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Reactivity of three pyrimidine derivatives, potential analgesics, by the DFT method and study of their docking on cyclooxygenases-1 and 2

Jean Missa Ehouman [1], Georges Stéphane Dembélé [1, 3, *], Mamadou Guy-Richard Koné [1, 3], Donourou Diabaté [2], Yafigui Traoré [1, 2] and Nahossé Ziao [1, 3]

[1] *Laboratory of Thermodynamics and Physical Chemistry of the Environment, NANGUI ABROGOUA University, Abidjan, Republic of Côte-d'Ivoire.*
[2] *Biological Sciences Department, Université Peleforo Gon Coulibaly, Korhogo, Republic of Côte-d'Ivoire.*
[3] *Ivorian Research Group in Disease Modeling (GIR2M), Abidjan, Ivory Coast.*

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Abstract

Three pyrimidine derivatives, namely 4-[4-(dimethylamino)phenyl]-6-(pyridin-4-yl)pyrimidin-2-amine (DMPN), 4-(4-aminophenyl)-6-[4-(dimethylamino)phenyl]pyrimidin-2-ol (DMPO) and 4-(4-aminophenyl)-6-[4-(dimethylamino)phenyl]pyrimidine-2-thiol (DMPS), with analgesic properties established by a QSAR study, were subjected to reactivity parameter studies using the DFT method, at the B3LYP/6-311++G(d,p) level of theory. Studies of the docking of these molecules to cyclooxygenases 1 (PDB ID: 5U6X) and 2 (PDB ID: 5F19) were also carried out using the CB-Dock online program. Reactivity parameter calculations revealed that the three derivatives are less stable to internal electron transfer, with the lowest energy gap ΔE ranging from 3.63 eV to 3.88 eV, compared with 6.03 eV for ibuprofen (IBP). These derivatives possess the lowest chemical hardnesses η from 1.81eV to 1.94eV versus 3.02eV for IBP and are better electrophiles than IBP with chemical electrophilicity index values ω from 3.20eV to 3.88eV versus 2.64eV for IBP. These derivatives possess greater chemical reactivity than ibuprofen. All three derivatives also feature electrophilic and nucleophilic attack sites. The docking results show that all three derivatives react with cyclooxygenases in the same areas of reactivity as most NSAIDs. These ligands are more active on COX-2 than COX-1, according to complex stability scores which are stronger with COX-2 than with COX-1. In addition, all three derivatives are more active on COX-2 than ibuprofen, with stability score values of -8.6 kcal/mol for DMPN, -9.2 kcal/mol for DMPO, -8.9 kcal/mol for DMPS and -7.6 kcal/mol for IBP. The DMPO ligand forms the most stable complex with COX-2. These three derivatives thus appear to be selective COX-2 inhibitors, with higher stability scores than ibuprofen. All three derivatives also have good absorption, distribution, metabolism and toxicity properties. They can therefore be used as drugs.

Keywords : Pyrimidine Derivatives; Analgesic; Molecular Docking; Cyclooxygenase; DFT.

1. Introduction

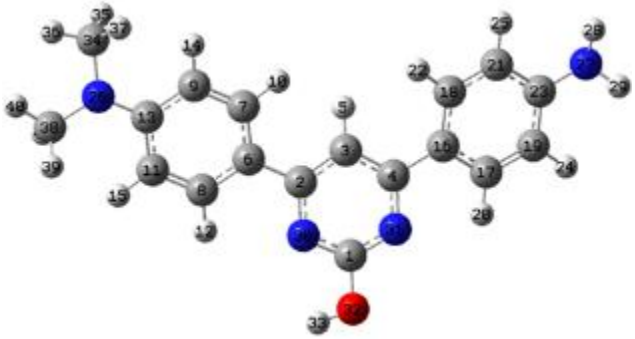
Pain has become a public health issue [1], given its impact on the social and economic productivity of those who suffer from it [2,3]. Various pain management programs exist in many countries, and several types of medication are used [4]. But these treatments are still plagued by numerous side-effects [5,6]. In the search for new, more effective drugs with fewer side effects, various derivatives are being synthesized and tested [7]. Structural modifications are also being made to products already on the market, such as tramadol [8], paracetamol [9], aspirin [10] and ibuprofen [11,12], to make them more effective and, above all, less prone to side effects. Derivatives containing the pyrimidine nucleus have also been synthesized and tested for their analgesic and anti-inflammatory properties [13,14]. The results obtained are very

* Corresponding author: Georges Stéphane Dembélé

encouraging [14,15]. Indeed, many natural or synthetic compounds containing the pyrimidine nucleus possess a wide range of interesting biological activities [16]. Non-steroidal anti-inflammatory drugs (NSAIDs) and other analgesics combat pain by inhibiting COX-1 or COX-2 cyclooxygenases. These enzymes are strongly implicated in the pain process [17,18]. Inhibition reactions of these enzymes can be selective or non-selective [19,20]. To determine the type of interaction possible between an inhibitor and the protein target, numerous electronic, energetic and structural parameters can be calculated.

DMPN

DMPO



DMPS

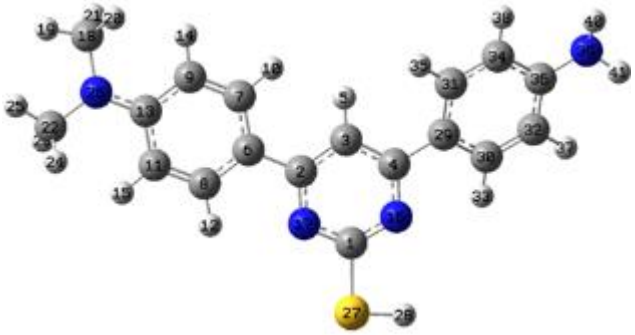


Figure 1 Structures of the three pyrimidine derivatives

These parameters are accessible by quantum chemical methods such as DFT [21,22]. Molecular docking is widely used in the design of new drugs. It determines the relative position of an organic molecule (the ligand) in the reactive zone of the target protein (the macromolecule). This reveals the types of interactions between the ligand and the amino acids forming the macromolecule. This process is very important in the design of new drugs. For example, docking studies are carried out in the search for improved analgesic and anti-inflammatory activities [23,24]. In addition, drug candidates must meet several criteria to be considered as drugs. These criteria are solubility, stability, pharmacokinetic and pharmacodynamic properties of absorption, distribution, metabolism, excretion and toxicity (ADMET). These various parameters are accessible from a druglikeness study based on the compounds' structures. The following three

tri-substituted pyrimidine derivatives : 4-[4-(dimethylamino)phenyl]-6-(pyridin-4-yl)pyrimidin-2-amine (DMPN), 4-(4-aminophenyl)-6-[4-(dimethylamino)phenyl]pyrimidin-2-ol (DMPO) and 4-(4-aminophenyl)-6-[4-(dimethylamino)phenyl]pyrimidine-2-thiol (DMPS) (Figure 1), have been identified as possessing improved analgesic properties compared with base molecules, using a QSAR model [15]. Our work focuses on these three derivatives (DMPs). As potential new analgesics, no data are available to date on their mode of action with the target proteins involved in the pain process. The aim of this work is to calculate the reactivity parameters of these compounds and to carry out their docking on cyclooxygenases-1 and 2 in comparison with ibuprofen (IBP), a current analgesic on the market.

2. Materials and methods

2.1. Geometry optimization

The program Gaussian 09 [25] with its graphical interface GaussView05 is used to build the molecular structures and perform the various calculations. Optimization of the molecular geometry is performed, followed by calculation of the vibrational frequencies. The level of theory employs the DFT method with the B3LYP hybrid functional. To take account of the inter-molecular interactions that are particularly important for this work, diffuse and polarization functions have been added to the Pople basis. Thus, the triple-dzeta split-valence (SVTZ) 6-311++G(d,p) was employed.

Based on the molecular structure optimized to the indicated level of theory, molecular reactivity parameters related to the frontier orbitals such as the energy of the highest occupied molecular orbital E_{LUMO} , that of the lowest vacant molecular orbital E_{LUMO} as well as the energy gap ΔE (ΔE=$E_{LUMO} - E_{HOMO}$) were calculated. Conceptual DFT global molecular reactivity parameters such as chemical hardness η, chemical softness σ, electronegativity χ as well as electrophilicity index ω were also calculated according to the expressions below [26].

$$\eta = \frac{\Delta E}{2} \quad (1)$$

$$\sigma = \frac{1}{\eta} \quad (2)$$

$$\chi = -\mu = \frac{E_{HOMO} + E_{LUMO}}{2} \quad (3)$$

$$\omega = \frac{\mu^2}{2\eta} \quad (4)$$

Local Fukui reactivity descriptors have been calculated for all atoms other than hydrogen atoms, in order to determine the electrophilic and nucleophilic attack sites of molecules [26]. These parameters are defined below:

$$f_k^+ = q_k(N+1) - q_k(N) \quad (5)$$

$$f_k^- = q_k(N) - q_k(N-1) \quad (6)$$

$$\Delta f = f_k^+ - f_k^- \quad (7)$$

$q_k(N)$ is the electron population of atom k in its neutral form, $q_k(N+1)$ the electron population of atom k in its anionic form and $q_k(N-1)$ the electron population of atom k in its cationic form. The positive sign of the dual descriptor Δf indicates an electrophilic site, while the negative sign indicates a nucleophilic site [26,27]. This descriptor is thus a good indicator of local sites of electrophilic and nucleophilic attack [28]. A visualization of molecular electrostatic potential energy surfaces can also indicate the electrophilic and nucleophilic attack zones of a molecule. These surfaces illustrate the spatial distribution of a molecule's electrical charges [29].

2.2. Molecular docking

The CB-Dock online program [30] for protein-ligand docking was used in this work. This program uses cavity detection to guide molecular docking with AutoDock Vina [30]. CB-Dock first identifies the active site by searching the entire surface of the protein [30]. The cavity selected is the one with the lowest overall binding energy. This indicates that the various bonds (hydrogen and hydrophobic bonds) are stronger or more numerous [31]. The program then performs

flexible docking with the optimized ligand form [24]. The structures of cyclooxygenases-1 and 2, enzymes involved in inflammatory and pain phenomena, are provided by the online "Protein Data Bank" (PDB) under the respective codes 5U6X [32] and 5F19 [33]. Proteins are prepared by CB-Dock prior to docking, removing all water molecules, starting ligands and heteroatoms. Discovery Studio 2021 enables docking results to be analyzed and visualized [34]. The choice of the CB-Dock program was guided by its speed and the quality of the results, which were judged to be of interest [35].

2.3. Drug-Likeness

Drug-likeness is a qualitative concept used in drug design to determine the efficacy of a drug candidate. It is estimated from the molecular structure even before the substance is synthesized and tested.

2.3.1. Lipinski's rule of five

According to the following empirical principles, enunciated by Christopher Lipinski and grouped together under the name of the "rule of five", this rule is the most widely used for the identification of "drug-like" compounds, a substance will be better absorbed or penetrated if [36,37] :

- Its molecular weight is less than or equal to 500 Da.
- It has fewer or 5 hydrogen bond donors HBD.
- It has fewer or 10 HBA hydrogen bond acceptors..
- Its log P value is less than or equal to 5.

The new molecular structures were analyzed with the SWISSADME server [38] (http://www.swissadme.ch/) to check whether or not the compounds complied with Lipinski's Rule of Five.

2.3.2. Prediction of Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET)

Human intestinal absorption (HIA) refers to the capacity of the human intestine to absorb the drug. The greater the percentage of human intestinal absorption, the better the human intestine absorbs the drug (from 0∼20% poor absorption; from 20∼70% average absorption, from 70 ∼100% strong absorption).

Caco-2(nm/s) and MDCK (nm/s) predict the intestinal permeability of a compound on Caco-2(<4 poor permeability, between 4 ∼70 medium permeability, >70 high permeability) and MDCK cells.

PPB (Plasma Protein Binding) refers to the degree to which drugs bind to proteins in the blood. The effectiveness of a drug can be affected by the degree to which it binds. The less a drug is bound, the more effectively it can cross cell membranes or diffuse. (<90 low binding, >90 high binding).

BBB (Blood–Brain Barrier) is the descriptor that indicates a compound's ability to penetrate the blood-brain barrier (BBB) and controls the passage of most compounds from the blood to the central nervous system (CNS) (<0.1 low absorption in the CNS, 0.1∼2 medium absorption in the CNS and >2 high absorption in the CNS).

Cytochromes P450 are key enzymes involved in the metabolism of various endogenous and exogenous molecules. They exist under several iso-forms (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) but the most important are the last two. Predicting the interaction of our best inhibitors with these iso-forms has also been essential, since inhibition of these iso-enzymes is certainly one of the main causes of drug interactions leading to toxic or adverse effects [39].

hERG (human Ether-à-go-go-Related Gene) is a gene encoding a voltage-dependent potassium channel that draws potassium out of the cell. Blocking this channel leads to fibrillations in cardiology, which can result in cardiac arrest.

AMES-Test (Salmonella typhimurium reverse Mutation Assay) is a simple method for testing the mutagenicity of a compound. It uses several strains of Salmonella typhimurium bacteria carrying mutations in genes involved in histidine synthesis, so that they require histidine for growth. This test consists in assessing the ability of a compound to induce a mutation enabling a return to growth on a histidine-free medium.

These parameters were determined using the online PreADMET server [40] (https://preadmet.bmdrc.kr/).

3. Results and discussion

3.1. Global molecular reactivity parameters

The three pyrimidine derivatives as well as ibuprofen, the reference molecule, were optimized at the B3LYP/6-311++G(d,p) level of theory with frequency calculations to avoid imaginary frequencies and to ensure that the molecule was in a stable state of minimum energy. The structure of each molecule and the optimized form with atom numbering are shown in Figure 1. Table 1 shows the results of the reactivity parameter calculations.

Table 1 Global molecular reactivity parameters $\boldsymbol{E_{HOMO}}$, $\boldsymbol{E_{LUMO}}$, ΔE, η, σ, χ, and ω calculated at B3LYP/6-311++G(d,p).

Molecules	**IBP**	**DMPN**	**DMPO**	**DMPS**
E_{HOMO} (eV)	-7.0065	-5.5673	-5.4686	-5.4579
E_{LUMO} (eV)	-0.9742	-1.9398	-1.5861	-1.6348
ΔE (eV)	6.0323	3.6276	3.8825	3.8231
η (eV)	3.0161	1.8138	1.9412	1.9116
σ (eV^{-1})	0.3316	0.5513	0.5151	0.5231
χ (eV)	3.9903	3.7536	3.5274	3.5464
ω (eV)	2.6396	3.8840	3.2047	3.2897

The values in Table 1 show that the three pyrimidine derivatives have the highest EHOMO energy values (-5.57eV to -5.46eV) and the lowest ELUMO energy values (-1.94eV to -1.59eV). These different values suggest that these three derivatives are the best electron donors and electron acceptors than ibuprofen respectively [36]. The three derivatives also have the lowest energy gap ΔE (3.63eV to 3.88eV). They therefore have lower kinetic stability and higher chemical reactivity than ibuprofen [37]. The global molecular reactivity descriptors of conceptual DFT are related to the energy gap and give deeper insight into the reactivity and stability of molecules. Table 1 shows that the three derivatives have the lowest chemical hardness η (1.81 eV to 1.94 eV) and the highest chemical softness σ (0.51eV^{-1} to 0.55eV^{-1}). These derivatives are therefore less stable and more reactive than ibuprofen [36]. We also note that the values of the electrophilicity indexes ω (3.20 eV to 3.88 eV) of the derivatives are the highest and those of the electronegativities χ (3.53eV to 3.75eV) are the lowest. These values show that pyrimidine derivatives are better electrophiles [38] and better electron donors than ibuprofen [26]. These overall reactivity descriptors show that the three pyrimidine derivatives studied are less stable to internal electron transfer and have greater chemical reactivity than ibuprofen.

3.2. Local molecular reactivity parameters

Table 2 shows the values of the Fukui local reactivity descriptors calculated by considering the analysis of natural NBO populations at the B3LYP/6-311++G (d, p) level.

Table 2 Local Fukui reactivity parameters $\boldsymbol{f_k^+}$(e), $\boldsymbol{f_k^-}$(e) and $\Delta\boldsymbol{f}$(e) of the three derivatives calculated at level B3LYP/6-311++G(d,p)

DMPN				DMPO				DMPS			
ATOMS	$\boldsymbol{f_k^+}$	$\boldsymbol{f_k^-}$	$\Delta\boldsymbol{f}$	ATOMS	$\boldsymbol{f_k^+}$	$\boldsymbol{f_k^-}$	$\Delta\boldsymbol{f}$	ATOMS	$\boldsymbol{f_k^+}$	$\boldsymbol{f_k^-}$	$\Delta\boldsymbol{f}$
C1	-0.0014	-0.0042	0.0028	C1	-0.0034	-0.0124	0.0090	C1	-0.0247	-0.0017	-0.0230
C2	-0.0966	0.0385	-0.1351	C2	-0.1032	0.0256	-0.1288	C2	-0.1197	0.0285	-0.1482
C3	0.0150	-0.0876	0.1026	C3	0.0305	-0.0887	0.1192	C3	0.0048	-0.0882	0.0930
C4	-0.0870	-0.0156	-0.0714	C4	-0.1012	0.0122	-0.1134	C4	-0.1317	0.0088	-0.1404
C6	0.0304	-0.1323	0.1627	C6	0.0263	-0.0907	0.1170	C6	0.0301	-0.0959	0.1260
C7	-0.0217	-0.0008	-0.0209	C7	-0.0392	-0.0013	-0.0380	C7	-0.0890	-0.0009	-0.0881

C8	-0.0490	-0.0002	-0.0488	C8	-0.0603	-0.0025	-0.0578	C8	-0.1046	-0.0008	-0.1038
C9	-0.0114	-0.0708	0.0593	C9	-0.0044	-0.0515	0.0471	C9	-0.0269	-0.0522	0.0253
C11	-0.0058	-0.0753	0.0695	C11	0.0018	-0.0596	0.0614	C11	-0.0337	-0.0614	0.0277
C13	-0.0574	-0.0028	-0.0546	C13	-0.0616	-0.0066	-0.0549	C13	-0.0875	-0.0057	-0.0818
C16	-0.0300	0.0256	-0.0556	C16	0.0156	-0.0528	0.0684	N16	-0.0604	-0.0296	-0.0308
C17	-0.0464	-0.0121	-0.0343	C17	-0.0477	-0.0075	-0.0402	N17	-0.0588	-0.0117	-0.0471
C18	-0.0349	-0.0059	-0.0290	C18	-0.0275	-0.0049	-0.0226	C18	-0.1164	0.0210	-0.1374
C19	-0.0274	-0.0120	-0.0154	C19	-0.0065	-0.0374	0.0309	C22	-0.1165	0.0212	-0.1376
C21	-0.0328	-0.0097	-0.0231	C21	-0.0125	-0.0288	0.0163	N26	-0.0262	-0.1435	0.1173
N24	-0.0777	-0.0805	0.0027	C23	-0.0631	-0.0293	-0.0337	S27	-0.0825	-0.0682	-0.0143
N25	-0.0929	-0.0090	-0.0839	N26	-0.0243	-0.1416	0.1173	C29	0.0287	-0.0410	0.0697
N26	-0.0278	-0.0464	0.0186	N27	-0.0302	-0.0779	0.0477	C30	-0.1012	-0.0075	-0.0937
C29	0.0062	0.0275	-0.0213	N30	-0.0774	-0.0168	-0.0606	C31	-0.0808	-0.0049	-0.0759
C33	0.0062	0.0275	-0.0213	N31	-0.0805	-0.0278	-0.0527	C32	-0.0361	-0.0334	-0.0027
N37	-0.0308	-0.1877	0.1569	O32	-0.0283	-0.0270	-0.0014	C34	-0.0429	-0.0256	-0.0173
N39	-0.0957	-0.0341	-0.0616	C34	0.0029	0.0206	-0.0177	C36	-0.0739	-0.0276	-0.0464
C1	-0.0014	-0.0042	0.0028	C38	0.0041	0.0209	-0.0168	H20	-0.0093	-0.0143	0.0049

The atoms with the lowest negative values of the dual descriptor Δf are the most favorable for electrophilic attack. These are: for the DMPN derivative C2, N25, C4 pyrimidine, N39 pyridine, C16 and C13; for DMPO it's C2, C4, N30 and N31 pyrimidine, C8, C13 and finally for DMPS we have C2, C4, C13 and C1. The heteroatoms O32 of the DMPO compound and S27 of the DMPS compound are also sites of electrophilic attack. The atoms most favorable to nucleophilic attack have the highest positive Δf values. These are C6, N37 of the dimethylamino group, C3, C11 and C9 for DMPN; N26 of the dimethylamino group, C3, C6, C16, C11 and N27 of the amine group for DMPO; and N39 of the amine group, N26 of the dimethylamino group, C6, C3, C29 and C11 for DMPS. The N26 nitrogen of the amine functional group of DMPN is a nucleophilic attack site. In all three derivatives, the nitrogen atom of the dimethylamino group is favorable to nucleophilic attack. All three derivatives therefore have both electrophilic and nucleophilic attack sites. They can bind to target proteins by hydrogen bonding or electrostatic interactions (Van Der Waals or other forces).

3.3. Molecular electrostatic potential energy surface

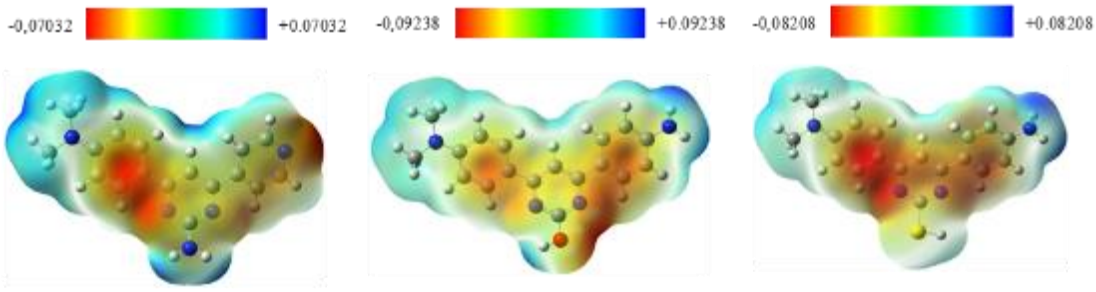


Figure 2 Electrostatic potential energy surfaces of the three derivatives

Several different colored zones are visible on the isodensity surfaces shown in Figure 2.

These colors change continuously from red, corresponding to areas with the most negative charges, to blue, corresponding to areas with the most positive charges. They thus make it possible to identify the areas most favorable

to electrophilic attack, in red, and the areas most favorable to nucleophilic attack, in blue [44]. The benzene ring of the dimethylaminophenyl group, rich in π electrons, is favorable to electrophilic attack in all three compounds. The same applies to the two nitrogen atoms of the pyrimidine nucleus, the benzene nucleus of the aminophenyl group, present in both DMPO and DMPS, as well as the pyridine nitrogen atom of DMPN and the oxygen atom of DMPO. Certain hydrogen atoms in the blue approach zones are favorable to nucleophilic attack. These include the hydrogen atoms of methyl groups, those of amine groups -NH2 and those of functional groups -OH and -SH. Isodensity surfaces show that derivatives can bind to target proteins via electrostatic interactions (Van Der Waals, π-bonds and others) or hydrogen bonds. Only the steric environment can favor one favorable site over another. Analysis of electrostatic potential energy surfaces leads to the same findings as those for local Fukui reactivity parameters seen above.

3.4. Molecular docking results

Optimized ligand forms at the B3LYP/6-311++G(d,p) level of theory were used for flexible dockings [24]. The docking of Ibuprofen (IBP), the reference molecule, was used to select the active site for each enzyme (COX-1 and COX-2). This site remains the same for all four ligands in each case. Cyclooxygenase-1 (PDB: 5U6X) is a dimer with two identical A and B units [32]. The active site belongs to monomer B. The center of this cavity is the point with coordinates x=42.181Å, y=154.727Å and z=-18.658Å. Cyclooxygenase-2 (PDB: 5F19) is also formed by a dimer composed of two A and B units. The active site belongs to monomer A. It is centered on the point with coordinates x=13.702Å, y=49.228Å and z=64.724Å. The interaction structures generated by Discovery Studio 2021 [34] are shown in Figures 3 and 4.

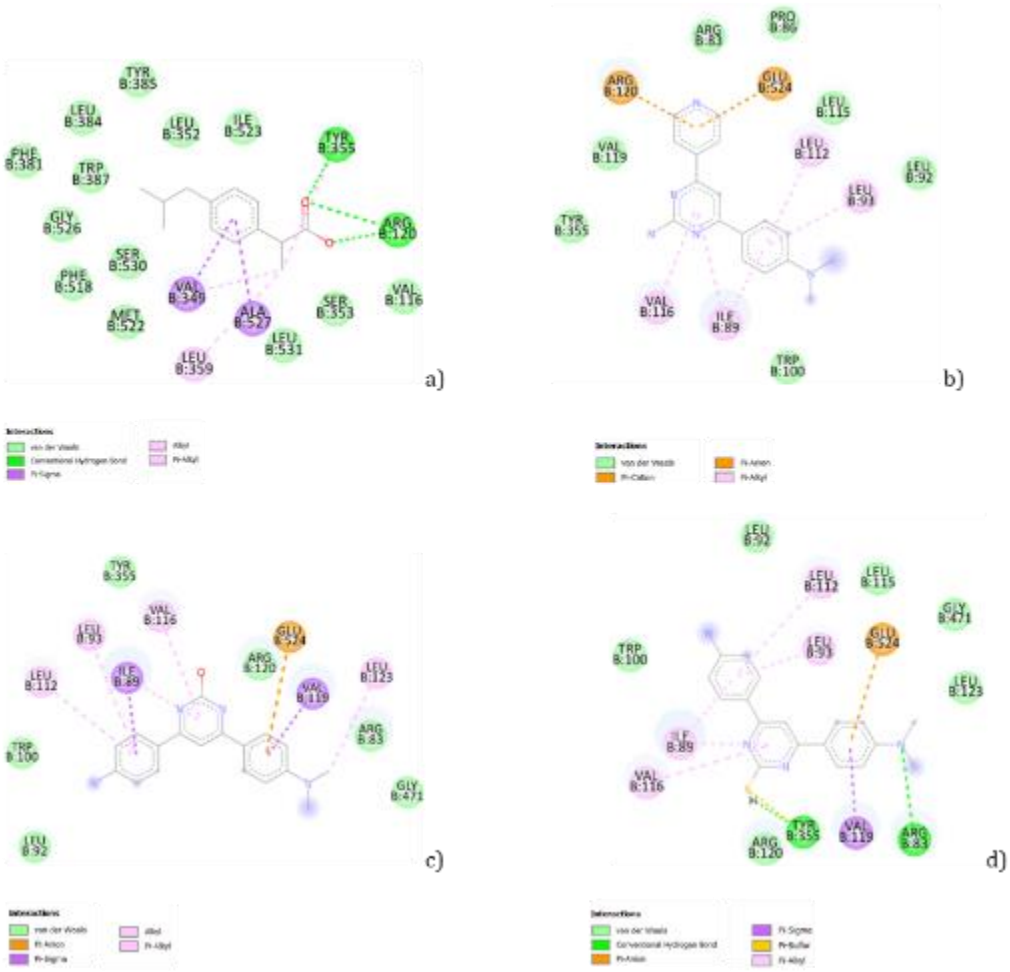


Figure 3 Structures of ligand interactions with amino acids of cyclooxygenase-1 (5U6X)

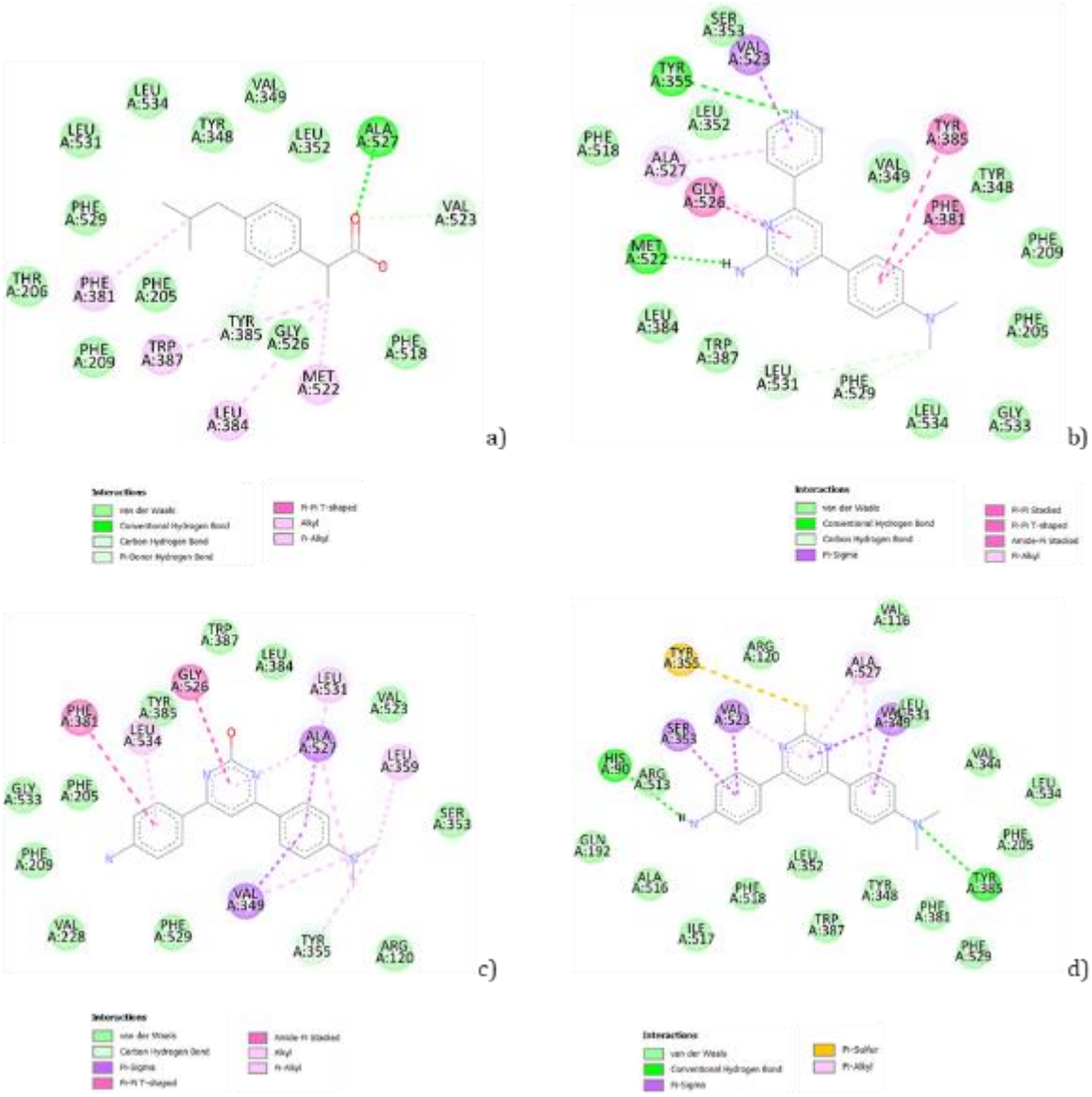


Figure 4 Structures of ligand interactions with cyclooxygenase-2 (5F19) amino acids

Tables 3 and 4 show the stability energies, interacting amino acids, types and bond lengths of the interactions of the various ligands with the amino acids of the target enzymes.

Table 3 Stability energy, amino acids, bond types and bond lengths for interactions of IBP, DMPN, DMPO and DMPS ligands with cyclooxygenase-1 (5U6X)

Ligand	stability score (kcal/mol)	Amino acids	type of interaction	Length (Å)	Ligand	stability score (kcal/mol)	Amino acids	type of interaction	Length (Å)
IBP	-7.4	Arg-120	LH	2.80	DMPO	-7.0	Glu-524	Pi-Anion	4.97
		Tyr-355	LH	3.00			Val-119	Pi-Sigma	3.47
		Arg-120	LH	3.29			Ile-89	Pi-Sigma	3.93
		Val-349	Pi-Sigma	3.40			Ile-89	Pi-Alkyle	5.08

		Ala-527	Pi-Sigma	3.68			Leu-93	Pi-Alkyle	5.12
		Tyr-355	Pi-Alkyle	4.55			Val-116	Pi-Alkyle	5.15
		Leu-359	Alkyle	4.48			Leu-112	Pi-Alkyle	5.49
		Val-349	Alkyle	5.01			Leu-123	Alkyle	4.43
DMPN	-6.8	Arg-120	Pi-Cation	3.71	DMPS	-6.8	Arg-83	LH	3.13
		Glu-524	Pi-Anion	3.76			Tyr-355	LH	2.92
		Ile-89	Pi-Alkyle	4.74			Glu-524	Pi-Anion	4.86
		Ile-89	Pi-Alkyle	4.96			Val-119	Pi-Sigma	3.69
		Val-116	Pi-Alkyle	5.02			Tyr-355	Pi-Soufre	5.10
		Leu-93	Pi-Alkyle	5.30			Ile-89	Pi-Alkyle	4.81
		Leu-112	Pi-Alkyle	5.38			Ile-89	Pi-Alkyle	4.88
							Val-116	Pi-Alkyle	5.06
							Leu-93	Pi-Alkyle	5.16
							Leu-112	Pi-Alkyle	5.26

Table 4 Stability energy, amino acids, bond types and bond lengths for interactions of IBP, DMPN, DMPO and DMPS ligands with cyclooxygenase-2 (5F19)

Ligand	**stability score (kcal/mol)**	**Amino acids**	**type of interaction**	**Length (Å)**	**Ligand**	**stability score (kcal/mol)**	**Amino acids**	**type of interaction**	**Length (Å)**
IBP	-7.6	Ala-527	LH	3.08	DMPO	-9.2	Phe-381	Pi-Pi T	5.01
		Val-523	C-LH	3.45			Gly-526	Amide-Pi	3.89
		Tyr-385	Pi-DLH	3.53			Leu-534	Pi-Alkyle	4.81
		Tyr-385	Pi-Pi T	5.11			Tyr-355	Pi-Alkyle	5.04
		Trp-387	Pi-Alkyle	4.89			Leu-531	Pi-Alkyle	5.08
		Phe-381	Pi-Alkyle	4.96			Tyr-355	Pi-Alkyle	5.15
		Met-522	Alkyle	4.60			Ala-527	Pi-Alkyle	5.19
		Leu-384	Alkyle	4.78			Leu-359	Alkyle	4.27
DMPN	-8.6	Met-522	LH	2.06			Ala-527	Alkyle	4.31
		Tyr-355	LH	3.30			Val-549	Alkyle	5.00

		Phe-529	C-LH	3.57	DMPS	-8.9	His-90	LH	3.03
		Leu-531	C-LH	3.58			Tyr-385	LH	3.28
		Val-523	Pi-Sigma	3.51			Val-523	Pi-Sigma	3.31
		Tyr-385	Pi-Pi T	5.82			Ser-353	Pi-Sigma	3.50
		Phe-381	Pi-Pi S	5.44			Val-349	Pi-Sigma	3.64
		Gly-526	Amide-Pi	3.84			Val-523	Pi-Sigma	3.79
		Ala-527	Pi-Alkyle	4.52			Val-349	Pi-Sigma	3.97
		Ala-527	Pi-Alkyle	4.54			Tyr-355	Pi-Soufre	5.10
DMPO	-9.2	Tyr-355	C-LH	3.58			Ala-527	Pi-Alkyle	4.09
		Val-349	Pi-Sigma	3.40			Val-523	Pi-Alkyle	4.89
		Ala-527	Pi-Sigma	3.46			Ala-527	Pi-Alkyle	5.06

The stability of the IBP molecule (Figure 3a) in the active site of cyclooxygenase-1 (PDB: 5U6X) is ensured by three conventional hydrogen bonds, two with Arg-120 and one with Tyr-355. π-Sigma bonds with Ala-527 and Val-349 and many other hydrophobic interactions (π-Alkyl, Alkyl and Van Der Waals) participate in this stabilization. DMPN stability in the same site (Fig. 3b) is ensured by a π-cationic bond with Arg-120 and a π-anionic bond with Glu-524. π-Alkyl and Van Der Waals bonds are also present. As for the DMPO compound, according to figure 3c, it establishes a π-anionic bond with Glu-524, two π-Sigma bonds with Ile-89 and Val-119 and other hydrophobic π-Alkyl, Alkyl and Van Der Waals bonds. The DMPS compound shown in Figure 3d is stabilized by two conventional hydrogen bonds with Tyr-355 and Arg-83, a π-anionic bond with Glu-524, a π-Sigma bond with Val-119 and a π-Sulfur bond with Tyr-355. There were also π-alkyl and Van Der Waals bonds. The stability energies of the three pyrimidine derivatives DMPN (-6.8 kcal/mol), DMPO (-7.0 kcal/mol) and DMPS (-6.8 kcal/mol) are lower than those of the IBP molecule (-7.4 kcal/mol). This suggests that pyrimidine derivatives may be less active on COX-1 than IBP [45].

For docking on cyclooxygenase-2 (PDB : 5F19); as shown in Figure 4 and Table 4, the stability of the IBP molecule in the active zone (Figure 4a) is ensured by a conventional hydrogen bond between the carbonyl group and Ala-527, a carbon-hydrogen bond between the same group and Val-523, a π-hydrogen bond between the phenyl group and Tyr-385, T-shaped π-π hydrophobic bonds with Tyr-385, π-alkyl with Trp-387 and Phe-381, alkyl with Met-522 and Leu-384 as well as Van Der Waals bonds. The DMPN compound (Figure 4b) establishes two conventional hydrogen bonds between a hydrogen atom of the amine group and Met-522 on the one hand, and between the pyridine nitrogen atom and Tyr-355 on the other. This ligand establishes two carbon-hydrogen bonds with Phe-529 and Leu-531, a π-sigma bond with Val-523, an S-shaped π-π bond with Phe-381, a T-shaped π-π bond with Tyr-385, an amide-π bond with Gly-526, two π-alkyl bonds with Ala-527 as well as Van Der Waals bonds. The DMPO compound, according to Figure 4c, is stabilized by a carbon-hydrogen bond with Tyr-355, two π-sigma bonds with Val-349 and Ala-527. DMPO further establishes a T-shaped π-π bond with Phe-381, an amide-π bond with Gly-526, five π-alkyl bonds with Ala-355, Leu-531, Leu-534 and Ala-527, three alkyl bonds with Leu-359, Ala-527 and Val-549 and Van Der Waals interactions. As for the DMPS compound, according to figure 4d, it is stabilized by two conventional hydrogen bonds established on the one hand between a hydrogen atom of the amine group and His-90 and on the other hand between the nitrogen atom of the dimethylamino group and Tyr-385. DMPS forms five π-sigma bonds with Val-349, Val-523 and Ser-353, one π-sulfur bond with Tyr-355, three π-alkyl bonds with Ala-527 and Val-523 as well as Van Der Waals bonds. The stability energies of the DMPN (-8.6 kcal/mol), DMPO (-9.2 kcal/mol) and DMPS (-8.9 kcal/mol) derivatives are higher than that of the IBP molecule (-7.6 kcal/mol). These different values for stability energies in the same COX-2 active site are relatively significant. They suggest that the three pyrimidine derivatives DMPN, DMPO and DMPS may inhibit COX-2 more significantly than Ibuprofen [45].

Previous studies have shown the role of the amino acids Arg-120 and Tyr-355 in the inhibition of cyclooxygenases by IBP [46,47]. The docking of other inhibitors with COX-2 (PDB: 5F19) shows the involvement of the same amino acids encountered in this study [48-50]. Numerous other studies have also shown the involvement of several of the amino acids cited in this work in the inhibition of COX-1 [51] and COX-2 cyclooxygenases [52]. The higher values of the stability energies of derivatives with COX-2 compared with COX-1 could demonstrate the selective character of the inhibitory

potentials for COX-2 [53]. This result is corroborated by the involvement of certain amino acids such as His-90, Val-523, Ser-353 and Arg-513 in COX-2 inhibition. In fact, these amino acids are located in a pocket of the COX active zone, access to which is specific to selective COX-2 inhibitors [54,55]. The DMPO derivative is more selective for COX-2 than the other two. The negative signs of the various stability energies show that the interaction reactions of ligands on cyclooxygenases-1 and 2 are spontaneous [21].

3.5. Druglikeness prediction

To check whether the pyrimidine derivatives studied are good drugs, druglikeness prediction criteria were calculated. We used the Lipinski rule predicted from the SWISSADME server [38] and the ADMET prediction from the PreADMET server [40].

3.5.1. The Lipinski rule (Rule of five)

The variables characterizing Lipinski's rule, namely molar mass (M), hydrogen donor number (HBD), hydrogen acceptor number (HBA) and lipophilicity (MlogP), were determined. The values of these parameters are given in Table 5.

Table 5 Lipinski parameters of new ligands with DMPs.

Molecules	M(g/mol)	HBD	HBA	MlogP
Règle	<500	<5	<10	<4.15
DMPN	291.35	1	3	1.32
DMPO	292.34	1	4	1.32
DMPS	308.40	0	3	1.75

Analysis of the results in the table shows that all the molecules have molar mass values of less than 500 g/mol, hydrogen donor values of less than 5 and hydrogen acceptor values of less than 10. This result implies that all the DMPs pass through the cell membrane. Also, all DMPs have MlogP values below 4.15, reflecting good water solubility, improved gastric tolerance, efficient elimination by the kidneys and good permeability across the cell membrane. In short, DMPs comply with Lipinski's rule of thumb, and can therefore be administered orally according to Lipinski.

3.5.2. Predicting absorption, distribution, metabolism, excretion and toxicity (ADMET) of DMPs

A good drug candidate must be rapidly and completely absorbed from the gastrointestinal tract, distributed specifically to its site of action in the body, metabolized in a way that does not impair body functions, and eliminated appropriately without causing harm [56]. Prediction of the pharmacological properties of absorption, distribution, metabolism, excretion and toxicity of new molecules was carried out using the PreADMET online server. These parameters are listed in Table 6.

Analysis of the table shows that, in terms of absorption, the three DMP derivatives have high intestinal absorption (70<HIA<100%). This means that the human intestine can assimilate these compounds. In terms of distribution, DMPO and DMPS (PPB>90) have a high degree of binding to blood proteins, compared with DMPN and IBP. DMPO and DMPS molecules can easily pass into the bloodstream. In addition, DMPN and DMPO have low absorption in the central nervous system, compared with DMPS and IBP, which have medium absorption. These low levels of absorption may reduce side effects such as dizziness and nausea (rare) caused by IBP. In terms of metabolism, DMPs have virtually no effect on the inhibition of cytochrome P450 iso-forms. Inhibition of these enzymes is a major source of undesirable drug interactions, since changes in CYP enzyme activity can affect drug metabolism. Finally, for the toxicity test, the compounds studied present medium risks for hERG inhibition, so they are non-carcinogenic for rats and mutagenic like IBP according to the AMES test. Nevertheless, they are carcinogenic to mice, except for the compound DMPS. These results indicate that the molecules studied have good absorption, distribution, metabolism and toxicity properties. These compounds can therefore be used as drugs.

Table 6 Prediction of absorption, distribution, metabolism, excretion and toxicity (ADMET) parameters of DMPs and IBP.

	Absorption			**Distribution**		**Metabolism**						**Toxicity**			
Molecules	HIA	Caco-2	MDCK	PPB	BBB	CYP2D6 Inhibition	CYP2D6 substrat	CYP2C19 Inhibition	CYP2C9 Inhibition	CYP3A4 Inhibition	CYP3A4 Substrat	hERG Inhibition	Carcinogenicity		Mutagenicity
													Mouse	Rat	(AMES-Test)
DMPN	96.603	23.688	15.664	81.847	0.017	No	No	No	No	No	No	Medium Risk	Positive	Negative	Mutagen
DMPO	95.815	22.618	9.939	90.129	0.028	No	No	No	No	No	No	Medium Risk	Positive	Negative	Mutagen
DMPS	98.871	45.043	8.633	94.340	0.140	No	No	No	No	No	Faible	Medium Risk	Négative	Negative	Mutagen
IBP	98.383	21.205	136.48	88.246	1.267	No	No	No	Yes	No	No	Low Risk	Négative	Negative	Mutagen

HIA (%) is the percentage of human intestinal absorption (from 0 ~ 20% poor absorption; from 20 ~ 70% average absorption, from 70~100% high absorption). Caco-2(nm/s) and MDCK (nm/s) predict the intestinal permeability of a compound on Caco-2(<4 poor permeability, between 4 ~70 medium permeability, >70 high permeability) and MDCK cells. PPB (Plasma Protein Binding %) predicts the degree of drug binding to proteins in the blood (<90 low binding, >90 high binding). BBB (Blood-Brain Barrier %) predicts penetration of the blood-brain barrier (<0.1 low absorption in the Central Nervous System (CNS), 0.1~2 medium absorption in the CNS and >2 high absorption in the CNS). P450 cytochromes (CYP2D6, CYP2C19, CYP2C9 and CYP2A4) are important in the oxidative metabolism of compounds. hERG (human Ether-à - go - go-Related Gene) is an ion (potassium) channel that draws potassium out of its cell. AMES-Test (Salmonella typhimurium reverse Mutation Assay) predicts the mutagenic potential of a molecule.

4. Conclusion

Density Functional Theory (DFT) using the B3LYP/6-311++G(d,p) level of theory was employed to calculate chemical reactivity parameters such as frontier orbital energies, conceptual DFT reactivity parameters, Fukui parameters as well as electrostatic potential energy surfaces, to elucidate the reactivities of the three pyrimidine derivatives DMPN, DMPO and DMPS as well as ibuprofen. Docking of the various ligands with the two proteins 5U6X (COX-1) and 5F19 (COX-2) was carried out using the online program CB-Dock. Calculations showed that the three pyrimidine derivatives are more chemically reactive than ibuprofen, and have both electrophilic and nucleophilic attack sites. Docking showed that the three pyrimidine derivatives inhibit COX-2 (5F19) more preferentially and more strongly than ibuprofen. The stability energies of the various complexes with COX-2 (5F19) are -8.6 kcal/mol for DMPN, -9.2 kcal/mol for DMPO, -8.9 kcal/mol for DMPS and -7.6 kcal/mol for IBP. These three derivatives thus appear to be selective COX-2 inhibitors, with higher stability scores than ibuprofen. This work corroborates the definite analgesic character of these three pyrimidine DMP derivatives. The calculation of ADMET properties established that these three derivatives can be good drugs. This study shows that a synthesis of the three derivatives DMPN, DMPO and DMPS, followed by in vivo tests, is necessary to verify the theoretical results obtained.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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