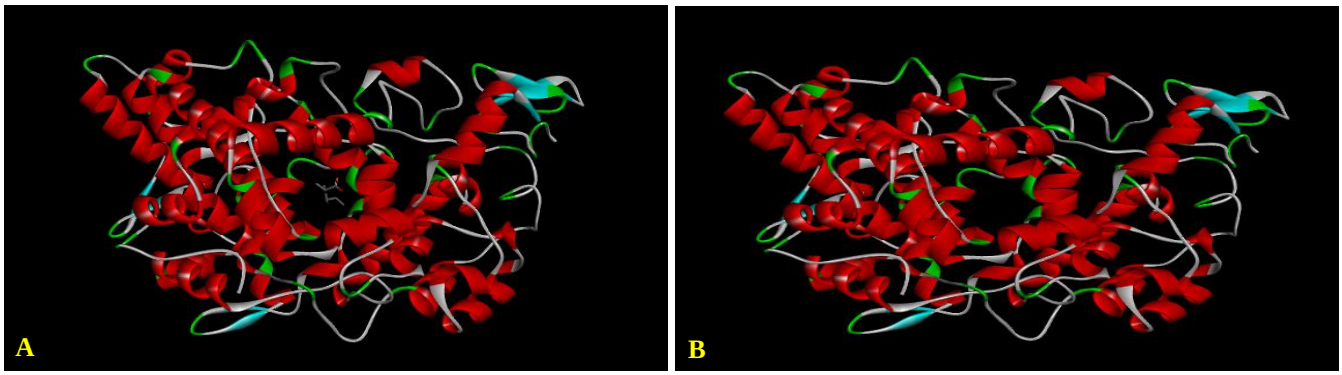

FIG. 1: COX2 POCKET (1A: BINDING IBUPROFEN, 1B FREE)

Interactions between COX2-Ibuprofen have been illustrated in Fig. 2a and 2b.

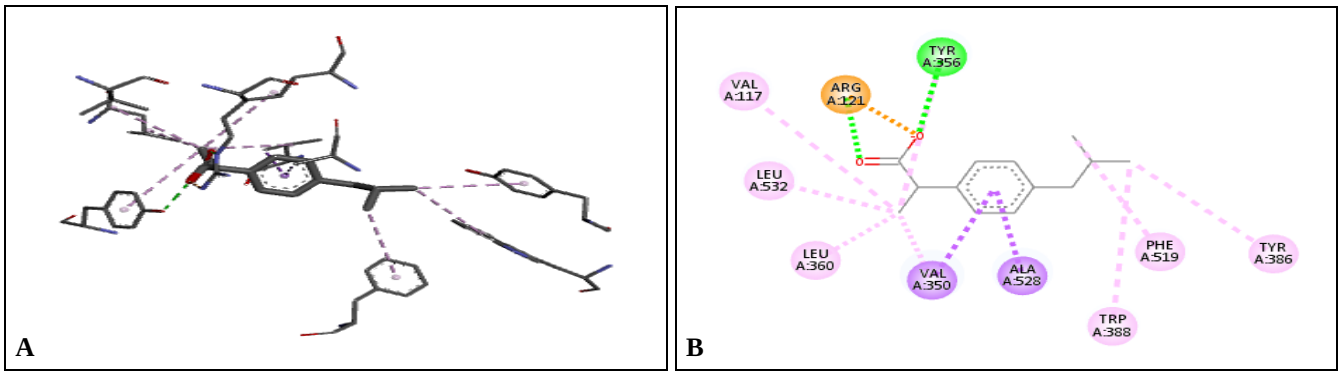

FIG. 2: A AND B: RECEIVER-LIGAND INTERACTIONS ON A 2D DIAGRAM

Coordinates of the Grid Box (X, Y, Z points, dimensions and spacing) used for the Ibuprofen-COX2 model (shown in **Fig. 3**) have been noted to help identify the Hydroxychloroquine-COX2 interaction. Prepared hydroxychloroquine must first enter through the open area called the “hall” **Fig. 4**.

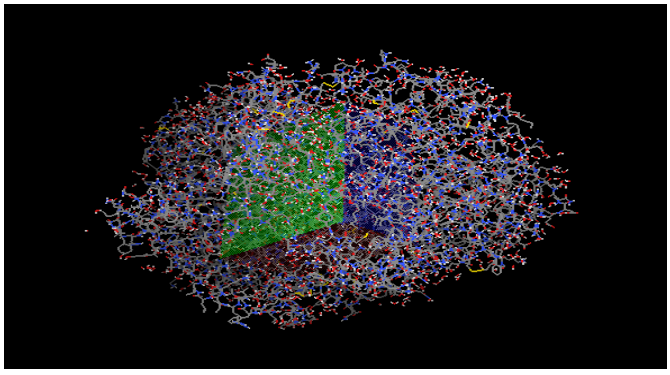

FIG. 3: GRID BOXIBUPROFEN-COX2

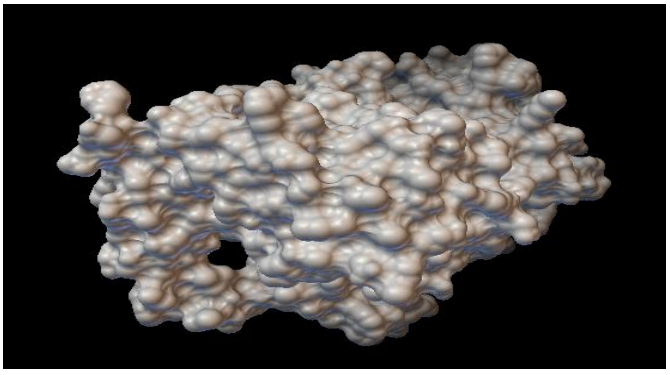

FIG. 4: PREPARED RECEPTOR (COX2)

Molecular Docking Simulations: Molecular docking was chosen as the first choice as a method of discrimination of the hydroxychloroquine-COX2 interaction due to the ability to simulate the binding of small compounds in the active site of enzymes, based on *in-silico* prediction of putative competitors [24].

In our work, the active site was identified in the A chain of COX2 of ibuprofen bound COX2 structure. Our simulations involved only a single monomer, as some works [25], or others that have focused on a smaller fragment of protein, as the membrane-binding domain [26], or the membrane binding domain and helices comprising active site of cyclooxygenase [27]. In addition, previous studies have indicated that many NSAIDs (including IBP) bind tightly to a single monomer of COX-2 dimer and allosterically inhibit the oxygenation of the substrate in the partner monomer [28, 29].

Binding affinities of the different poses in the COX2 active site A validation docking of crystal structure of murine (mu) COX-2 in complex with Ibuprofen (IBP) [14]. (PDB ID: 4PH9) (http: / /www.rcsb.org/pdb) was performed on Autodock vina after viewing on discovery studio and treatment of the ligand and receptor. Results are shown in **Table 1**.

TABLE 1: RESULTS OF THE BEST MOLECULAR DOCKING POSES: IBUPROFEN-COX2

Mode	Affinity (kcal / mol)	Distribution of RMSD	Best mode RMSD
1	-7,3	22,181	23,884
2	-6,4	22,236	23,890
3	-6,3	21,384	22,730
4	-6,3	22,279	23,833
5	-6,2	14,475	15,595
6	-6,1	23,055	24,780
7	-6,1	16,577	17,580
8	-6,0	25,852	27,421

Complex formed by Ibuprofen (pose 1) -COX 2 of experimental model of Orlando and al has the lowest energy value and shows the best mooring score (Affinity: -7.3 (kcal/mol) with a root mean square deviation of (RMSD = 23.884). Docking results of all hydroxychloroquine poses with the target (COX 2) are shown in **Table 2**.

TABLE 1: DOCKING RESULTS OF ALL HYDROXYCHLOROQUINE POSES WITH THE TARGET (COX 2)

Mode	Affinity (kcal / mol)	Distribution of RMSD	Best mode RMSD
1	-7,6	1,816	2,542
2	-7,3	4,402	8,010
3	-7,3	2,936	4,617
4	-6,5	3,815	5,854
5	-6,2	11,579	14,151
6	-6,1	10,063	13,409
7	-6,0	12,299	15,319
8	-6,0	13,774	16,865

Mann-Whitney test **Table 3** reveals that the median affinity of the Hydroxychloroquine/COX2 complex (-6,350 kcal/mol) is significantly higher than the median affinity of the complex of the model crystallized by Orlando *et al*., in 2015: Ibuprofen /COX2 (-6,250 kcal/mol), (P-Value= 0.6712), IC (95%): (-0.200; 1.200).

Pose that has the lowest binding energy of the docking score will be considered the best pose and inhibits the target receptor because lower binding energy corresponds to higher binding affinity [30]. Comparison of the pose-complex scores revealed that the complex formed by Hydrochloroquine (pose1) -COX 2 has the lowest energy value and gives the best docking score (Affinity: -7.6 (kcal/mol) with the best root mean square deviation (RMSD = 2.542). Hydroxychloroquine -COX 2 complex can be considered to be stable with a higher binding affinity.

Based on these docking results, we can classify hydroxychloroquine as a good inhibitor of the COX2 enzyme. 3D representations of the best pose interactions between poses and receptor were visualized using Discovery studio software. The COX2-hydroxychloroquine poses formed are shown in **Fig. 5**.

TABLE 3: PARAMETER OF THE MANN-WHITNEY TEST

Complex	Median affinity (kcal/mol)	Average affinity (kcal/mol)	Standard deviation (kcal/mol)	Maximum affinity (kcal/mol)	Minimum affinity (kcal/mol)	Coefficient of variation
Hydroxychloroquine/COX2	-6,350	-6,625	0,6671	-7,6	-6,0	0 ,1
Ibuprofene/COX2	-6,250	-6,3375	0,4103	-7,3	-6,0	0,06

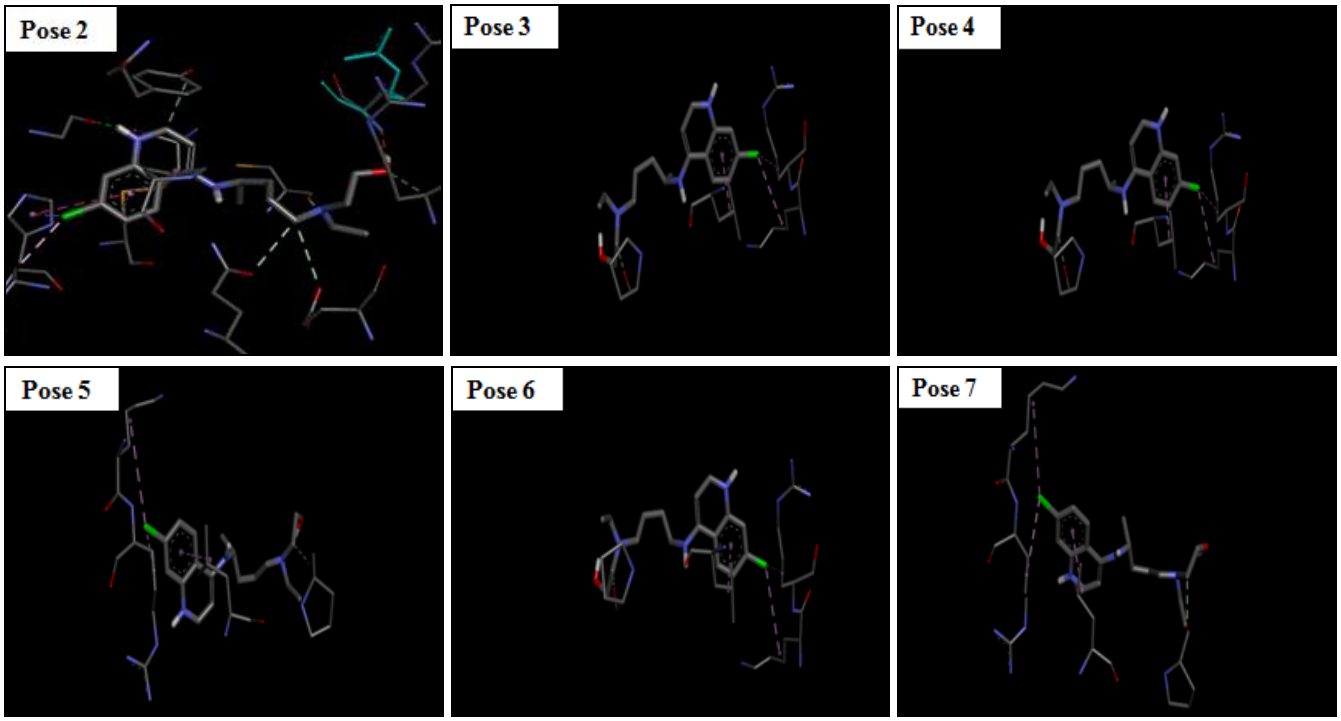

FIG. 5: COX2-POSES COMPLEXES FORMED (COX2 BOUND TO HYDROXYCHLOROQUINE)

Binding mode observed for the best pose with receptor pocket is shown in **Fig. 6, 7**.

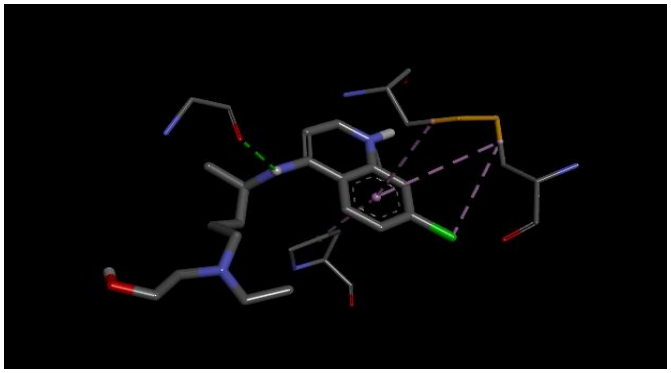

FIG. 6: 3D VIEW OF HYDROXYCHLOROQUINE (POSE1) - COX2 INTERACTION

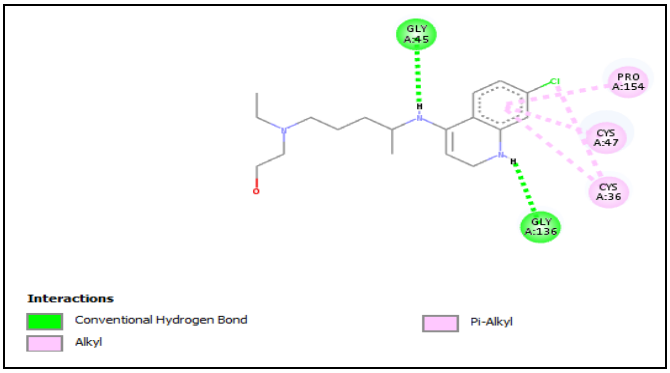

FIG. 7: 2D VIEW OF HYDROXYCHLOROQUINE (POSE1) - COX2 INTERACTION

Types of interactions, bonds, and amino acids involved in the Hydroxychloroquine-COX2 complex are shown in **Table 4**. Hydroxychloroquine binds to COX2 through two hydrogen bonds (in H-GLY136 and H-GLY45) and four hydrophobic bonds: Cl -CYS36, 0 -CYS36, 0 -CYS47, and 0 -PRO154) at different distances (2.68043) A, 2.46642 A, 4.47781 A, 5.06953 A, 4.66876 A and 4.23862 A, respectively). Glycine with a hydrogen (H) radical is devoid of any hydrophobic or hydrophilic character. GLY45 and GLY136 bind to hydroxychloroquine through hydrogen bonds, H-GLY136 and H-GLY45) . Cysteine is hydrophobic, but its SH group binds to another cysteine to produce a strong (covalent) bond, the disulfide bridge. CYS36 and CYS47 are involved in hydrophobic bonds with hydroxychloroquine. Interestingly, CYS36 is doubly committed, with oxygen and chlorine atoms of hydroxychloroquine. Although the physiological significance of chloride binding at this position is unknown, chloride ions have previously been used to identify the molecular oxygen binding site in various proteins, including dioxygenases [31, 32]. This raises the possibility that the chloride ion may be indicative of the position of molecular oxygen prior to incorporation into COX substrates. In addition, proline (PRO154), which is highly hydrophobic, binds like CYS36 and CYS47 to the oxygen atom of hydroxychloroquine. Arylcarboxylic acid inhibitors bind in one of two orientations of the COX active site [12]. Flurbiprofen binds canonically with its hydrogen paired carbo-

xylate fraction to the Arg-120 and Tyr-355 residues constriction site [12]. In contrast, diclofenac binds in an inverted orientation in which its carboxylate is hydrogen-bonded to the side chains of Tyr-385 and Ser-530 [11]. Ibuprofen occupies an area of the enzyme between the opening of the substrate channel and the top of the active site. A total of thirteen contacts is made between the isobutyl group of ibuprofen and Trp-387, Met-522, Val-523, Gly-526, Ala-527, and Ser-530. In addition, the α-methyl group of ibuprofen makes contact with Val-349 and Leu-359 [14]. Rofecoxib binds to the active site of cyclooxygenase in the same general conformation as observed for celecoxib [16]. Rofecoxib makes a total of 42 contacts with residues lining the cyclooxygenase channel [33].

TABLE 4: TYPES OF INTERACTIONS, BONDS AND AMINO ACIDS INVOLVED: HYDROXYCHLOROQUINE-COX2

Nom	Distance	Catégories	Types	From	From chemistry	To	To chemistry	Angle DHA	Angle HAY
:UNK0:H5 - A:GLY136:O	2,68043	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:H5	H-Donor	A:GLY136:O	H-Acceptor	104,688	154,153
:UNK0:H31 - A:GLY45:O	2,46642	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:H31	H-Donor	A:GLY45:O	H-Acceptor	136,328	138,467
:UNK0:Cl - A:CYS36	4,47781	Hydrophobic	Alkyl	:UNK0:Cl	Alkyl	A:CYS36	Alkyl		
:UNK0 - A:CYS36	5,06953	Hydrophobic	Pi-Alkyl	:UNK0	Pi-Orbitals	A:CYS36	Alkyl		
:UNK0 - A:CYS47	4,66876	Hydrophobic	Pi-Alkyl	:UNK0	Pi-Orbitals	A:CYS47	Alkyl		
:UNK0 - A:PRO154	4,23862	Hydrophobic	Pi-Alkyl	:UNK0	Pi-Orbitals	A:PRO154	Alkyl		

Prediction of Bioactivity: The diversity of possible drug targets (each of which requires a different combination of corresponding molecular characteristics) is so enormous. To predict the bioactivity of hydroxychloroquine for different receptors, we used Molinspiration (https://www.molinspiration.com/). This server predicts the bioactivity score for the most important therapeutic targets such as GPCR receptors, kinase inhibitors, ion channel modulators, enzymes, and nuclear receptors [20]. The bioactivity of a compound was decided based on the bioactivity score. If the bioactivity score is> 0, it is an active compound while <-5.0 is an inactive compound, and the range between -5.0 and 0.0 corresponds to moderately active compounds [34].

TABLE 5: PREDICTION OF THE BIOACTIVITY OF HYDROXYCHLOROQUINE AND OTHER NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

Molecules	GPCR Ligand	Ion channel	Kinase inhibitor	nuclear receptor	Protease inhibitor	Enzyme inhibitor
hydroxychloroquine	0,35	0,30	0,44	-0,12	0,12	0,15
Ibuprofen	-0,17	-0,01	-0,72	0,05	-0,21	0,12
Diclofenac	0,14	0,20	0,17	0,09	-0,10	0,25
Naproxène	-0,11	-0,06	-0,38	0,14	-0,26	0,15
Piroxicam	-0,42	-0,57	-0,50	-0,73	-0,04	0,18
Célécoxib	-0,06	-0,27	0,01	-0,28	-0,06	0,17

Hydroxychloroquine was the only one to show high activity against more than one target receptor. (GPCR, kinase inhibitors, ion channel modulators, protease inhibitor, and enzyme inhibitor) in **Table 5** (bioactivity greater than zero). In drug discovery, activity against multiple targets means that a molecule will have multiple side effects. Further proof of our docking results, hydroxychloroquine is a COX2 enzyme inhibitor (bioactivity as an enzyme inhibitor : 0.15), like other NSAIDs whose enzymatic inhibitory biactivity is between 0.12 and 0.25. Hydroxychloroquine is active on GPCR ligand receptors (bioactivity : 0.35). Impact of GPCR structures on drug discovery is very important. Structure-based drug design has been applied to an increasing number of GPCR targets over the last decade [35]. Hydroxychloroquine was also active on modulating ion channel receptors (bioactivity : 0.30). Ligand-dependent ion channels are also pharmacological targets for the development of new therapies [36]. For receptors of kinase inhibitors, the bioactivity of hydroxychloroquine is 0.44). Receptor tyrosine kinases are key regulators of cellular processes and their role in the pathophysiology of many diseases is well recognized [37]. The structure of hydroxychloroquine, which differs from that of nonsteroidal anti-inflammatory drugs, tends to show activity against

a wide variety of biological targets. On this fact, it is sometimes said that such a compound has a promiscuous behavior. Several drugs may be promiscuous, especially in the case of drug reuse [38]. which could explain the cardiovascular effects related to the repositioning of hydroxychloroquine in hypertensive patients during COVID 19 infection.

Pharmacokinetics of Hydroxychloroquine: The SwissADME database (Swissadme.ch/index.php) was used to compare physicochemical data and pharmacokinetic properties of hydroxychloroquine with certain anti-inflammatory drugs. The smiles (chemical structure) of each structure were down-loaded from the Drugbank database (Drugbank.ca/). First, we checked whether the molecules comply with the conditions of the following five Lipinski rules [39]): molecular weight: ≤ 500, number of hydrogen bond donors: ≤ 5, number of hydrogen bond acceptors: ≤ 10, lipophilicity (in LogP): ≤5. Lipinky's rule analysis with SwssADME in **Table 6**.

TABLE 6: LIPINKY'S RULE FOR HYDROXYCHLOROQUINE AND CERTAIN NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

Molecules	Formula	Molecular weight (g / mol)	Number of H Acceptors	Number of H donors	LogP
Hydroxychloroquine	$C_{18}H_{26}ClN_3O$	335.87	3	2	3.87
Ibuprofen	CC (C) CC1 = CC = C(C = C1) C (C) C (O) = O	206.28	2	1	3.5
Diclofenac	OC (= O) CC1 = CC = CC = C1NC1 = C (Cl) C = CC = C1Cl	296.15	2	2	4.98
Naproxène	COC1 = CC2 = C (C = C1) C = C (C = C2) [C @ H] (C) C (O) = O	230.26	3	1	3.29
Piroxicam	CN1C (C (= O) NC2 = NC = CC = C2) = C (O) C2 = C (C = CC = C2) S1 (= O) = O	331.35	5	2	2.2
Célécoxib	CC1 = CC = C (C = C1) C1 = CC (= NN1C1CC = C (C = C1) S (N) (= O) = O) C (F) (F) F	381.37	7	1	3.99

As much as other NSAIDs, hydroxychloroquine respects the lipinski rule **Table 6**. Ibuprofen, diclofenac, naproxen, piroxicam and cecoxib have the following molecular weights, respectively: 335.87, 206.28, 296.15, 230.26, 331.35, 381.37), g /mol, they all have a molecular weight ≤ 500 g/ mol. By comparing the lipophilicity (LogP) values of our ligands, we observed that they all have values below 5 and therefore can be easily absorbed. However, all ligands have a number of hydrogen bond donors: ≤ 5, a number of hydrogen bond acceptors: ≤ 10.

The fact that hydroxychloroquine is taken into account for the management of COVID-19 patients clearly highlights the need to better understand its pharmacokinetics. The bioavailability after oral administration of hydroxychloroquine can reach 80% with a plasma peak of approximately 2 to 4 hours. Its large volume of distribution (200 to 800 L / kg) explains its long half-life, which could range from 30 to 60 days [40, 41]. Hydroxychloroquine are metabolized *via* CYP-450 enzymes into other active compounds, which are responsible for extensive pharmacological actions and increased toxicity.

Hydroxychloroquine is mainly excreted *via* the kidneys (60%) as unchanged or metabolized, and the remainder (40%) is usually eliminated by the liver, feces and skin or stored in other lean body tissues [42].

CONCLUSION: In view of the cardiovascular effects reported in hypertensive patients, particular attention should be paid to repositioned hydroxy-chloroquine in the treatment of COVID 19. Our results reveal for the first time in atomic detail how hydrochloroquine, one of the most controversial drugs binds to a new molecular target (COX2) with a mooring core (Affinity: -7.6 (kcal/mol) and a root mean square deviation (RMSD = 2.542). Hydroxychloroquine has been shown to be promiscuous. Its structure, which differs from that of non-steroidal anti-inflammatory drugs, tends to show activity against a wide variety of biological targets. This discovery improves the understanding of its mechanism of action and the adverse effects attributed to it.

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CONFLICTS OF INTEREST: No direct or indirect interest (of a financial or other nature) with a private, industrial or commercial organization relationship with the subject presented.

REFERENCES:

1. Vellingiri B, Jayaramayya K, Iyer M, Narayanasamy A, Govindasamy V, Giridharan B, Ganesan S, Venugopal A, Venkatesan D, Ganesan H, Rajagopalan K, Rahman P, Cho SG, Kumar NS and Subramaniam MD: Covid-19: A promising cure for the global panic. Sci Total Environ 2020; 725: 138277.
2. Chen J, Lu H, Melino G, Boccia S, Piacentini M, Ricciardi W, Wang Y, Shi Y and Zhu T: Covid-19 infection: The china and italy perspectives. Cell Death Dis 2020; 11: 438.
3. WHO. Who announces covid-19 epidemic a pandemic. World Health Organization, Genève, Suisse 2020.
4. Ferner RE and Aronson JK: Chloroquine and hydroxychloroquine in covid-19. Bmj 2020; 369: m1432
5. Zhai P, Ding Y, Wu X, Long J, Zhong Y and Li Y: The epidemiology, diagnosis and treatment of covid-19. International Journal of Antimicrobial Agents 2020; 55: 105955.
6. Yao X, Ye F, Zhang M, Cui C, Huang B, Niu P, Liu X, Zhao L, Dong E, Song C, Zhan S, Lu R, Li H, Tan W and Liu D: *In-vitro* antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory syndrome coronavirus 2 (sars-cov-2). Clin Infect Dis 2020; 71: 732-39.
7. Devaux CA, Rolain JM, Colson P and Raoult D: New insights on the antiviral effects of chloroquine against coronavirus: What to expect for covid-19? International Journal of Antimicrobial Agents 2020; 55: 105938.
8. Guastalegname M and Vallone A: Could chloroquine /hydroxychloroquine be harmful in coronavirus disease 2019 (covid-19) treatment? Clin Infect Dis 2020; 71: 888-89.
9. Day M: Covid-19: Ibuprofen should not be used for managing symptoms, say doctors and scientists. BMJ 2020; 368: m1086.
10. Kiefer JR, Pawlitz JL, Moreland KT, Stegeman RA, Hood WF, Gierse JK, Stevens AM, Goodwin DC, Rowlinson SW, Marnett LJ, Stallings WC and Kurumbail RG: Structural insights into the stereochemistry of the cyclooxygenase reaction. Nature 2000; 405: 97-101.
11. Rowlinson SW, Kiefer JR, Prusakiewicz JJ, Pawlitz JL, Kozak KR, Kalgutkar AS, Stallings WC, Kurumbail RG, Marnett LJ. A novel mechanism of cyclooxygenase-2 inhibition involving interactions with ser-530 and tyr-385. Journal of Biological Chemistry 2003; 278: 45763-769.
12. Duggan KC, Walters MJ, Musee J, Harp JM, Kiefer JR, Oates JA and Marnett LJ: Molecular basis for cyclooxygenase inhibition by the non-steroidal anti-inflammatory drug naproxen. Journal of Biological Chemistry 2010; 285: 34950-959.
13. Wang JL, Limburg D, Graneto MJ, Springer J, Hamper JRB, Liao S, Pawlitz JL, Kurumbail RG, Maziasz T and Talley JJ: The novel benzopyran class of selective cyclooxygenase-2 inhibitors. Part 2: The second clinical candidate having a shorter and favorable human half-life. Bioorganic & Medicinal Chemistry Letters 2010; 20: 7159-63.
14. Orlando BJ, Lucido MJ and Malkowski MG: The structure of ibuprofen bound to cyclooxygenase-2. J Struct Biol 2015; 189: 62-66.
15. Rowlinson SW, Kiefer JR, Prusakiewicz JJ, Pawlitz JL, Kozak KR, Kalgutkar AS, Stallings WC, Kurumbail RG and Marnett LJ: A novel mechanism of cyclooxygenase-2 inhibition involving interactions with ser-530 and tyr-385. J Biol Chem 2003; 278: 45763-769.
16. Wang JL, Carter J, Kiefer JR, Kurumbail RG, Pawlitz JL, Brown D, Hartmann SJ, Graneto MJ, Seibert K and Talley JJ: The novel benzopyran class of selective cyclo-oxygenase-2 inhibitors-part i: The first clinical candidate. Bioorganic & Medicinal Chem Letters 2010; 20: 7155-58.
17. Zhou D, Dai SM and Tong Q: Covid-19: A recommendation to examine the effect of hydroxychloroquine in preventing infection and progression. Journal of Antimicrobial Chemotherapy 2020; 75(7): 1667-70.
18. Yan Y, Zou Z, Sun Y, Li X, Xu K-F, Wei Y, Jin N and Jiang C: Anti-malaria drug chloroquine is highly effective in treating avian influenza a h5n1 virus infection in an animal model. Cell Research 2013; 23: 300-02.
19. Gaillard T: Evaluation of autodock and autodock vina on the casf-2013 benchmark. Journal of Chemical Information and Modeling 2018; 58: 1697-1706.
20. Ertl P, Rohde B and Selzer P: Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. J Med Chem 2000; 43: 3714-17.
21. Mitchener MM, Hermanson DJ, Shockley EM, Brown HA, Lindsley CW, Reese J, Rouzer CA, Lopez CF and Marnett LJ: Competition and allostery govern substrate selectivity of cyclooxygenase-2. Proceedings of the National Academy of Sciences 2015; 112: 12366-371.
22. Dong L, Sharma NP, Jurban BJ and Smith WL: Pre-existent asymmetry in the human cyclooxygenase-2 sequence homodimer. Journal of Biological Chemistry 2013; 288: 28641-655.
23. Picot D, Loll PJ and Garavito RM: The x-ray crystal structure of the membrane protein prostaglandin h2 synthase-1. Nature 1994; 367: 243-49.
24. Wu CH, Arighi CN and Ross KE: Protein Bioinformatics: From Protein Modifications and Networks to Proteomics, Springer New York. Methods in Molecular Biology 2017; 472.
25. Cukier RI and Seibold S: Molecular dynamics simulations of prostaglandin endoperoxide h synthase-1. Role of water and the mechanism of compound i formation from hydrogen peroxide. The Journal of Physical Chemistry B 2002; 106: 12031-44.
26. Nina M, Bernèche S and Roux B: Anchoring of a monotopic membrane protein: The binding of prostaglandin H_2 synthase-1 to the surface of a phospholipid bilayer. Eur Biophys J 2000; 29: 439-54.
27. García-Nieto R, Pérez C and Gago F: Automated docking and molecular dynamics simulations of nimesulide in the cyclooxygenase active site of human prostaglandin-endoperoxide synthase-2 (cox-2). J Comput Aided Mol Des 2000; 14: 147-60.
28. Dong L, Vecchio AJ, Sharma NP, Jurban BJ, Malkowski MG and Smith WL: Human cyclooxygenase-2 is a sequence homodimer that functions as a conformational heterodimer. J Biol Chem 2011; 286: 19035-46.
29. Duggan KC, Hermanson DJ, Musee J, Prusakiewicz JJ, Scheib JL, Carter BD, Banerjee S, Oates JA and Marnett

LJ: (r)-profens are substrate-selective inhibitors of endocannabinoid oxygenation by cox-2. Nat Chem Biol 2011; 7: 803-09.
30. Simon L, Imane A, Srinivasan K, Pathak L and Daoud I: *In-silico* drug-designing studies on flavanoids as anticolon cancer agents: Pharmacophore mapping, molecular docking, and monte carlo method-based qsar modeling. Interdisciplinary Sciences: Computational Life Sciences 2017; 9: 445-58.
31. Colloc'h N, Gabison L, Monard G, Altarsha M, Chiadmi M, Marassio G, Sopkova-de Oliveira Santos J, El Hajji M, Castro B, Abraini JH and Prangé T: Oxygen pressurized x-ray crystallography: Probing the dioxygen binding site in cofactorless urate oxidase and implications for its catalytic mechanism. Biophys J 2008; 95: 2415-22.
32. Steiner RA, Janssen HJ, Roversi P, Oakley AJ and Fetzner S: Structural basis for cofactor-independent dioxygenation of n-heteroaromatic compounds at the alpha/beta-hydrolase fold. Pro Nat Aca Sci U S A 2010; 107: 657-62.
33. Marnett LJ: The coxib experience: A look in the rearview mirror. Annu Rev Pharmacol Toxicol 2009; 49: 265-90.
34. Balakrishnan N and Raj NK: *In-silico* studies on new indazole derivatives as gsk-3î² inhibitors". International J of Pharmacy and Pharmaceutical Sci 2015; 7: 295-99.
35. Congreve M, de Graaf C, Swain NA and Tate CG: Impact of GPCR structures on drug discovery. Cell 2020; 181: 81-91.
36. Lara CO, Burgos CF, Moraga-Cid G, Carrasco MA and Yévenes GE: Pentameric ligand-gated ion channels as pharmacological targets against chronic pain. Frontiers in Pharmacology 2020; 11: 167.
37. Pottier C, Fresnais M, Gilon M, Jérusalem G, Longuespée R and Sounni NE: Tyrosine kinase inhibitors in cancer: Breakthrough and challenges of targeted therapy. Cancers 2020; 12: 731.
38. Gupta MN, Alam A and Hasnain SE: Protein promiscuity in drug discovery, drug-repurposing and antibiotic resistance. Biochimie 2020; 175: 50-57.
39. Lipinski CA, Lombardo F, Dominy BW and Feeney PJ: Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 2001; 46: 3.
40. Rainsford K, Parke AL, Clifford-Rashotte M and Kean W: Therapy and pharmacological properties of hydroxy-chloroquine and chloroquine in treatment of systemic lupus erythematosus, rheumatoid arthritis and related diseases. Inflammopharmacology 2015; 23: 231-69.
41. Yamada Y, Hidefumi K, Shion H, Oshikata M and Haramaki Y: Distribution of chloroquine in ocular tissue of pigmented rat using matrix-assisted laser desorption/ ionization imaging quadrupole time-of-flight tandem mass spectrometry. Rapid Communications in Mass Spectro-metry 2011; 25: 1600-08.
42. Kalia S and Dutz JP: New concepts in antimalarial use and mode of action in dermatology. Dermatologic Therapy 2007; 20: 160-74.

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