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ECHAHID HAMMA LAKHDAR UNIVERSITY OF EL-OUED

FACULTY OF NATURAL AND LIFE SCIENCES

DEPARTMENT OF CELLULAR AND MOLECULAR BIOLOGY

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THEME

Comprehensive Computational Exploration: Design, Molecular Docking ,MD Simulation ,ADMET Analysis and Toxicity Prediction of Novel Analogues Based on Diclofenac for Improved Anti-Inflammatory Proprieties

Presented by:

- Bouhamed Abd El-Raouf
- Bekkouche Omar
- Necib Brahim El-Khalil

Before the jury composed of :

President:	**Dr, Laoufi Hayat**	**Université d'El Oued.**
Examiner:	**Dr. Ghania Ahmed**	**Université d'El Oued.**
Promoter:	**Dr. LANEZ Elhafnaoui**	**Université d'El Oued.**
Co-Promoter :	**Dr. BENAMOR Mohmmed Larbi**	**Université d'El Oued.**

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Abstract

Résumé

خلاصة

Abstract

In This study aims to design novel derivatives based on diclofenac, a well-known nonsteroidal anti-inflammatory drug (NSAID), to enhance its anti-inflammatory properties. towards cyclooxygenase-(COX) using in silico approaches .Virtual screening was performed using SwissADME and ProTox web servers. The results showed good ADMET properties for the selected compounds.Molecular docking study showed that all compounds were active against the main COX where DI3 and DI 6 interacted the best with lowest binding free energy equal to -10.6 and -10.24 kcal/mol. However, only DI16 have shown a good binding energy equal to -7.04 with cyclooxygenase . The predicted IC50 values were comparatively similar to the IC50 of standard compounds.

Keywords: diclofenac, cyclooxygenase ADMET, Molecular docking.

Résumé

Cette étude vise à concevoir de nouveaux dérivés à base de diclofénac, un anti-inflammatoire non stéroïdien (AINS) bien connu, pour améliorer ses propriétés anti-inflammatoires. vers la cyclooxygénase-(COX) par des approches in silico Le dépistage virtuel a été effectué à l'aide des serveurs Web SwissADME et ProTox. Les résultats ont montré de bonnes propriétés ADMET pour les composés sélectionnés.Une étude d'amarrage moléculaire a montré que tous les composés étaient actifs contre la COX principale, où DI3 et DI 6 interagissaient le mieux avec une énergie libre de liaison la plus faible égale à -10,6 et -10,24 kcal/mol. Cependant, seuls les DI16 ont montré une bonne énergie de liaison égale à -7,04 avec la cyclooxygénase. Les valeurs CI50 prévues étaient comparativement similaires à la CI50 des composés standards.

Mots clés : diclofénac, cyclooxygénase ADMET, amarrage moléculaire.

خلاصة

تهدف هذه الدراسة إلى تصميم مشتقات جديدة تعتمد على الديكلوفيناك، وهو عقار معروف مضاد للالتهابات غير الستيرويدية (NSAID)، لتحسين خصائصه المضادة للالتهابات نحو انزيمات الأكسدة الحلقية- (COX) عن طريق نهج السيليكو .تم إجراء الفحص الافتراضي باستخدام خوادم الويب SwissADME وProTox. أظهرت النتائج خصائص ADMET جيدة للمركبات المختارة. أظهرت دراسة الالتحام الجزيئي أن جميع المركبات كانت نشطة ضد COX الرئيسي، حيث تفاعل DI3 وDI6 بشكل أفضل مع أدنى طاقة حرة ملزمة تساوي -10.6 و-10.24 كيلو كالوري/مول. ومع ذلك، أظهر DI16 فقط طاقة ربط جيدة تساوي -7.04 مع إنزيمات الأكسدة الحلقية كانت قيم IC50 المتوقعة مشابهة نسبيًا لقيم IC50 للمركبات القياسية.

الكلمات المفتاحية: ديكلوفيناك، إنزيمات الأكسدة الحلقية ADMETالالتحام الجزيئي.

Summary

List of figures

List of Tables

Introduction

Introduction

Computational investigation is a powerful tool in drug design and development, integrating various techniques such as molecular design, molecular docking, molecular dynamics (MD) simulation, ADMET (absorption, distribution, metabolism, excretion and toxicity) analysis, and toxicity prediction. This study aims to take advantage of these techniques to design new derivatives based on diclofenac, a well-known nonsteroidal anti-inflammatory drug (NSAID), to enhance its anti-inflammatory properties. This process is done by:

-Designing derivatives of the novel:

The process begins with designing new diclofenac derivatives using molecular design tools.

-Molecular docking:

After the design phase, molecular docking studies are performed to evaluate the interactions between the new derivatives and potential biological targets.

-Molecular dynamics (MD) simulation:

MD simulations are used to study the stability of designed compounds in biological environments over time.

-ADMET analysis:

ADMET analysis is performed to evaluate the pharmacokinetic properties of the designed derivatives. This includes evaluating how well compounds are absorbed into the body, distributed through tissues, metabolism, excretion, and potential toxicity.

-Toxicity prediction:

Finally, computational models are used to predict the potential toxicity of new derivatives.

First part
Bibliographic synthesis

Chapter I

Cyclooxygenase

(COX)

1. Cyclooxygenase (COX)

1.1 Definition

The enzyme Prostaglandin endoperoxide synthase, also known as cyclooxygenase (COX), is responsible for converting arachidonic acid into the potent lipid mediators called prostaglandins and thromboxane. There are two COX isoforms - COX-1 and COX-2 - which were discovered in order. These end products play a crucial role in diverse pathophysiological processes such as inducing vascular inflammation during tissue damage or infection, regulating body temperature through fever perception, providing relief from pain sensation, facilitating haemostasis of blood vessels when required while protecting gastric mucosa against harmful effects of stomach acids besides having the ability to induce childbirth or regulate kidney function; all making them targets for nonsteroidal anti-inflammatory drugs .**(Turini and DuBois. 2002; Botting and Botting. 2008)**

1.2. Biosynthesis of prostaglandins

Prostanoids (PG's) are end products of fatty acid metabolism produced via the COX pathway. PG's have long been known to behave as important physiological and pathological mediators implicated in a number of therapeutic areas of interest including inflammation, pain, pyrexia, cancer, glaucoma, male sexual dysfunction, osteoporosis, cardiovascular disease, labor and asthma **(Abramovitz and Metters. 1998**).

Arachidonic acid (AA), an unsaturated 20-carbon fatty acid embedded in cell membranes as a phospholipid ester, is the precursor for PG synthesis (Figure 1). In response to a wide variety of stimuli, free AA is released which is subsequently converted via COX, lipoxygenase (LOX) and cytochrome P450 enzyme catalysis to various lipid mediators known collectively as eicosanoids **(Smith, DeWitt, and Garavito. 2000)**. In the COX pathway, the two known COX isoforms. catalyse the first committed step in the biosynthesis of PG's, thromboxane (TxA) and other eicosanoids (**Abramovitz and Metters. 1998; Smith et al. 2000; Fitz Gerald and Patrono .2001).**

The production of these eicosanoids is dependent on **t**he availability of AA. The release of AA from membrane phospholipids is mediated by either secretory (sPLA2) or cytoplasmic (cPLA2) phospholipases. Once AA is released, the COX isoforms catalyse two sequential reactions. The initial COX reaction converts AA to prostaglandin. G2 (PGG2). The subsequent peroxidase (POX) reaction reduces PGG2 to prostaglandin H2 (PGH2) which is then converted by various cell specific isomerases and synthases to produce five biologically active primary PG's that include prostaglandin D2 (PGD2), prostaglandin E2 (PGE2), prostaglandin F2α (PGF2α), prostacyclin

(PGI2) and thromboxane A2 (TxA2) as illustrated in (Figure 1). These products act as secondary messengers by interacting with prostanoid G-protein coupled receptors and other receptors **(Thuresson et al. 2001).**

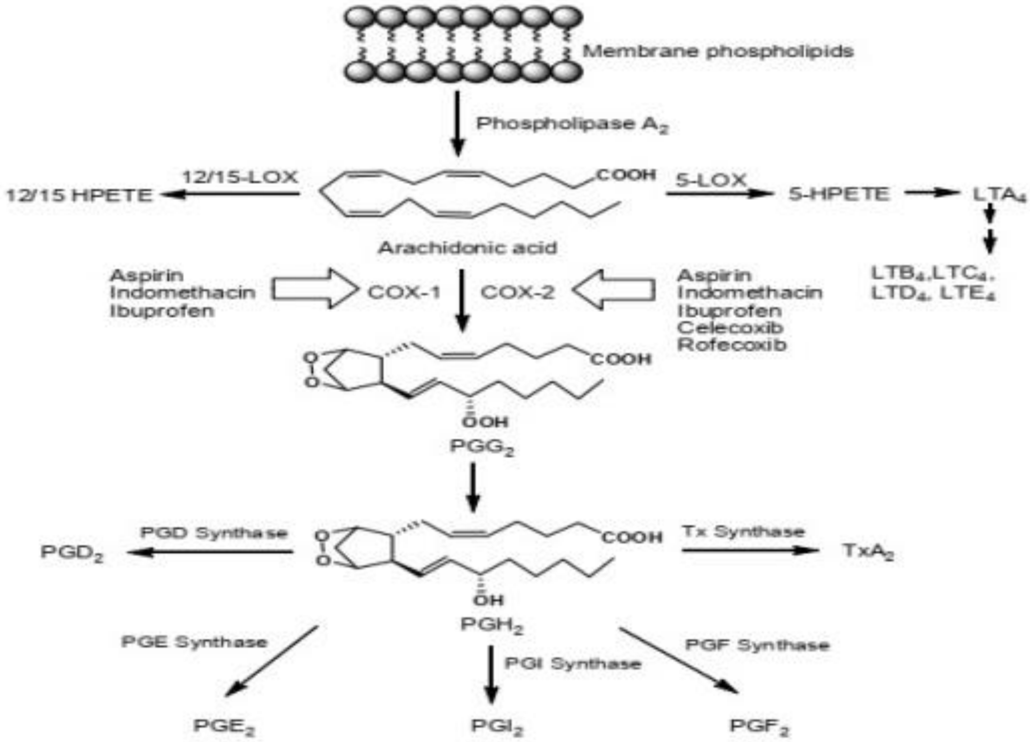

Figure 1 : Representative biosynthetic pathway of prostaglandin (PG) biosynthesis from arachidonic acid (AA) via COX-1/COX-2 isoform catalysis. The NSAIDs aspirin, indomethacin and ibuprofen are nonselective inhibitors of COX isozymes whereas celecoxib and rofecoxib exhibit selective COX-2 inhibition **(Praveen Rao and Knaus. 2008).**

1.3. Structure

COX-1 and COX-2 are the products of two distinct genes, which in humans are localized on chromosomes 9 and 1, respectively **(Yokoyama and Tanabe. 1989; Funk and al. 1991)**. The COX-1 gene is about 22 kb in length with 11 exons and is transcribed as a 2.8 kb mRNA . The COX-2 gene is about 8 kb long with 10 exons and it is transcribed as 4.6, 4.0 and 2.8 kb mRNAs variants **(Hla and Neilson .1992; Tay et al. 1994)**. Sequence analysis of the COX-2 5´-flanking region has revealed several potential transcription regulatory elements, including a TATA box, a NF-IL-6 motif, two AP- 2 sites, three Sp1 sites, two NF-kB sites a CRE motif and an E-box **(Morita. 2002).**

The amino acid sequences of COX-1 and COX-2 from a single species are about 60% identical. COX-1 is a glycoprotein that in its native, processed form has 576 amino acids with an apparent molecular mass of 70 kDa **(Funk et al. 1991; Jones et al. 1993).** The cDNA for COX-2 encodes a polypeptide that, before cleavage of the signal sequence, contains 604 amino acids with an apparent molecular mass of 70 kDa **(Tanabe and Tohnai. 2002; Tay an al. 1994).** The main difference is that the COX-1 protein contains a 17 amino acid sequence near its amino terminus that is not present in COX-2. In contrast, COX-2 contains a 18 amino acid sequence near its carboxyl terminus that is not present in COX-1 **(Funk and al. 1991; Jones and al. 1993; Tanabe and Tohnai .2002)**.

However, and as already mentioned, the two COX isoforms catalyze identical reactions and exhibit the same kinetic constants for the conversion of arachidonic acid to prostanoids. The most striking distinctions between COX-1 and COX-2 are the differential regulation of their expression and their tissue distribution. COX-1 is ubiquitous and is constitutively expressed throughout the gastrointestinal system, the kidneys, the vascular smooth muscle and platelets **(Morita. 2002**). COX-1 is presumably involved in the housekeeping functions of PGs, such as the cytoprotective effects in the gastric mucosa, the integrity of platelet function and the maintenance of renal perfusion. Conversely, COX-2 is undetectable in most tissues, but its expression can be induced by a variety of stimuli related to inflammatory response. COX-2 is, therefore, commonly referred to as the inducible COX isoform because, like other immediate-early genes, it can be rapidly upregulated in response to growth factors and cytokines **(Morita. 2002)**. This has led to the supposition that the inducible COX-2 isoform is responsible for the synthesis of PGs involved in inflammatory response, whereas COX-1-derived PGs are involved in preserving the physiological functions of these prostanoids. However, this distinction is not entirely accurate, since COX-1 can be induced or upregulated under certain conditions and COX-2 has been consistently shown to be constitutively expressed in organs, such as the brain and the kidneys(**Clària .2003).**

1.4. Active site

COX-1 and COX-2 are homodimers of 70 kDa subunits and dimerization is required for structural integrity and catalytic activity **(Xiao; Chen and Kulmacz .1998).** Sheep COX-1 was one of the first membrane proteins to be crystallized and to have its structure solved **(Picot; Loll and Garavito .1994)**, and there are now many COX crystal structures available, including several with bound inhibitors. The asymmetric unit of the ovine COX-1/flurbiprofen crystals was shown to contain two identical monomers exhibiting extensive contacts in a large subunit interface **(Picot**

and al. 1994). Each subunit contained a cyclooxygenase and a peroxidase active site, with inhibitor bound only in the cyclooxygenase active site. Although it has been assumed that both subunits are active simultaneously, recent work suggests that substrate or inhibitor binding in thecyclooxygenase active site at one subunit precludes binding of another molecule at the other subunit. **(Yuan and al. 2006)**

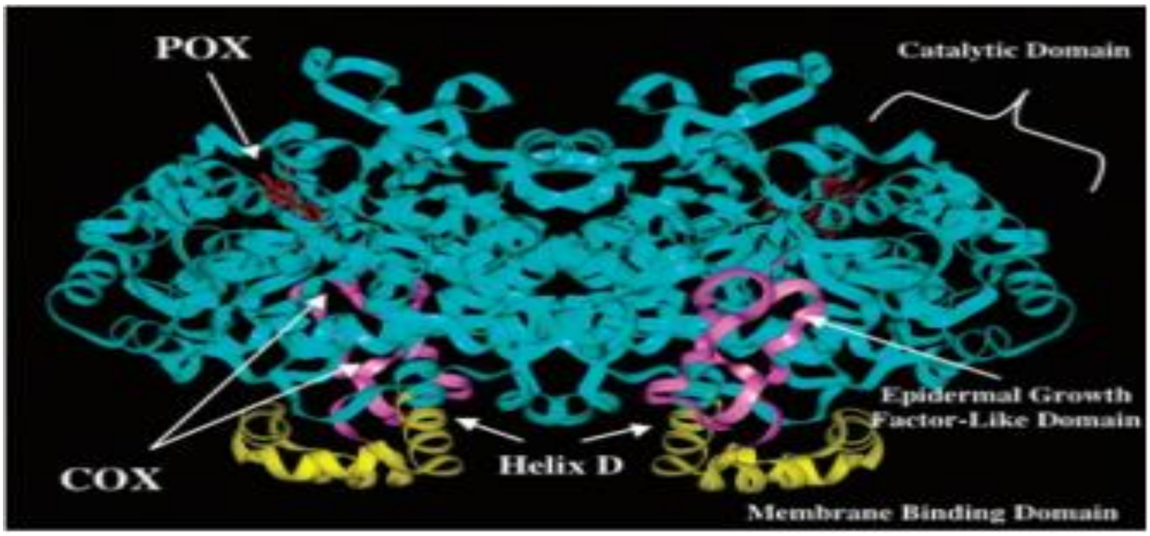

Figure 2: Structural representation of the murine COX-2 dimer **(Blobaum and Marnett .2007)**

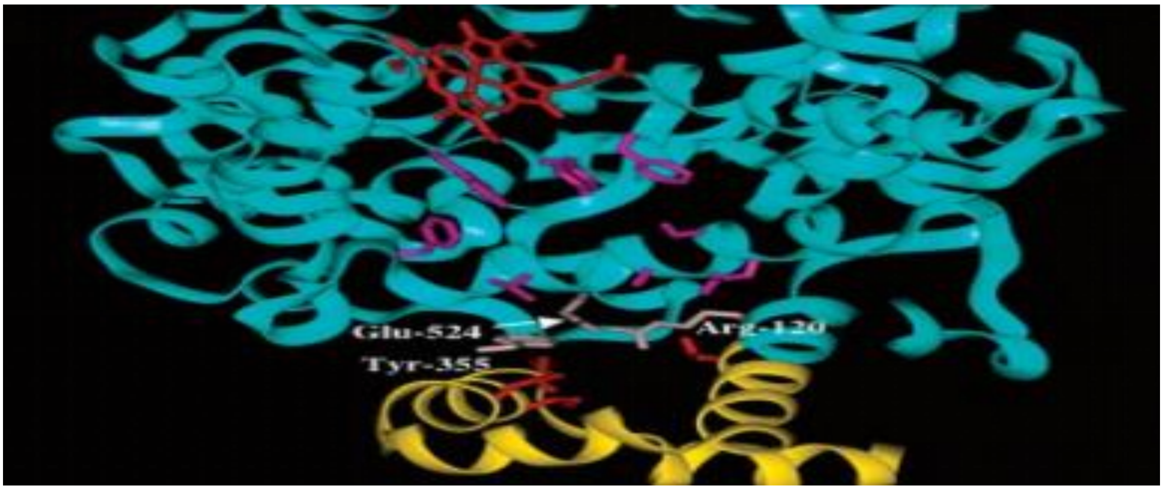

Figure 3 : Cyclooxygenase active site of murine COX-2 **(Blobaum and Marnett. 2007)**

Each monomer of COX consists of three structural domains: a short N-terminal epidermal growth factor domain, a membrane binding domain, and a large, globular C-terminal catalytic domain (Figure 2)**.(Picot and al. 1994 Kurumbail and al. 1996; Luong and al. 1996)** The COX and peroxidase (POX) active sites are located on opposite sides of the catalytic domain with the heme prosthetic group positioned at the base of the peroxidase site. The epidermal growth factor domain and catalytic domain create the dimer interface and place the two membrane binding domains on the same face of the homodimer about 25 Å apart **(Thuresson and al. 2001; Garavito and Mulichak. 2003).** The membrane binding domain of cyclooxygenase is composed of four amphipathic R helices, with hydrophobic and aromatic residues that project from the helices to create a surface that interacts with one face of the lipid bilayer**.(Picot & al. 1994)** Three of the four helices lie in the same plane, whereas the last helix (helix D) projects up into the catalytic domain**.(Garavito and Mulichak. 2003)** The catalytic domain constitutes the majority of the COX monomer and is the site of substrate binding and NSAID action. The entrance to the COX active site occurs at the base of the membrane binding domain and leads to a long hydrophobic channel that extends deep into the interior of the catalytic domain (Figure 3). (**Picot & al. 1994).**

The COX channel narrows at the interface between the membrane binding domain and the catalytic domain to form a constriction composed of three residues (Arg-120, Tyr-355, and Glu-524) that separates the “lobby” from the active site. The COX-1 and COX-2 active sites are very similar but differ in the presence of a side pocket in COX-2 located above the Arg-120/Tyr-355/Glu-524 constriction. This COX-2 side pocket is bordered by Val-523 (isoleucine in COX-1) and contains a conserved Arg-513 (His-513 in most COX-1) at the base of the pocket**.(Kurumbail & al. 1996; Luong & al. 1996)** The solvent accessible surface in the COX-2 active site is larger than that of COX-1 (Figure 4) because of the one Val-523 to Ile substitution in the active site and several key substitutions in the secondary shell (Arg-513 to His and Val-434 to Ile). In addition, the last helix of the membrane binding domain (helix D) is positioned differently in COX-2 and shifts the location of Arg-120 at the constriction site, allowing for a larger solvent accessible surface at the interface between the membrane binding domain and the COX active site in COX-2.

Crystal structures and molecular models of ovine COX-1 (oCOX-1) and murine COX-2 (mCOX-2) complexed with arachidonic acid indicate that the carboxylic acid of the substrate ion-pairs to the guanidinium group of Arg-120 and hydrogen bonds to Tyr-355. The aliphatic backbone projects up into the top of the cyclooxygenase active site from the hydrophobic channel and then makes a sharp bend in the vicinity of Tyr- 385 (Figure 5)**.(Thuresson & al. 2001; Rowlinson & al. 1999)** In these structures, the vast majority of contacts between the substrate and various protein residues in the active site involve van der Waals interactions. The ω-end of arachidonic acid binds in a narrow channel at the top of the active site and is surrounded by six aromatic amino acids.

Mutation of Gly-533 at the top of this channel seals off the channel and abolishes the oxygenation of arachidonic acid but not that of fatty acids with shorter carbon chains. (**Kiefer & al. 2000)**

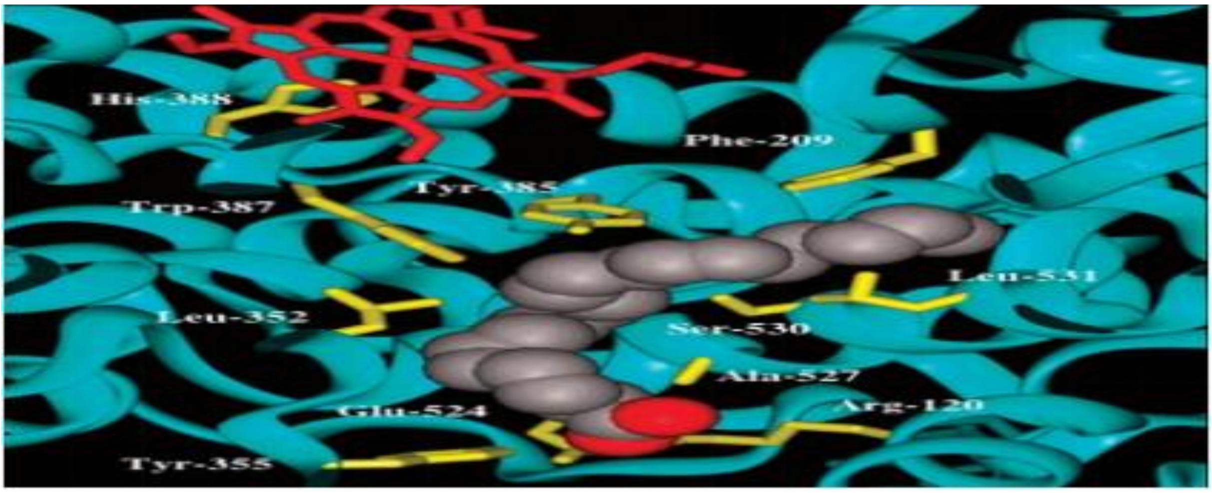

Figure 4 : Arachidonic acid bound in the active site of oCOX-1 **(Blobaum and Marnett;. 2007)**

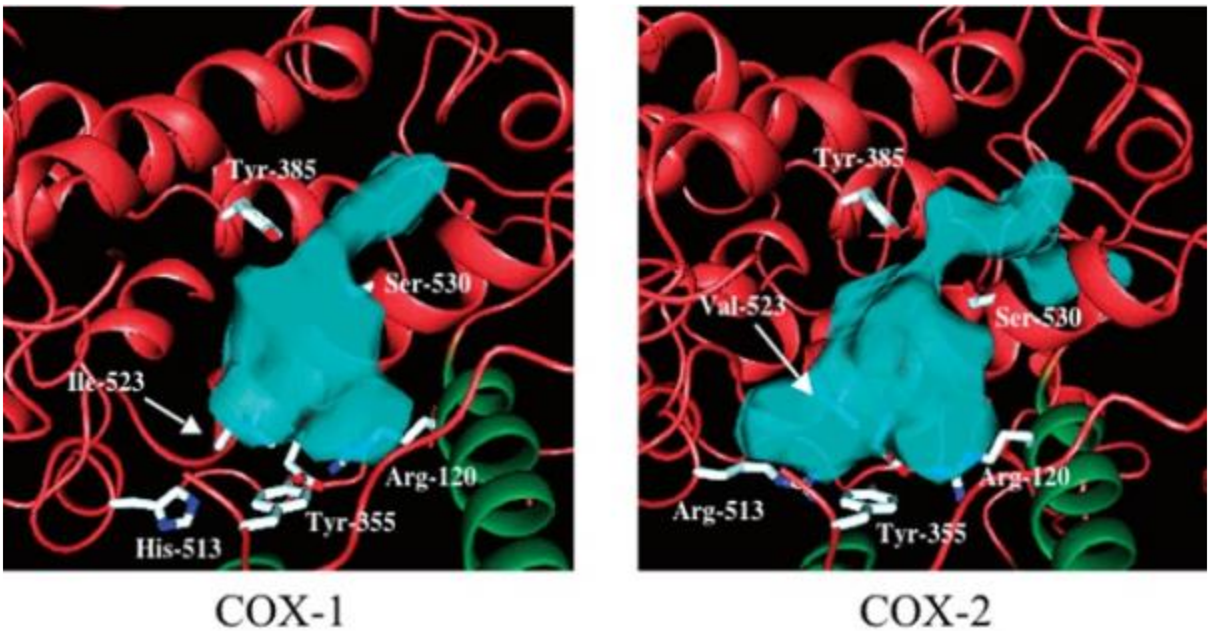

Figure 5: Solvent accessible surfaces in COX-1 and COX-2. The catalytic domains of the COX proteins are shown as red in a ribbon diagram with the membrane binding domains (predominantly helix D) shown in green. Residues lining the COX active site of both proteins are shown in white with the solvent accessible surfaces in the active site designated as translucent light-blue. **(Blobaum and Marnett .2007).**

1.5. Mechanism of action

Ruf and co-workers **(Dietz; Nastainczyk and Ruf. 1988)** first proposed a mechanism that included the requirement of peroxide-dependent heme oxidation to initiate a mechanistically coupled oxygenase reaction in the spatially separate cyclooxygenase active site in the protein. In this mechanism (Figure 6), a hydroperoxide reacts with the heme iron to effect a two-electron oxidation to yield compound **I** (an oxyferrylheme radical cation), which rapidly carries out an intramolecular electron transfer from Tyr-385 to the heme to form a tyrosyl radical at position 385 (intermediate I). **(Smith and al. 2000; Tsai and Kulmacz. 2000)** When the COX active site is occupied by arachidonic acid, the tyrosyl radical initiates the cyclooxygenase reaction by abstracting the 13-pro(*S*) hydrogen to yield an arachidonyl radical**. (Tsai and Kulmacz. 2000)** The fatty acid radical then reacts with molecular oxygen to produce an 11-hydroperoxyl radical, which cyclizes to form the endoperoxide moiety of PGH2. The addition of another equivalent of oxygen at carbon 15, followed by reduction, yields PGG2 and leads to tyrosyl radical regeneration. PGG2 diffuses from the COX active site to the POX active site where it is reduced to PGH2. Although the POX reaction is generally considered the second step in the formation of product, the COX reaction is absolutely dependent on POX activity for its activation. In short, one turnover of the POX reaction is required to provide the tyrosyl radical for initiation of the COX reaction, which continues to turn over, in the presence of fatty acid substrate, until radical-induced inactivation occurs (Figure 6). The productive conformation of arachidonic acid observed in crystal structures positions the 13-pro(*S*) hydrogen of the substrate 2.3 Å from the phenolic oxygen on Tyr-385, ideally located for abstraction by the tyrosyl radical produced during catalysis.(Kulmacz 1998) Interestingly, a crystal structure of mCOX-2 is available in which a mixture of structures was observed containing substrate (AA) and product (PGH2) bound in the cyclooxygenase active site.**(Thuresson & al. 2001)** Although the product species is ambiguous (it could be PGG2 or PGH2), the carboxylate of the molecule is positioned near Arg-120 and Tyr-355 and the ω-end is bound in the top channel, similar to arachidonic acid.**(Kiefer & al. 2000)** This conformation of product, in which the PGG2/PGH2 species hydrogen-bonds with the constriction site residues and bends in an L-shaped fashion at Tyr-385, suggests that arachidonic acid was positioned properly for catalysis. The mixed structure cocrystal also yielded a conformer of arachidonic acid that is bound in an inverted configuration with its carboxylate hydrogen-bonded to Tyr-385 and Ser-530. This binding conformation of arachidonic acid in the active site is thought to reflect a noncatalytic or inhibitory binding mode where no viable products can be produced. High-resolution crystal structures of mCOX-2 with arachidonate and eicosapentaenoic acid (EPA) also have revealed an inhibitory or nonproductive binding mode of substrate in the active site.41 Mechanistic studies have confirmed that this binding mode is not viable for catalysis. (**Thuresson & al. 2001)**

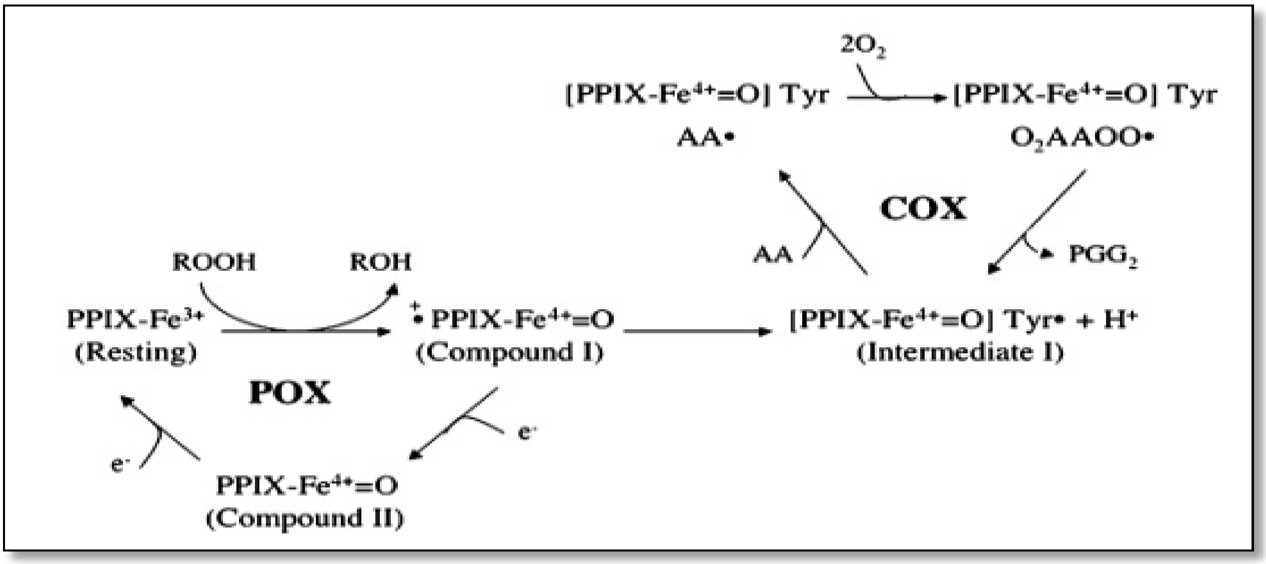

Figure 6: Reaction mechanism for COX enzymes. The COX reaction is peroxide-dependent and requires that the heme group at the peroxidase site undergo a two-electron oxidation. A tyrosyl radical is generated from the POX reaction and initiates the COX reaction, which then becomes autocatalytic in the presence of substrate, until radical induced inactivation occurs **(Blobaum and Marnett. 2007)**

Chapter II

Diclofenac derivatives

2. Diclofenac derivatives

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely prescribed pharmaceutical agents in the world and these drugs are commercially in sodium or potassium salt form. **(Mahmut .2018)**

Diclofenac is a proven, commonly prescribed nonsteroidal anti-inflammatory drug (NSAID) that has analgesic, anti-inflammatory, and antipyretic properties, and has been shown to be effective in treating a variety of acute and chronic pain and inflammatory conditions. As with all NSAIDs, diclofenac exerts its action via inhibition of prostaglandin synthesis by inhibiting cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) with relative equipotency. However, extensive research shows the pharmacologic activity of diclofenac goes beyond COX inhibition, and includes multimodal and, in some instances, novel mechanisms of action (MOA). (Gan**. 2010)**

2.1. Diclofenac Proprieties:

- Non-steroidal anti-inflammatory drugs or NSAIDs, from the arylcarboxylic acids group, whose analgesic, anti-inflammatory, antipyretic and antiplatelet aggregation effects are linked to the inhibition of cyclo-oxygenase 1 and 2 (Cox-1 and Cox-2)

- Rapid digestive absorption, 99% binding to plasma proteins, hepatic metabolism, urinary elimination in the form of metabolites, onset of action of approximately 20 to 30 minutes IM, 30 to 60 minutes rectally, 1 to 2 hours orally (**Vital . 2010)**

2.2. Indications

- Long-term symptomatic treatment of chronic inflammatory rheumatism and certain painful and disabling osteoarthritis

- Short-term symptomatic treatment of acute attacks of abarticular rheumatism, osteoarthritis, microcrystalline arthritis, low back pain, severe radiculalgia post-operative pain or pain of neoplastic origin (ketoprofen), renal colic attacks, trauma, essential dysmenorrhea, adjuvant treatment of mani - inflammatory infections in stomatology or dental or ENT surgery **(Vital. 2010)**

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2.3. Side effects

- Digestive (10%): nausea, gastralgia, vomiting, dyspepsia, diarrhea, hemorrhage

- Occult digestive, local intolerance (rectally), more rarely peptic ulcer, perforation and patent digestive hemorrhage, colitis, proctitis Allergic: pruritus, skin rashes, angioedema, asthma attack, even shock

-Anaphylactic, exceptionally Lyell or Stevens-Johnson syndrome Neuro-psychic (51%) (dase-dependent): dizziness, headache, more rarely visual disturbances, drowsiness, tinnitus, asthenia, insomnia, psychological disorders, aseptic meningitis

-Miscellaneous: oedema, hypertension, elevated transaminases, exceptionally hepatitis, acute renal failure, oliguria, nephrotic syndrome, drug-induced cystitis, anemia, leukopenia, agranulocytosis (requiring immediate cessation) **(Vital. 2010)**

2.4. Precautions for use

- Use with caution in cases of dehydration, hypertension, history of ulcer, hiatal hernia or digestive hemorrhage, cardiac, hepatic or renal failure, in the elderly and in drivers and users of machines.

- Immediate cessation of treatment in the event of an allergic reaction or other serious disorders Pregnancy salicylates or NSAIDs in the 3rd trimester of pregnancy expose the foetus to sometimes fatal disorders (pulmonary arterial hypertension, renal failure) Pediatrics: the use of NSAIDs in pediatrics requires extreme caution and must be reserved, for certain authors, for the treatment of juvenile rheumatoid arthritis. **(Vital . 2010)**

- Gastrointestinal topicals: interval of 2 hours or more between oral doses **(Vital. 2010)**

2.5. Drugs interactions

- Combinations not recommended: combined NSAIDs, anticoagulants, lithium, high dose methotrexate, high-dose salicylates, enoxacin (with fenbufen), fenoprofen, ticlopidine, IUDs

- Combinations to monitor : antihypertensives, corticosteroids, digoxin (with ibuprofen), diuretics, alpha interferon, SSRIs, prostaglandin analogs, thrombolytics, low dose methotrexate (15 mg/week)

- Gastrointestinal topicals : interval of 2 hours or more between oral doses **(Vital. 2010)**

2.6. Mechanism of action:

Diclofenac inhibits cyclooxygenase-1 and -2, the enzymes responsible for production of prostaglandin (PG) G2 which is the precursor to other PGs., **(Brunton LL. 2018**)

These molecules have broad activity in pain and inflammation and the inhibition of their production is the common mechanism linking each effect of diclofenac.

PGE2 is the primary PG involved in modulation of nociception.**(Chen .2013)** It mediates peripheral sensitization through a variety of effects. **(Brunton . 2018)**

(Chen. 2013) PGE2 activates the Gq-coupled EP1 receptor leading to increased activity of the inositol trisphosphate/phospholipase C pathway. Activation of this pathway releases intracellular stores of calcium which directly reduces action potential threshold and activates protein kinase C (PKC) which contributes to several indirect mechanisms. PGE2 also activates the EP4 receptor, coupled to Gs, which activates the adenylyl cyclase/protein kinase A (AC/PKA) signaling pathway. PKA and PKC both contribute to the potentiation of transient receptor potential cation channel subfamily V member 1 (TRPV1) potentiation, which increases sensitivity to heat stimuli. They also activate tetrodotoxin-resistant sodium channels and inhibit inward potassium currents. PKA further contributes to the activation of the P2X3 purine receptor and sensitization of T-type calcium channels. The activation and sensitization of depolarizing ion channels and inhibition of inward potassium currents serve to reduce the intensity of stimulus necessary to generate action potentials in nociceptive sensory afferents. PGE2 act via EP3 to increase sensitivity to bradykinin and via EP2 to further increase heat sensitivity. Central sensitization occurs in the dorsal horn of the spinal cord and is mediated by the EP2 receptor which couples to Gs. Pre-synaptically, this receptor increases the release of pro-nociceptive neurotransmitters glutamate, CGRP, and substance P. Post-synaptically it increases the activity of AMPA and NMDA receptors and produces inhibition of inhibitory glycinergic neurons. Together these lead to a reduced threshold of activating, allowing low intensity stimuli to generate pain signals. PGI2 is known to play a role via its Gs-coupled IP receptor although the magnitude of its contribution varies. It has been proposed to be of greater importance in painful inflammatory conditions such as arthritis. By limiting sensitization, both peripheral and central, via these pathways NSAIDs can effectively reduce inflammatory pain.

PGI2 and PGE2 contribute to acute inflammation via their IP and EP2 receptors. **(Brunton LL. 2018)**, (**Hirata T. 2012)** Similarly to β adrenergic receptors these are Gs-coupled and mediate vasodilation through the AC/PKA pathway. PGE2 also contributes by increasing leukocyte adhesion to the endothelium and attracts the cells to the site of injury. **(Brunton LL. 2018)**

PGD2 plays a role in the activation of endothelial cell release of cytokines through its DP1 receptor.11 PGI2 and PGE2 modulate T-helper cell activation and differentiation through IP, EP2, and EP4 receptors which is believed to be an important activity in the pathology of arthritic conditions. By limiting the production of these PGs at the site of injury, NSAIDs can reduce inflammation.

PGE2 can cross the blood-brain barrier and act on excitatory Gq EP3 receptors on thermoregulatory neurons in the hypothalamus. **(Brunton LL. 2018)**

This activation triggers an increase in heat-generation and a reduction in heat-loss to produce a fever. NSAIDs prevent the generation of PGE2 thereby reducing the activity of these neurons.

2.7. Structure

Diclofenac is a monocarboxylic acid consisting of phenylacetic acid having a (2,6-dichlorophenyl)amino group at the 2-position. It has a role as a non-narcotic analgesic, an antipyretic, an EC 1.14.99.1 (prostaglandin-endoperoxide synthase) inhibitor, a xenobiotic, an environmental contaminant, a drug allergen and a non-steroidal anti-inflammatory drug. It is a secondary amino compound, an amino acid, a dichlorobenzene, an aromatic amine and a monocarboxylic acid. It is functionally related to a phenylacetic acid and a diphenylamine. It is a conjugate acid of a diclofenac(1-).

Diclofenac was the product of rational drug design based on the structures of [phenylbutazone], [mefenamic acid], and [indomethacin]. The addition of two chlorine groups in the ortho position of the phenyl ring locks the ring in maximal torsion which appears to be related to increased potency. It is often used in combination with [misoprostol] to prevent NSAID-induced gastric ulcers. **(Alfred. 1986)**

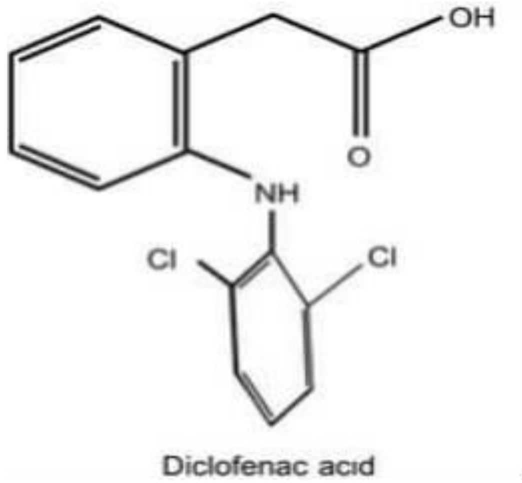


Figure 7 : Structure of diclofenac **(Altman, R. 2015).**

2.8.Pharmacodynamics

Diclofenac reduces inflammation and by extension reduces nociceptive pain and combats fever It also increases the risk of developing a gastrointestinal ulcer by inhibiting the production of protective mucus in the stomach **(Brunton LL. 2018)**

2.9. Metabolism

Diclofenac undergoes oxidative metabolism to hydroxy metabolites as well as conjugation to glucuronic acid, sulfate, and taurine The primary metabolite is 4'-hydroxy diclofenac which is generated by CYP2C9. This metabolite is very weakly active with one thirtieth the activity of diclofenac. Other metabolites include 3'-hydroxy diclofenac, 3'-hydroxy-4'methoxy diclofenac, 4',5-dihydroxy diclofenac, an acyl glucuronide conjugate, and other conjugate metabolites. **(Roque.1999)**

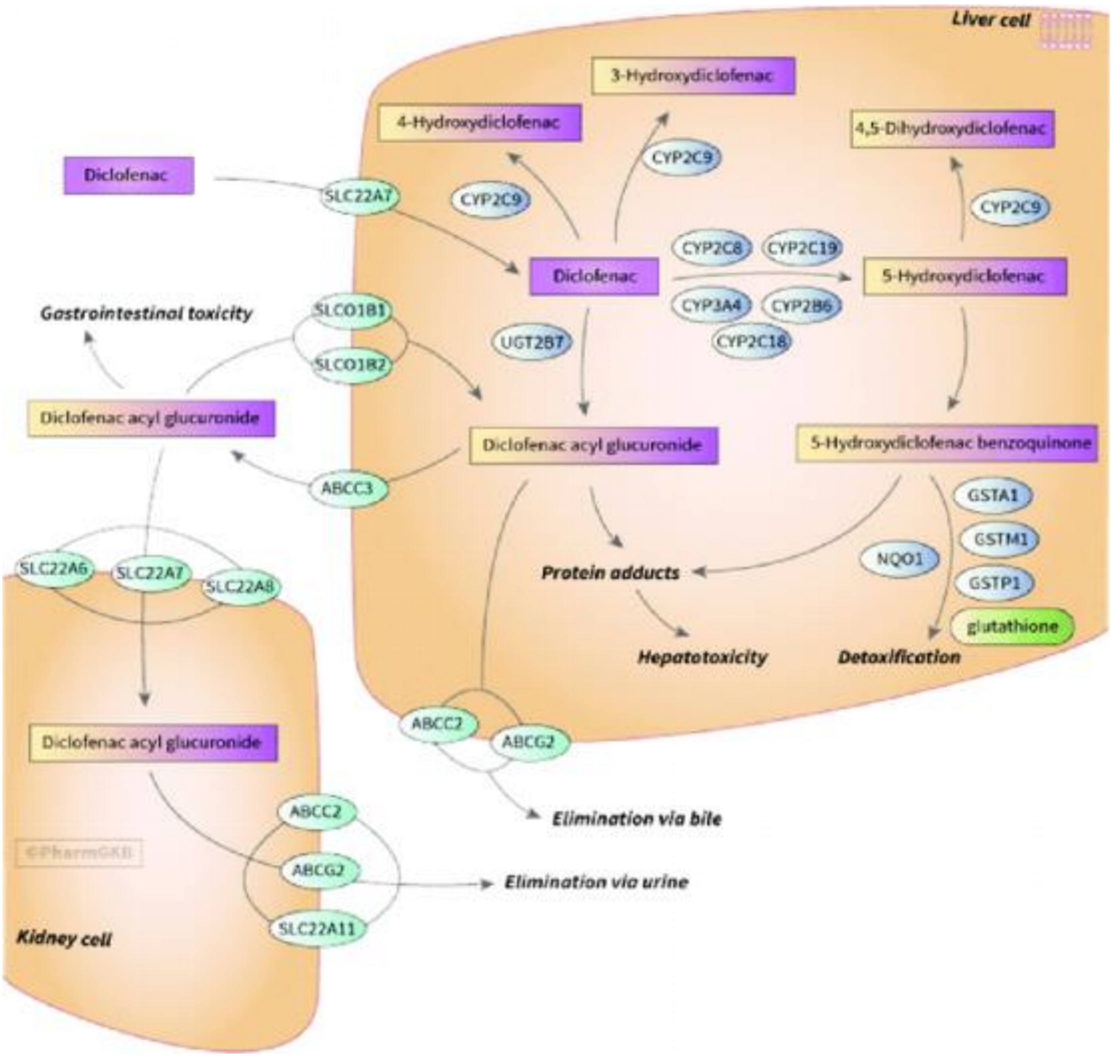


Figure 8: Stylized diagram showing diclofenac metabolism and transport in the liver.

(Božina and Nada. 2021).

2.10. Toxicity

Diclofenac, a 2-arylacetic acid, nonsteroidal anti-inflammatory drug, has been reported to cause adverse hepatic effects in certain individuals. To discriminate among possible mechanisms of hepatotoxicity, we examined the effects of diclofenac on human and rat hepatocytes and hepatic cell lines (HepG2, FaO), investigated the major biochemical events in the course of diclofenac cytotoxicity (calcium homeostasis, lipid peroxidation, and mitochondrial dysfunction), and investigated whether cytotoxicity could be related to drug metabolism by cytochrome P-450. Acute diclofenac-induced toxicity in hepatocytes was preluded by a decrease in ATP levels, whereas no significant oxidative stress (decrease in glutathione and lipid peroxidation) or increase in intracellular calcium concentration could be observed at early incubation stages. Diclofenac was more cytotoxic to drug metabolizing cells (rat and human primary cultured hepatocytes) than to nonmetabolizing cell lines (HepG2, FaO). Despite the fact that diclofenac itself was effective in impairing ATP synthesis by mitochondria, we found evidence that toxicity was also related to drug metabolism and was reduced by the addition of cytochrome P-450 inhibitors (proadifen and ketoconazole) to culture medium. The in vitro cytotoxicity correlated well with the formation by hepatocytes of 5-hydroxydiclofenac and, in particular,N,5-dihydroxydiclofenac, a minor metabolite first characterized in this article. Hepatic microsomes showed the ability to both oxidize 5-hydroxydiclofenac toN,5-dihydroxydiclofenac and back reduce the latter to 5-hydroxydiclofenac with the consumption of NADPH. The experimental results suggest that the toxic effect of diclofenac on hepatocytes may be caused by drug-induced mitochondrial impairment, together with a futile consumption of NADPH**. (Roque .1999)**

Chapter III

In silico approaches

3. In silico approaches

3.1. Generality

Since the early of 1980s the molecular docking**(Kuntz. 1982)** become the most important method used to study the interaction of a small molecule (ligand) with a macromolecule (receptor)**(Leonardo.2015)** or more clearly used to predict the favorite orientation of binding between molecules**(Sergio.2006)** to have the stable complex **(Kitchen. 2004)** which leaded to predict the strength of liaisons or associations and the binding affinity between two molecules.

3.2. Molecular docking

Molecular docking is the primary method for simulation of molecular interaction. This method is able to give insights into the interaction at the atomic level, offering the opportunity to fully characterize the binding site of each molecule. Except the conformation of the complex and the orientation of the small molecule, molecular docking provides information regarding the affinity of each ligand**(McConkeyetal.2002)**. Docking is conducted in two basic steps: first, the determination of a wide conformational area where the ligand can occupy the target with different orientations and second, the calculation of the energy associated with each conformational state**(Huang and Zou.2010).**Molecular docking allows to know how a ligand (small molecule) interacts with a receptor (macromolecule) and to calculate the binding energy between them. It also tells which candidate ligand will interact best with a target receptor.**(LeonardoG2015)**

The docking consists of two distinct sections. The first section consists of search algorithms, these algorithms are able to generate a large number of possible structures and to determine the binding mode. Among these algorithms: genetic algorithm, the Monte Carlo method and the second section is devoted to the function of scoring, which are mathematical methods used to estimate the interaction power and binding affinity between two molecules after have been through the docking stage. The best result for docking is the receptor-ligand adduct which have the lowest energy.

3.3.Different types of molecular docking

There are two types of molecular docking: the first type is called rigid docking which consists in obtaining the preferential conformation of a receptor-ligand system by considering each of the two molecules maintain a fixed internal geometry. In this case, the relaxation of the internal geometry of each entity, interacting in the complex, is not taken into account. However, it is quite conceivable that the receptor and ligand structures are modified during the molecular docking process in order to optimize the interaction between the two entities, in this case, docking is called flexible docking**(Mulliken RS. 1952)**.

A very large number of molecular docking software are already available. Among these,we will mention for example Autodock **(Globisch C.2006)(OpreaTI.2005)** , MDV **(Kathryn.2012)**, or Hex **(RitchieDW.2000)**etc. They differ from each other on how to represent the molecular system and how to determine the docking score (score function). Two approaches are mainly used for modeling the receptor-ligand system. In this research project, we used the AutoDock version 4.2software.

3.4 Theory of mollecular docking

- **Docking glossary**

Docking: is a computational simulation of the binding of the receptor to a ligand**(Gregory.2008)**.

Receptor: is the receiving molecular and it is a protein or other biopolymer **(Brooijmans 2003)**.

Ligand: is the small molecule binds to a receptor**(Brink . 2009).**

The binding mode is the confirmation and the direction of the candidate ligand and the receptor when they joined each other **(Gao. 2018)**.

The pose is the favorites (candidates) binding mod**(Aumentado. 2018)**.

Scoring: is the calculation of the number of intramolecular interactions to delineated the correct pose **(Zongxi . 2018)**.

Ranking: is the process of ligand classification according to its favorite of interaction with the receptor using the binding free energy**(Gomes. 2018)**.

- **The basic theory:**

Two interrelated steps are employed to realize the subject of the molecular docking using computational methods**(Lengauer. 1996)**: the first one is a determination of binding mods by counting the number of orientations and conformations of the ligand in the active site of the receptor**(Lindert. 2016)** in the second step the scoring conformation used to rank this confirmation **(Ramírez, D. 2016).**

The sampling algorithms **(Yves . 2006)** use to reproduce the binding mods and the scoring function used to classify the confirmation in increasing of the favorite interaction order**(Sheng-.2010)**.

Sampling algorithms : because of the enumerating conformation of ligands-receptor**(Till´e,Y. 2010)**, it is so expensive to generate computationally all the binding mods**(Ullrichb.2017)**, this is the time to developed the samplings algorithms **(Kathryn. 2012)** such as Matching algorithms, LUDI, MCSS,Monte Carlo, Genetic algorithms**(Akhter.2016)**, Molecular dynamics and Incremental construction for using in molecular docking software **(F.H.Allen. 2007)**.

The scoring function **(JieLiu. R.2015)** is a mathematical function used to determine the binder from inactive compounds **(Huang.2010)** by the classification of the confirmation**(Shamsara,J.2014)** after the calculation of binding affinity and the strong association between ligand-receptor **(Böhm. 1994)** and adopting assumptions and simplifications**(AN. 2006)**.

3.5. AutoDock molecular docking software

In this software the docking is based on the trajectory simulation, is more precise: from a random initial position, outside the active site, the ligand explores the site studied by the successive repetition of movements and evaluations of the ligand-receptor interaction. The movements are performed by translation, rotation and conformational changes. The interaction energy is calculated by an energy function. The movements of the next cycle are guided by the energy variations induced by the movements of previous cycles. The algorithm stops when it finds the ideal ligand position in the receiver. These techniques take better account of the flexibility of the ligand and allow the exploration of larger regions.

3.5.1. Theory of autodock

A semiempirical free energy force field is used during the docking simulation process**(Zhiqiang.2012)**. the force field evaluates conformation in 2 steps, the first step estimation of the intramolecular energy of transformation from the unbounded to the bounded stats of ligands receptor conformation **(Joost.2005)**, the second one is the evaluation of the intramolecular energy of combining the ligand and the receptor in the bound state**(Gregory. 2008)**

$$\Delta G = \Delta G_{vdw} + \Delta G_{hbond} + \Delta G_{elec} + \Delta G_{tor} + \Delta G_{sol}$$

Where : ΔG_{vdw} is the energy of dispersion/repulsion.

ΔG_{hbond} is the energy of hydrogen bonding.

ΔG_{elec} is the energy of electrostatics interaction.

ΔGtor a term which reflects the increase in energy of the system due to the restriction of the free rotors of the ligand and the restriction of rotation and translation of the ligand during complexation at the receptor.

ΔGsol a term related to the entropy which describes the variations of the energy of the system during the disolvatation of the ligand at the time of the complexation with the receptor.

3.6. Molecular modelling

In order to achieve molecular docking, the ligand structures must be optimized by molecular modeling, in what follows a theoretical insight into molecular modeling. Molecular modeling is an application of theoretical methods and computational methods to solve problems involving molecular structure and chemical reactivity. These methods can be relatively simple and usable quickly or on the contrary they can be extremely complex and require hundreds of hours of computer time, even on a super-computer. In addition, these methods often use very sophisticated in fographic means that greatly facilitate the transformation of impressive quantities of numbers into some easily interpretable graphic representations. Different approaches can be envisaged in the context of molecular modelling tools. While those of classical mechanics, which are economical in terms of computing time, make it possible to process large molecular systems, quantum methods (semi-empiricalor density functional theory) are able to calculate the electronic properties of the systems. For this reason, these approaches have been used in this study.

3.6.1 .Molecular mechanics (MM)

Molecular mechanics appeared in 1930 **(Andrews. 1930)**, but developed from 1960s, with advances in accessibility and performance of computers. It makes it possible to determine the energy of a molecule according to its atomic coordinates and to look for minima of the energy corresponding to stable conformers **(Hetényi C. 2003)(Keseru . 1999)**.

Modeling techniques based on quantum mechanics suffer from a major inconvenient: they are very expensive in terms of computation time and are therefore applicable only to molecular systems of small size. In the end, the time required to process a system by ab initio methods is approximately proportional to the fourth power of the number of electrons it contains. The use of these techniques can be problematic for the study of macromolecular objects such as an enzyme in interaction with an inhibitor or for the characterization of large-scale metallo-organic complexes, such as those which are the subject of the invention of this research. The main idea of this method is to establish, by the choice of the energetic functions and the parameters which they contain, a mathematical model, the "field of force", which represents as well as possible the variations of the potential energy with molecular geometry. However, there is still no single model for simulating all aspects of molecular behavior, but a set of models **(Magali T. 2004)**.

3.6.2 Quantum methods

- **The theory of the functional density (DFT)**

In the formalism of the theory of the density functional the energy is expressed as a function of the electronic density. The first to express energy as a function of density was L.H. Thomas (1927), E. Fermi (1927, 1928) and P.A. Dirac (1930) on the model of non-interacting electron gas. The goal of the DFT methods is to determine functionalities that make it possible to relate electronic density to energy **(Parr RG.1989)**. The DFT really started with the fundamental theorems of Hohenberg and Kohn in 1964**(Hohenberg P. 1964),** which establish a functional relation between the energy of the ground state and its electronic density. The two theorems show the existence of a density functional which makes it possible to calculate the energy of the ground state of a system.

3.6.3. Semi-empirical methods

Semi-empirical methods are used to model large molecular systems. They are based on two approximations, the first is to consider only the valence layer (the valence electrons that intervene in the chemical bonds and thus define the properties of the system). The second cancels the multi-center electronic repulsion integrals. Using parameters adjusted to the experimental results, they can lead sometimes to important errors in the evaluation of the total energies **(TsiliouS, 2012)**. AutoDock based incremental docking protocol to improve docking of large ligands.

3.7. Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties

Drug discovery and development is a very complex and costly attempt, which includes disease selection, target identification and validation, lead discovery and optimization, preclinical and clinical trials

The properties of absorption, distribution, metabolism, excretion Figure 3.1, and toxicity (ADMET) are phenomena that are closely related to the fate of a chemical in the human body. Each of the properties of ADMET will reflect the outcome of a chemical compound when interacting with various organs in the body. Prediction of the ADMET properties from a compound is essential, especially for foreign chemical compounds that are consumed in the long term or large concentrations. Information about the properties of ADMET from a compound is mainly needed in the development of a new drug compound, where the information can be used to predict various pharmacokinetic phenomena of these compounds, which can then be used as necessary information in the further development of new drug compounds .Computational strategies play vital roles in early stage of drug discovery and expected to minimize the risk of toxicity. The pharmacokinetic activity and toxicity can be assessed using computational algorithms to organize, analyze, model, simulate, visualize or predict chemical toxicity. Predicted toxicity in silico is performed prior to in-vitro and in-vivo testing to minimize time and cost **(Mohammad R. 2019).**

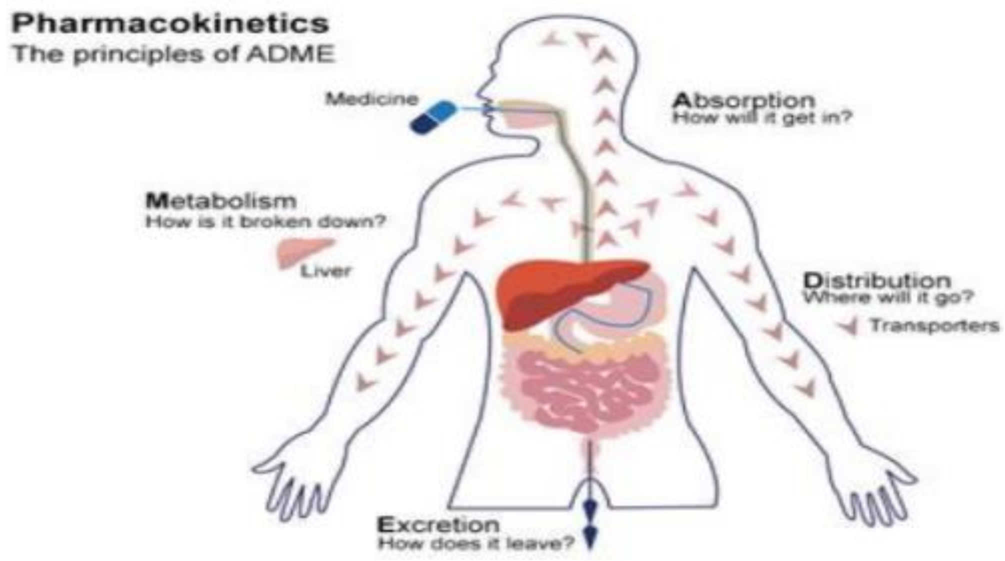


Figure 9 : The principles of ADME.

3.7.1. Physicochemical Properties

- **Lipophilicity**

Lipophilicity, most commonly referred to as the LogP, represents the ratio at equilibrium of the concentration of a compound between two phases, an oil and a liquid phase **(Bohnert, T. 2012).**

Lipophilicity is a physicochemical parameter that has to be widely taken into account when developing new drugs since it has been reported to have a significant influence on various pharmacokinetic properties such as the absorption, distribution, permeability, as well as the routes of drugs clearance**(van de Waterbeemd, H. 2000)**. It has been increasingly demanded to develop drugs with high lipophilicity in order to fulfill the required selectivity and potency of drugs. Such demands have basically arisen as a result of the lipid nature of biological targets. On the other hand, suitable drug formulations have to reflect a good aqueous solubility as well as an acceptable degree of lipophilicity in order to assess the best oral absorption along with the required deposition and activity.

- **Hydrogen Bonding**

Hydrogen bonding is considered the driving factor that plays an obvious role in the partitioning of the biologically active compounds. Hydrogen bonding reflects the interaction between the H-bond (HB) acceptor target and the H-bond (HB) donor compound or vice versa **(Schwobel,J.2011)**.

- **Solubility**

Aqueous solubility is a fundamental property that is nearly involved in every stage of drug development due to its role in the determination of drug uptake, transfer, and elimination from the body**(Balakin,K.2006)**. Intrinsic solubility can be defined as the drug's thermodynamic solubility at a pH value where the drug is found to be completely in the unionized form **(Bergstrom,C.2016).** Drugs' efficiency is primarily dependent on their aqueous solubility, therefore ,drugs with poor solubility or low dissolution rates will be eliminated before entering the blood circulation and hence without giving the required pharmacological activity **(Balakin,K.2006).**The solubility of chemical compounds is influenced by two important factors, namely, the lipophilicity and the tightness of the crystalline structure, and it should be noted that both parameters are related to the solubility in an inverse relationship **(Bohnert,T.2012)**.it is also proven as a key factor in the determination of a drug's oral absorption.

- **Permeability**

Permeable drugs primarily cross biological barriers including the intestinal epithelial and the Blood Brain Barries (BBB) by the mechanism of passive diffusion, where substances are transported by the effect of a concentration gradient. Basically, there are two types of passive diffusion, one is the paracellular transport while the other is the transcellular transport mechanism; other drugs are being transported by either the carrier-mediated or the P-pg mediated transport **(Hou,T. 2006)**. Drug permeability is described by the hydrogen bonding parameter as mentioned in various studies, and the majority of results have shown that less importance is associated with hydrogen bond (HB) acceptor descriptors when predicting the permeability of the human intestinal epithelium **(Refsgaard, H. 2005).**

Excretion refers to the process by which the body gets rid of the waste/toxic products. The drug excretion process can be achieved by either the kidney and/or the liver where drugs are eliminated in the form of urine or bile, respectively. The most important factor that determines the proper drug removal mechanism is the molecular weight, where substances of relatively small molecular weights are mainly removed through urine**(Lamberti,G. 2016).**

3.7.2. The pharmacokinetic profile

The pharmacokinetic profile of a drug substance is determined by various parameters including tissue distribution. The prediction of drug distribution throughout the body is basically divided into three main areas of examination, which are the BBB permeability, the volume of distribution (VD), and the plasma protein binding (PPB). All of the three areas have an observable role in the determination of drug suitable regimens, the effective plasma concentration, and the permeability across the BBB, which in turn helps in predicting CNS targets, side effects, and non-CNS therapies as well **(Lombardo,F. 2003)**.

A number of aspects are being optimized during the assessment of a drug's metabolism profile at the early stages, and these aspects include the metabolic routes, stability, and interactions along with the kinetics of metabolizing enzymes as well. These aspects were shown to be essential for the selection of the suitable drug candidates during the development and discovery of pharmaceutical drugs **(Pelkonen, O. 2005).**The cytochrome P450 (CYP) is considered to be the most influential enzyme in the drug metabolism. Also essential is the knowledge about interaction of molecules with cytochromes P450 (CYP). This superfamily of isoenzymes is a key player in drug elimination through metabolic biotransformation**(Testa B.2007)**. It has been suggested that CYP and P-gp can process small molecules synergistically to improve protection of tissues and organisms**(van Waterschoot RA. 2011**) One can estimate that 50 to 90% (depending on the authors) of therapeutic molecules are substrate of five major isoforms (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) **(Wolf.C.2000)**Inhibition of these isoenzymes is certainly one major cause of pharmacokinetics-related drug-drug interactions**(Huang SM. 2008)** leading to toxic or other unwanted adverse effects due to the lower clearance and accumulation of the drug or its metabolites**(Kirchmair J. 2015)**.

3.7.3. Drug-likeness

As defined earlier, "drug-likeness" assesses qualitatively the chance for a molecule to become an oral drug with respect to bioavailability. Bioavailability is an important property designating the quantity or fraction of the ingested dose of a chemical compound that is absorbed , and strongly influenced by the physicochemical properties of the compounds, especially by their hydrophilicity and solubility **(R.P. Heaney, 2001)**.The Lipinski rule of five **(Lipinski, C.2001),** Ghose,,**(Ghose, A. K. 1999)**, Veber **(Veber, D. 2002),** Egan **(Egan, W. 2000)** and Muegge **(Muegge, I. 2001), this** rules were used for drug likeness pre-screening studies. Lipinski's Rule of Five is a rule of thumb to evaluate drug likeness, or determine if a chemical compound with acertain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule was formulated by Christopher A. Lipinski in 1997, based on the observation that most medication drugs are relatively small and lipophilic molecules**(Lipinski,C. 2001).** The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion ("ADME"). However, the rule does not predict if a compound is pharmacologically active. Lipinski's Rule of Five states that in general an orallyactive drug has:

- ❖ Not more than 5 hydrogen bond donors (OH and NH groups)
- ❖ Not more than 10 hydrogen bond acceptors (notably N and O)
- ❖ A molecular weight under 500 g/mol
- ❖ A partition coefficient log P less than 5

Note that all numbers are multiples of five, which is the origin of the rule's name. Ghose' filter defines drug-likeness constraints as follows: calculated log P is between -0.4 and 5.6, molecular weight is between 160 and 480, molar refractivity is between 40 and 130, and the total number of atoms is between 20 and 70**(Ghose, A. 1999).**

Veber's role base that majority of compounds with a good bioavailability in had less than 10 rotable bonds (ROTB) and polar surface area less than 140 A0 **(Veber, D. 2002)**.

The Egan rule considers good bioavailability for compounds with $0 \geq$ TPSA ≤ 131.6 Å2 and -1≥ logP ≤ 5.88**(Egan, W. 2000)**.

3.7.4. Toxicity Profile

In silico prediction methods that are specialized for the prediction of drugs' toxicity can be classified into methods that predict the systemic toxicity and the other methods specifically predict the toxicity for a certain organ. However, other in silico models that are concerned with predicting the carcinogenicity as well as the genotoxicity are considered to be more complicated **(Roncaglioni, A. 2013)**.

Second part
Experimental part

Chapter I

Materials and methods

Materials and Methods

1. Introduction

Drug discovery is the process through which potential new therapeutic entities are identified, using a combination of computational, experimental, translational, and clinical model**s .** Despite advances in biotechnology and understanding of biological systems, drug discovery is still a lengthy, costly, difficult, and inefficient process with a high attrition rate of new therapeutic discovery. Drug design is the inventive process of finding new medications based on the knowledge of a biological target. In the most basic sense, drug design involves the design of molecules that are complementary in shape and charge to the molecular target with which they interact and bind. Drug design frequently but not necessarily relies on computer modeling techniques and bioinformatics approaches in the big data era **.**

Modern drug discovery involves the identification of screening hits, medicinal chemistry and optimization of those hits to increase the affinity, selectivity (to reduce the potential of side effects), efficacy/potency, metabolic stability (to increase the half-life), and oral bioavailability **.**Once a compound that fulfills all of these requirements has been identified, it will begin the process of drug development prior to clinical trials **.**

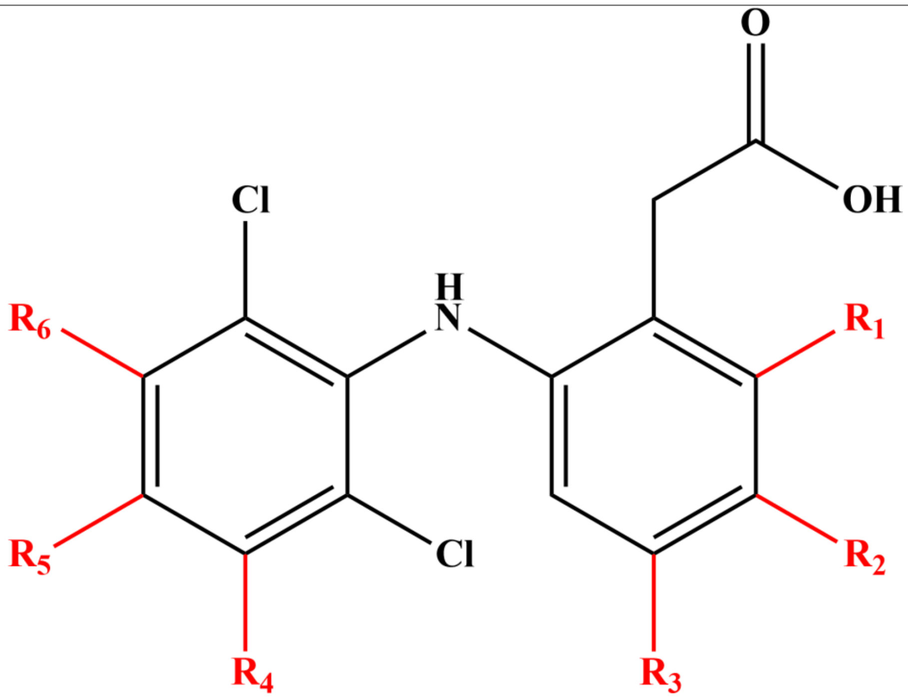
In this project we aim to evaluate the activity of a new series of potentially new diclofenac derivatives towards cyclooxygenase isoform (COX-1 and COX-2) using in silico approach.

2 Combinatorial library

For creating combinatorial library of diclofenac, SmiLib v2.0 was used for rapid combinatorial library enumeration. SmiLib v2.0 offers the possibility to construct very large combinatorial libraries using the flexible and portable SMILES format. The software needs three fragments, the building blocks, the scaffolds and the linkers **(Schüller *et al.*, 2007).** The diclofenac was used as scaffolds by adding the roots as shown in Table1**.**The functional groups (NO_2, CN, OH, $COCH_3$, SCH_3, NH_2) were used as building blocks, also we used an empty linker. The compounds and the functional groups were set in the SMILES format.

After the enumeration, 1252 compounds were created, the screening tools, SwissADME and Protox-III were used to filter the compounds depending on their ADMET propertie

Tableau 1 : The Compounds of ferroquine and the Functional groups.



	R_1	R_2	R_3	R_4	R_5	R_6
Diclofenac 1	NO_2	$COCH_3$	NH_2	SCH_3	NO_2	OH
Di2	CN	NH_2	NH_2	$COCH_3$	NO_2	OH
Di3	OH	NH_2	CN	NO_2	$COCH_3$	NO_2
Di4	OH	NH_2	CN	NO_2	SCH_3	$COCH_3$
Di5	NO_2	$COCH_3$	OH	CN	OH	NO_2
Di6	$COCH_3$	OH	$COCH_3$	NO_2	CN	SCH_3
Di7	NH_2	OH	$COCH_3$	SCH_3	OH	$COCH_3$
Di8	NO_2	NH_2	$COCH_3$	NO_2	$COCH_3$	OH
Di9	NO_2	NH_2	OH	$COCH_3$	OH	$COCH_3$
Di10	$COCH_3$	CN	OH	NO_2	CN	$COCH_3$
Di11	$COCH_3$	OH	NO_2	OH	CN	OH
Di12	NO_2	OH	$COCH_3$	CN	$COCH_3$	$COCH_3$
Di13	NO_2	OH	$COCH_3$	$COCH_3$	OH	CN
Di14	NH_2	$COCH_3$	CN	CN	OH	NO_2
Di15	NH_2	OH	$COCH_3$	$COCH_3$	OH	CN
Di16	NH_2	OH	NO_2	$COCH_3$	$COCH_3$	$COCH_3$
Di17	CN	$COCH_3$	OH	$COCH_3$	OH	OH
Di18	NO_2	$COCH_3$	NO_2	CN	OH	$COCH_3$
Di19	$COCH_3$	OH	OH	OH	NO_2	NO_2
Di20	SCH_3	OH	$COCH_3$	NO_2	OH	OH

3. ADMET screening

The compounds were submitted to SwissADME and Protox webservers to analyze their overall drug score and toxicity risks, compared to the available drugs used **(Ranjith et al., 2019; Drwal et al., 2014).** The ADMET properties including aqueous solubility, blood brain barrier

(BBB), CYP binding, intestinal absorption and hepatotoxicity were evaluated for these molecules.

3.1. Screening for pharmacokinetics and drug-likeness

Pharmacokinetics and drug-likeness prediction for the compounds were performed by the online tool SwissADME **(Ranjith et al., 2019)** of Swiss Institute of Bioinformatics (http://www.sib.swiss) to evaluate individual ADME behaviors of those compounds **(Zoete et al., 2016)**. 2D structural models were drawn in ChemBioDraw Ultra version 15.0 (Cambridge Software) and were then copied as SMILES to be analyzed in the SwissADME webserver. The analysis task was done to check whether those compounds were inhibitor of isoforms of Cytochrome P450 (CYP) family, CYP1A2 and CYP2D6. In addition, pharmacokinetics (such as gastro intestinal absorption, P-glycoprotein and Blood brain barrier) and drug-likeness prediction Lipinski, Ghose and Veber rules and bioavailability score were evaluated **(Lipinski, 2004; Ghose et al., 1998; Al Wasidi et al., 2020).** The Lipinski, Ghose and Veber rules were applied to assess druglikeness to predict whether a compound is likely to be a bioactive according to some important parameters such as molecular weight, LogP, number of Hydrogen-bond acceptors and hydrogen-bond donors. The SwissADME tool used vector machine algorithm (SVM) **(Riyadi et al., 2021) w**ith fastidiously cleaned large datasets of known inhibitors/non-inhibitors as well as substrates/non-substrates.

3.2. Screening For Toxicity properties

The webserver ProTox-III was used for the prediction of toxicity of chemicals, we present it that incorporates molecular similarity, pharmacophores, fragment propensities and machine-learning models for the prediction of various toxicity endpoints; such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes pathways and toxicity targets. The predictive models are built on data from both in vitro assays (e.g. Tox21 assays, Ames bacterial mutation assays, hepG2 cytotoxicity assays, Immunotoxicity assays) and in vivo cases (e.g. carcinogenicity, hepatotoxicity).

The prediction results for the acute toxicity and toxicity targets are generated instantly. The result page will show the predicted median lethal dose (LD50) in mg/kg weight, toxicity class, and prediction accuracy as well as average similarity along with three most similar toxic compounds from the dataset with the known rodent oral toxicity value. The predicted toxicity targets information, if available will be shown with the name of the target as well as the average Fit and similarity of the input compound with the pharmacophore and known ligands of the respective targets.

We choose the following very important proprieties to select the best molecules from the combinatorial library because it shows how dangerous these compounds are to the human health and their side effects:

- **Hepatotoxicity:** Drug-induced hepatotoxicity is a significant cause of acute liver failure and one of the major reasons for the withdrawal of drugs from the market **(Siramshetty et al., 2016).** Drug-induced liver injury (DILI) is either a chronic process or a rare event. However, prediction of DILI is important and one of the safety concerns for the drug developers, regulators and clinicians **(Liu et al., 2015).**
- **Carcinogenicity:** Chemicals that can induce tumors or increase the incidence of tumors are referred as carcinogens **(Kroes et al., 2004).**
- **Mutagenicity:** Chemicals that cause abnormal genetic mutations such as changes in the DNA of a cell are referred as mutagens **(Ames et al., 1973).** Such changes can cause harm to the cells and result in certain disease, e.g. cancer.
- **Cytotoxicity:** Prediction of cytotoxicity is important to screen compounds that can cause undesired and desired cell damage, the latter as in the case of the tumor cells **(Zhang et al., 2014)**.
- **Immunotoxicity:** The adverse effect of xenobiotics on the immune system is called immunotoxicity **(Schrey et al., 2017).**

4. Structural Optimization

The geometries of the studied ligands were first optimized by molecular mechanics (MM), then they were fully re-optimized by the DFT/ B3LYP method with the 6-311G++ (d,p) basis set using Gaussian 09W program package **(Frisch et al., 1988).**

5. Protein selection

The downloading of two protein COX-1 (PDB ID: 5U6X) **(Cingolani et al., 2017)** and COX-2 (PDB ID: 1PXX) **(Rowlinson et al., 2003)** was made from the data base Brookhaven Protein Data Bank (www.rcsb.org/pdb)

6. Molecular docking

The crystal three dimensional structures of the two proteins (PDB ID : 5U6X) and (PDB ID: 1PXX) were selected from protein data bank **(Rose *et al.*, 2017).** The targets receptors were first prepared, all water molecules, ligands and cofactors were deleted and the active site was defined using discovery studio visualize **(Diego, 2007).** The PDB file of 5U6X contains 2 chains, so one chain B were deleted and only chain A was kept to speed up and simplify calculations. Moreover, the PDB files of 1PXX contains 4 chains (A, B, C, D) For COX-2; were three chains B, C and D eliminated, so one chain A was kept to speed up. The PDB files of ligands were saved as PDBQT files after adding the polar hydrogen atoms. Grid boxes were generated using the Auto Grid tool, the grid parameters summarized in Table 2**.** For docking calculations, Lamarckian genetic algorithms were used. All docking experiments consisted of 15 docking runs with 150 individuals and 2,500,000 energy evaluations. The other parameters were left to their default values. The best conformation was selected with the lower docking energy **(Khennoufa *et al.*, 2021; Laraoui *et al.*, 2023)** and was used in the docking analysis using Protein-Ligand Interaction Profiler (PLIP) **(Salentin *et al.*, 2015).**

Tableau 2: Parameters of grid.

Parameter	5U6X	1PXX
Center x	-12.415	125.974
Center y	12.303	133.411
Center z	69.983	139.686
Spacing (Å)	0.375	0.4
Size (Å)	50×50×50	60×60×60

7. Prediction of inhibitory concentration

To predict the IC_{50} concentration of lead compounds, the standards inhibitors of cyclooxygenase (diclofenac) were docked into the same targets (COX-1 and COX-2), the binding affinity vary from -4.86 to -9.47. Standard inhibitors IC_{50} of diclofenac (1.4 mM), were retrieved from in vitro experiments performed elsewhere (**Zacher *et al.*, 2008).** Linear regression analysis was performed through Microsoft office Excel software to generate the pIC_{50}.

Chapter II

Results and Discussion

Results and Discussion

1. ADMET screening

1.1. Physicochemical properties

The physicochemical properties such as solubility and lipophilicity play a major role of whether a drug can progress to be a successful drug candidate (Kwong, 2017). The lipophilicity property of the compounds portray an important role for molecular discovery activities in multifarious domains (Pliška *et al.*, 1996). The parameters considered to measure the score are lipophilicity (0.7 <X Log p< 5), molecular weight (MW) (150 < MW <500 g/mol), solubility (0 < log S <-6). The physicochemical properties of our compounds are shown in Table 3. The results of the logP and logS values of all the designed compounds indicating, that they have a reasonable absorbency, showed the least lipophilicity and moderately water soluble except the compound Diclofenac 1 & Diclofenac 9 which are poorly soluble. Additionally, all compounds with limited complexity defined as fewer than 8 rotatable bonds except Diclofenac 3 and Diclofenac 4.Another physicochemical characteristic of great importance obtained with respect to the acid-base character of the molecule, determined by the ability to accept and donate protons H^+ (Avdeef, 2012). (Lipinski *et al.*, 2012) inferred that molecules that exhibit a lower number of hydrogen bond donor atoms - sum of hydrogen bond donor atoms O-H and N-H (HBD) and a higher number of hydrogen bond acceptor atoms - sum of hydrogen bond acceptor atoms O and N (HBA) have the most favorable ADME/Tox profile. In this Study all compounds have the higher number of hydrogen bond acceptor and lower number of hydrogen bond donor, so they have the most favorable ADMET profile.

1.2. Druglikeness and pharmacokinetics

- **Druglikeness**

According to Lipinski's rule of five (Lipinski, 2004), 10 compounds (Di2, Di8, Di9, Di10, Di11, Di12, Di13, Di15, Di16, Di19) had a good druglikeness satisfying all five criteria under this rule. This proves these ligands can serve as qualified drug candidates. According to Ghose's filter (Al Wasidi *et al.*, 2020), 5 compounds (Di9, Di12, Di13, Di15, Di19) had a good druglikeness implementing the four criteria under this rule. Also, all the compounds had a good druglikeness depending on rule of Veber (Zekri *et al.*, 2022), characterized by high polarity (TPSA≤140), and good flexibility (number of rotatable bonds≤10). Same like Egan's filter ,all the ligands are highly druglikeness (Rishton, 2003). The most important rules of drug likeness e.g. Lipinski, Ghose, Veber, Egan, Muegge showed a score of 55%, indicating good bioavailability for all compounds which means a good druglikeness, Table 4.

Table 3: Physicochemical Properties of the studied compounds.

Molecule	Water Solubility (Log *S*)	Lipophilicity (Consensus Log $P_{o/w}$)	Molar refractivity	H-bond acceptor	H-bond donor	Rotatable bonds	Molecular weight
Di1	-6.60	1.88	118.49	6	3	6	505.29
Di2	-5.25	2.60	129.28	5	2	7	498.7
Di3	-5.23	2.91	137.45	5	1	8	521.77
Di4	-4.88	3.06	146.54	5	0	9	547.81
Di5	-4.69	2.49	129.28	5	2	7	573.85
Di6	-5.01	2.65	128.37	5	2	7	521.77
Di7	-5.19	2.47	122.89	5	2	6	521.77
Di8	-5.04	2.34	120.20	5	3	6	504.75
Di9	-6.03	2.36	114.94	5	3	5	495.74
Di10	-4.81	2.23	120.20	6	3	6	478.71
Di11	-5.13	2.55	119.29	6	3	6	495.74
Di12	-5.31	2.20	113.81	6	3	5	495.74
Di13	-5.15	2.24	111.11	6	4	5	478.71
Di14	-4.98	2.67	118.18	4	2	6	469.70
Di15	-5.11	2.95	117.35	3	2	6	479.74
Di16	-5.26	2.71	122.07	4	2	6	463.74
Di17	-5.09	2.90	127.55	4	2	7	488.75
Di18	-4.63	2.82	127.26	4	1	7	505.77
Di19	-5.37	2.88	128.46	4	2	7	505.77
Di20	-4.59	2.48	119.37	5	3	6	505.77

- **Pharmacokinetics**

The drug development process includes ADMET evaluation. As the drug is absorbed by the system, it encounters several membrane barriers such as gastrointestinal epithelial cells; hepatocyte membrane, blood capillary wall, restrictive organ barriers (e.g. Blood-brain-barrier), glomerulus, and the target cell (Singh *et al.*, 2022). A molecule is said to be less skin permeant if the value of log Kp is more negative (Potts *et al.*, 1992).

Table 4: Druglikeness properties of the studied compounds.

Molecule	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability Score
Di1	0	1	0	0	0	0.55
Di2	1	1	0	0	0	0.55
Di3	1	2	0	0	0	0.55
Di4	1	2	0	0	0	0.55
Di5	1	1	0	0	0	0.55
Di6	1	1	0	0	0	0.55
Di7	1	1	0	0	0	0.55
Di8	0	1	0	0	0	0.55
Di9	0	0	0	0	0	0.55
Di10	0	1	0	0	0	0.55
Di11	0	1	0	0	0	0.55
Di12	0	0	0	0	0	0.55
Di13	0	0	0	0	0	0.55
Di14	0	0	0	0	0	0.55
Di15	0	0	0	0	0	0.55
Di16	0	1	0	0	0	0.55
Di17	1	1	0	0	0	0.55
Di18	1	1	0	0	0	0.55
Di19	1	1	0	0	0	0.55
Di20	0	0	0	0	0	0.55

From the ADMET results in Table 5, all compounds are found to be the least skin permeate Kp. The absorption of the molecule in the intestine is explained by the gastrointestinal (GI) parameter, it is reported as high which means all the compounds are highly absorbed in the intestine HIA .It is physiological barrier which restricts the passage of most of the compounds from the blood to brain, thus having a brain protecting property (Nisha *et al.*, 2016). The compound Di15 was predicted to be blood-brain-barrier (BBB) permeant, and therefore, this compound may suitable for central nervous system therapy, the rest show negative response for blood brain barrier (BBB). P-glycoprotein (P-gp) is an efflux drug transporter that serves as a biological barrier that protects cells from the harmful effects of drugs by transporting toxins and xenobiotics out of cells (Goodman, 1996), also all of the compounds are substrates to P-gp except Di3 and Di4. In the family of CYP enzymes, the CYP3A4 was the most important enzyme on account of metabolizing 50% of all drugs by itself and the CYP2C9 enzyme mainly metabolizes several clinically used drugs such as celecoxib and diclofenac (de Andrés *et al.*, 2021). Almost all of molecules returned as noninhibitors for CYP isoenzymes except for Di1 for CYP2C19 and Di1 and Di4 for CYP2C9, Di16 for CYP3A4. The Boiled-egg plot between TPSA and LogP shown in Figure 1 allows to evaluate passive gastrointestinal absorption (HIA) and brain penetration (BBB), the white region is for high probability for passive absorption by GIT and the yellow region (yolk) is for high probability of brain penetration. In addition, the points are colored in blue, if predicted as actively effluxes by P-gp (PGP+) and in red if predicted as non-substrate of P-gp (PGP-) (Daina *et al.*, 2016). In this study prediction, all compounds except Di1 are within the prediction site, among them Di15 is within yolk (high brain penetration) of the BOILED-Egg and the rest are in white of the BOILED-Egg (high passive absorption of GIT). The Di3 and Di4 are depicted as red indicating non-substrate of P-gp, the rest in blue actively effluxes by P-gp.

Table 5: Pharmacokinetics properties of the studied compounds.

Molecule	GI absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Log Kp (skin permeation) cm/s
Di1	High	No	Yes	No	Yes	Yes	No	No	-6.32
Di2	High	No	Yes	No	No	No	No	No	-6.93
Di3	High	No	No	No	No	No	No	No	-7.06
Di4	High	No	No	No	No	Yes	No	No	-7.41
Di5	High	No	Yes	No	No	No	No	No	-7.32
Di6	High	No	Yes	No	No	No	No	No	-7.10
Di7	High	No	Yes	No	No	No	No	No	-6.96
Di8	High	No	Yes	No	No	No	No	No	-6.97
Di9	High	No	Yes	No	No	No	No	No	-6.28
Di10	High	No	Yes	No	No	No	No	No	-7.12
Di11	High	No	Yes	No	No	No	No	No	-6.90
Di12	High	No	Yes	No	No	No	No	No	-6.77
Di13	High	No	Yes	No	No	No	No	No	-6.77
Di14	High	No	Yes	No	No	No	No	No	-6.61
Di15	High	Yes	Yes	No	No	No	No	No	-6.27
Di16	High	No	Yes	No	No	No	No	Yes	-6.65
Di17	High	No	Yes	No	No	No	No	No	-6.78
Di18	High	No	Yes	No	No	No	No	No	-6.97
Di19	High	No	Yes	No	No	No	No	No	-6.59
Di20	High	No	Yes	No	No	No	No	No	-7.01

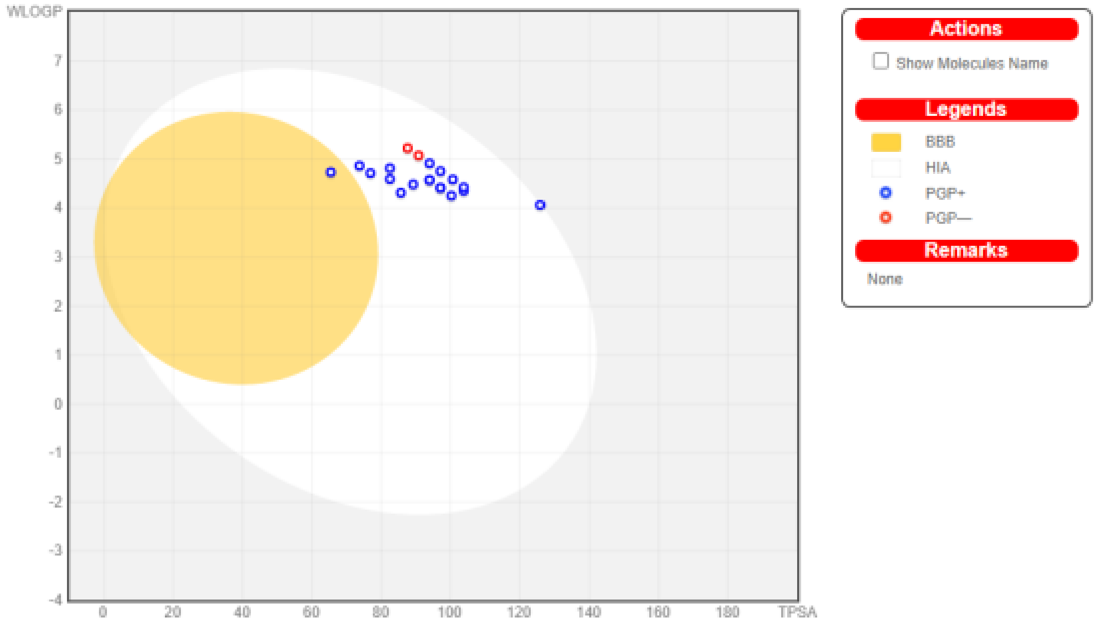


Figure 10: The Boiled-egg plot of the studied compounds.

1.3. Medicinal Chemistry

Similarly, these compounds showed no PAINS (Pan Assay Interference compounds or frequent hitters or promiscuous compounds) so the molecules show potent response in assays irrespective of the protein targets, notably such compounds are reported to be active in many different assays, which can be considered as potential starting points for further exploration. On other hand all the compounds show 2 to 5 Brenkalert, considers compounds that are less small and more hydrophobic and not those defined by “Lipinski’s rule of 5” to widen opportunities for lead optimization. This was after exclusion of compounds with potentially mutagenic, reactive and unfavorable groups such as nitro groups, sulfates, phosphates, 2-halopyridines and thiols. The concept of lead likeness designed to provide leads with tremendous affinity in high throughput screening (HTS) that avow for exploitation of additional interactions in the lead optimization phase (Rishton, 2003). Leads are exposed to chemical modifications that will most likely decrease size and increase lipophilicity which is less hydrophobic than drug like molecules. Lead optimization has been done by rule-based method consisting of molecules with molecular weight in between 250 and 350 Da, log P $\leq$3.5 and are greatly considered as superior to those of drug like compounds and therefore lead like (Hann *et al.*, 2012; Hann *et al.*, 2012). All the compounds flouted brenks rule, and all failed Lead likeness criteria of molecular weight. All the newly designed compounds expose synthetic accessibility score in range of 3.70 to 4.02 suggesting that these compounds are facility synthesizable.

Table 6. Medicinal chemistry of the studied compounds

Molecule	PAINS	Brenk	Leadlikeness	Synthetic accessibility
Di1	0	5	2	3.80
Di2	0	3	2	3.86
Di3	0	3	3	3.94
Di4	0	2	2	4.02
Di5	0	4	1	3.91
Di6	0	4	1	3.84
Di7	0	4	1	3.77
Di8	0	4	1	3.75
Di9	0	5	2	3.75
Di10	0	3	1	3.79
Di11	0	3	1	3.75
Di12	0	3	1	3.72
Di13	0	3	1	3.70
Di14	0	4	2	3.76
Di15	0	4	2	3.70
Di16	0	3	2	3.67
Di17	0	3	2	3.75
Di18	0	4	1	3.92
Di19	0	2	2	3.70
Di20	0	3	1	3.71

1.4. Toxicity

The Protox online server also predicts five toxicological endpoints such as cytotoxicity, mutagenicity, carcinogenicity, hepatoxicity, immunotoxicity. Toxicity classes are defined according to the globally harmonized system of classification of labelling of chemicals (GHS), the Globally Harmonized System. LD_{50} values are given in [mg/kg]:

- class 1, fatal if swallowed (LD50≤5)
- class 2, fatal if swallowed (5<LD50≤50)
- class 3, toxic if swallowed (50<LD50≤300)
- class 4, harmful if swallowed (300<LD50≤2000)
- class 5, may be harmful if swallowed (2000<LD50≤5000)
- class 6, nontoxic (LD50≥5000)

All compounds the LD_{50} surrounded 300 and 2000, so all belong into class 4 except Di7, Di14, Di15 and Di18 that belongs to class 3. The Normally probability of prediction of toxicity model used ProTox-III webserver referenced bellow 0.7 so, all the compounds ranked immunotoxin active (red) because the probability of prediction is 0.85 to 0.99. Also, all compounds are within the limit concerning the mutagenicity except Di4 and Di19. The rest results showed that all compounds were predicted to be non-hepatotoxic, noncarcinogenic and noncytotoxic Table 7.

According to in silico physicochemical, pharmacokinetics, druglikeness and medicinal chemistry prediction and toxicity prediction, the best ligands selected for the molecular docking study are Di3, Di5, Di6, Di9, Di13, Di16, and Di19.

Table 7. Toxicity prediction of the studied compounds

Molecule	Class	Cytotoxicity	Mutagenicity	Immunotoxicity	Carcinogenicity	Hepatotoxicity
Di1	4	0.52	0.68	0.99	0.53	0.52
Di2	4	0.51	0.58	0.99	0.63	0.59
Di3	4	0.51	0.59	0.99	0.56	0.59
Di4	4	0.55	0.51	0.85	0.60	0.61
Di5	4	0.52	0.61	0.96	0.61	0.58
Di6	4	0.55	0.63	0.99	0.58	0.56
Di7	3	0.57	0.67	0.98	0.56	0.58
Di8	4	0.53	0.66	0.99	0.60	0.55
Di9	4	0.51	0.61	0.99	0.59	0.54
Di10	4	0.50	0.68	0.98	0.60	0.58
Di11	4	0.53	0.68	0.99	0.57	0.54
Di12	4	0.53	0.68	0.99	0.58	0.56
Di13	4	0.51	0.66	0.99	0.59	0.55
Di14	3	0.57	0.69	0.99	0.51	0.58
Di15	3	0.58	0.68	0.94	0.50	0.58
Di16	4	0.58	0.67	0.87	0.52	0.60
Di17	4	0.58	0.64	0.99	0.58	0.58
Di18	3	0.53	0.66	0.89	0.50	0.61
Di19	4	0.56	0.51	0.86	0.66	0.64
Di20	4	0.50	0.63	0.98	0.59	0.58

2. Molecular Docking

At the end of docking runs, diverse binding energies of the ligand were obtained with their respective conformations; the stable conformation, which corresponds to the lowest binding energy, was chosen as the best pose (Mouada *et al.*, 2019). The binding energy and binding constant K of the docked structures of all ligands with the two proteins are summarized in Table 8 The magnitude of the calculated binding energy indicates a high binding affinity between proteins and the studied ligands, the binding constant K was calculated using the equation:

$$\Delta G = -RT\ln K$$

Table 8: Binding free energies and binding constant values obtained by molecular docking approach.

Compounds	5U6X		1PXX	
	ΔG (Kcal/mol)	K ($M^{-1)}$)	ΔG (Kcal/mol)	K ($M^{-1)}$)
Di3	-10.6	57.7×10^6	-6.48	55.53×10^3
Di5	-8.65	2.54×10^6	-6.12	30.26×10^3
Di6	-10.24	31.44×10^6	-6.3	40.99×10^3
Di9	-9.67	12.03×10^6	--6.79	93.65×10^3
Di13	-8.1	0.852×10^6	-6.19	34.06×10^3
Di16	-8.66	2.19×10^6	-7.04	142.75×10^3
Di19	-9.58	10.33×10^6	-6.47	54.6×10^3

The results indicate that all the ligand have high binding affinity with studied receptor 5U6X, the best two ligands are Di3 and Di6 with binding free energies equal to -10.6, -10.24 Kcal/mol respectively. Moreover, for the receptor 1PXX, the ligand Di16 interacted the best with binding energy equal to -7.04 Kcal/mol. Moreover, the ligands Di3, Di6 and Di16 represents the. highest binding constant values K. The chosen ligand interacts with both receptors via hydrogen bonds and a hydrophobic bond.

The ligands interactions information with 5U6X and 1PXX are summarized in Table 9. The results showed that all compounds interacted via hydrogen bonds to the amino acids of the binding pocket of 5U6X. The best ligand Di13 interacted via three hydrogen bonds to Arg180, Thr190 and Thr190 amino acids of 5U6X and three hydrophobic bonds between the ligand. and Pro168, Gln189 and Gln189 of amino acids of 5U6X. Also, the results showed that all compounds interacted via hydrogen and hydrophobic bonds of 1PXX.

Table 9. Distances of formed bonds between ligands and 5U6X and 1PXX receptor's residues.

Adduct	Bond type	AA	Distance
Di3-5U6X	Hydrophobic interactions	LEU27A	3.78
		HIS41A	3.91
		MET25A	3.23
		GLU166A	3.16
	Hydrogen-bonds	GLY143A	2.99
		SER144A	3.18
		CYS145A	3.0
		GLU166A	3.05
		GLU166A	2.17
Di5-5U6X	Hydrophobic interactions	HIS41A	3.62
		MET49A	3.93
		MET165A	3.12
		GLN189A	3.23
	Hydrogen-bonds	GLY143A	2.99
		CYS145A	2.88
		GLU166A	2.86
Di6-5U6X	Hydrophobic interactions	LEU27A	3.89
		MET165A	3.12
		GLU166A	3.03
	Hydrogen-bonds	GLY134A	3.06
		CYS145A	2.77
		GLU166A	3.52
		GLU166A	2.68
Di9-5U6X	Hydrophobic interactions	PHE140A	3.98
		MET165A	3.03
		GLU166A	3.34
	Hydrogen-bonds	GLY143A	2.82
		SER144A	2.85
		SER144A	4.09
		CYS145A	2.81
		HIS164A	3.17
		GLU166A	3.07
		GLU166A	2.70
Di13-5U6X	Hydrophobic interactions	MET165A	3.28
		PRO168A	3.42
		GLN189A	3.75
		GLN189A	3.99
	Hydrogen-bonds	GLU166A	2.68
		GLU166A	3.07
		ARG188A	3.11
		THR190A	2.82
		THR190A	2.54

Di16-5U6X	Hydrophobic interactions	MET165A	3.48
	Hydrogen-bonds	GLY134A	2.98
		GLU166A	2.76
		GLU166A	2.87
		GLN192A	3.21
Di19-5U6X	Hydrophobic interactions	LEU27A	3.41
		GLU166A	3.40
	Hydrogen-bonds	GLY143A	2.82
		CYS145A	3.01
		GLU166A	2.81
		GLN189A	2.72
Di3-1PXX	Hydrophobic interactions	ILE589A	3.13
		LYS593A	3.17
		LEU758A	3.30
	Hydrogen-bonds	VAL588A	2.44
		THR591A	2.98
Di5-1PXX	Hydrophobic interactions	ILE589A	3.19
		LYS593A	3.21
		TRY598A	3.41
	Hydrogen-bonds	GLY590A	2.64
		THR591A	3.51
		THR591A	2.98
		LYS593A	2.79
Di6-1PXX	Hydrophobic interactions	LYS593A	3.70
		PHE594A	3.31
		LEU758A	3.40
		GLN815A	3.82
	Hydrogen-bonds	GLY590A	2.87
		LYS593A	4.08
		ASP865A	3.56
Di9-1PXX	Hydrophobic interactions	LYS593A	3.23
		TRP598A	3.31
	Hydrogen-bonds	GLY590A	2.87
		THR591A	2.85
		LYS593A	4.08
		CYS813A	2.88
		SER814A	2.81
Di13-1PXX	Hydrophobic interactions	LYS593A	3.67
		TRP598A	3.53
		LEU758A	3.33
	Hydrogen-bonds	VAL588A	3.80
		VAL588A	3.10
		GLY590A	3.30
		LYS593A	2.64
		ASP865A	3.16
Di16-1PXX	Hydrophobic interactions	PHE441A	3.25
		ILE548A	3.43
		ILE548A	3.77
		ILE548A	3.16

		ILE548A	3.89
		ARG836A	3.83
		ALA840A	3.52
		ARG858A	3.75
	Hydrogen-bonds	HIS439A	3.14
		ARG836A	3.24
		ASP845A	2.79
Di19-1PXX	Hydrophobic interactions	PHE441A	3.52
		ALA547A	3.87
		ILE548A	3.75
		VAL844A	3.20
		ASP845A	3.55
		ARG858A	3.93
	Hydrogen-bonds	TYR546A	3.81
		ASP845A	2.71
		ASP845A	3.30
		ARG858A	3.31
		ARG858A	3.30

3.Prediction of inhibitory concentration IC50

After the docking study, we attempted to predict the IC_{50} of the lead compounds. The docking results of reference compounds as well as their IC_{50} values against cyclooxygenase are shown in Table 10. The linear regression analysis showed a good correlation with correlation coefficient equal to 0.86 and 0.89 respectively with the enzymes COX-1 (5U6X) and COX-2 (1PXX).

The predicted IC_{50} values for the lead compounds were in the range of 5.363 to 2.924 mM and from 2.24 to 4.564 for COX-1 and COX-2 respectively, which were comparatively similar to the IC_{50} of standard compounds that range between 1.4 to 4.9 mM

Tableau.10: Binding free energies and IC50 of standard compounds values obtained by molecular docking approach

	5U6X		**1PXX**	
Comps	ΔG	IC_{50}	ΔG	IC_{50}
Di3	-10.6	5.363	-6.48	3.151
Di5	-8.65	1.101	-6.12	2.243
Di6	-10.24	4.169	-6.3	2.697
Di9	-9.67	2.279	-6.79	3.933
Di13	-8.1	2.924	-6.19	2.420
Di16	-8.66	1.068	-7.04	4.564
Di19	-9.58	1.982	-6.47	3.126

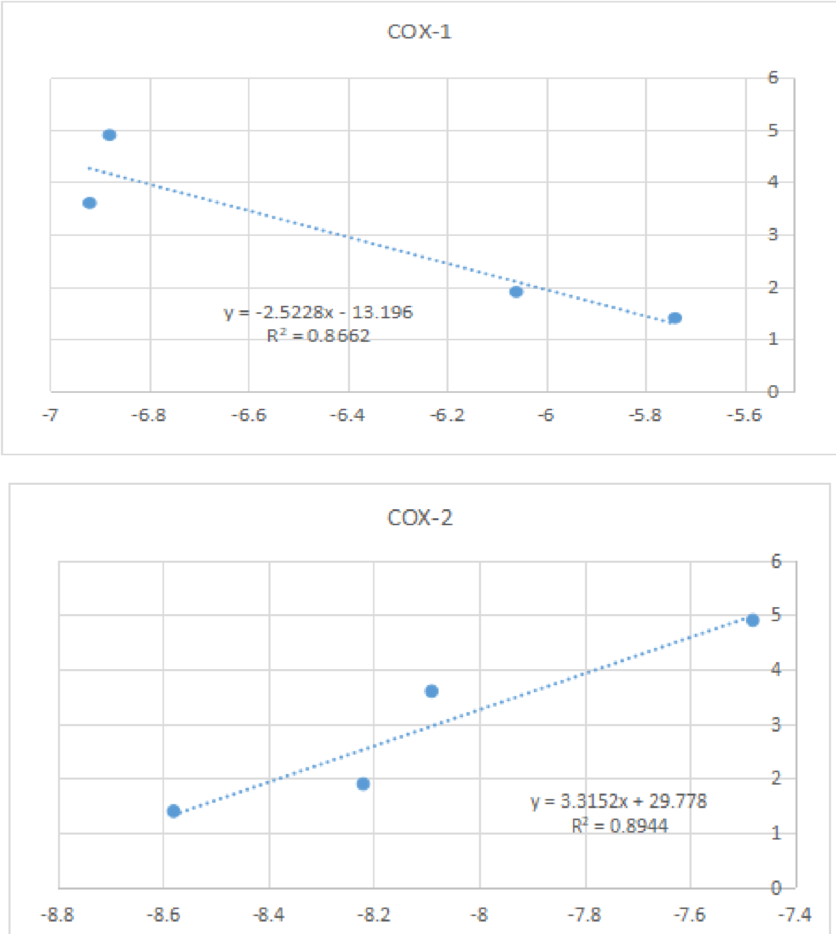


Figure 11 : The difference between binding free energies and IC50 of compounds values obtained by molecular docking approach.

Conclusion

Conclusion

In the present study, in silico approaches applicable for the molecules of diclofenac and study their effects on the target cyclooxygenase. After forming the combinatorial library on Smilib v2.0 by substation of the roots NO_2, CN, SCH_3, OH, NH_2 and $COCH_3$ on the base core of diclofenac, 1252 compounds were obtained. Virtual screening was performed using SwissADME and ProTox web servers, 20 compounds were selected, the ADMET properties were obtained which indicated a good druglikeness, pharmacokinetics, and physicochemical properties. The toxicity prediction showed that all compounds are immunotoxin, and the most within the limit of mutagenicity, however all compounds were predicted to be non-hepatotoxic, noncarcinogenic and noncytotoxic.

After the ADMET virtual screening, 7 compounds which showed better ADMET properties were selected for the molecular docking study. The compounds were docked into COX-1 and COX-2. The two compounds Di3 and Di6 with binding free energies equal to -10.6 and -10.24 Kcal/mol respectively are the best ligands interacted with the COX-1. For the receptor COX-2, the ligand Di16 interacted the best with binding energy equal to -7.04 Kcal/mol. The best ligand Di13 interacted via three hydrogen bonds to Arg180, Thr190 and Thr190 amino acids and three hydrophobic bonds between the ligand and Pro168, Gln189 and Gln189 amino acids of main protease, while all other compounds interacted via only hydrogen bonds to receptors. All ligands can be competitive inhibitors for receptors.

Furthermore, the IC_{50} values of new compounds were predicted using linear regression which were in the range from -5,363 to 2,924 for COX-1, and from 2,433 to 4,564 for COX-2. Finally, the studied compounds might be a good drug candidate for cyclooxygenase.

Perspectives:

The identification of compounds with favorable ADMET properties and activity against cyclooxygenase (COX) highlights their potential as promising anti-inflammatory agents. This research contributes to the growing field of applied biochemistry and drug development, providing valuable insights into the design and evaluation of new drugs with improved therapeutic efficacy and reduced side effects.

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