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Theoretical Analysis of the Reactive Sites of Non-steroidal Anti-inflammatory Drugs

Nora Okulik[1] and Alicia H. Jubert[2]

[1]Facultad de Agroindustrias, Universidad Nacional del Nordeste, Cte. Fernández 755, (3700) Roque Sáenz Peña, Chaco, Argentina.
[2] CEQUINOR, Centro de Química Inorgánica (CONICET-UNLP), Departamento de Química, Facultad de Ciencias Exactas y Facultad de Ingeniería, Universidad Nacional de la Plata, CC 962, (1900) La Plata, Argentina.

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Abstract

Motivation. Inflammation is a disease condition in which body tissues are affected by heat, redness, swelling and pain. The therapeutic effects of non-steroid anti-inflammatory drugs (NSAIDs) are well known regarding different diseases. Although there remain a number of other potential sites of action for anti-inflammatory agents, the mode of action of the NSAIDs is attributed primarily to the inhibition of PG synthesis, and more specifically, to the inhibition of the COX enzyme system. In an effort to gain a deeper insight on the properties of NSAIDs as Indometacine,{1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid}, Diclofenac,{[2-(2,6-dichlorophenyl) amino]-benzeneacetic acid}, and Niflumic acids, {2-3((3-trifluoromethyl)phenylamino)-3-pyridinecarboxylic acid}, that will provide knowledge of their action and thus be potentially helpful in the design of new drugs with therapeutic effects, we have performed theoretical studies for the rationale design of their activity mechanism.

Method. The conformational space of these compounds has been scanned using molecular dynamics and complemented with functional density calculations. The Molecular Electrostatic Potential Maps (MEPs) were obtained and analyzed and the corresponding topological study was performed in the frame of the AIM Bader's theory (atoms in molecules).

Results. The optimized geometries of Niflumic, Diclofenac and Indometacine acids were used to carry out the study of the topological properties of several Bond and Non Bonding Critical Points as well as to analyze the Molecular Electronic Potentials. Detailed information is reported herein or deposited as supplementary material.

Conclusions. The topological properties of the NSAIDs studied show that the C-C, C-N and C-H ring bonds are typical of covalent interactions. The C=O and O-H bonds in the carboxylate groups are strong shared interactions. Hydrogen bonds are only localized in the Niflumic acid. The oxygen atoms of the carboxylic groups are sites of the highest concentration of charge and it is assumed that these atoms shall be the preferred sites for an electrophilic attack.

Availability. Molekel is available at http://www.cscs.ch/molekel; AIM package is available at http://www.chemistry.mcmaster.ca/aimpac/

Keywords. NSAIDs; reactive sites; DFT; AIM theory.

1 INTRODUCTION

Inflammation is a disease condition in which body tissues are affected by heat, redness, swelling and pain [1]. There is a plethora of reviews and textbooks outlining the pathology of inflammation, including the sequence of events, network of mediators, e.g. prostaglandins (PGs), leukotrienes and cytokines, and the complex molecular mechanisms that are involved [2]. It is a key feature of a number of diseases and the clinical features of these diseases are described in standard medical text-books [3].

The therapeutic effects of non-steroid anti-inflammatory drugs (NSAIDs) are well known regarding different diseases [4] understood as those that do not have steroid molecular nucleus. These drugs have three main characteristics: a) Anti-inflammatory, b) Analgesic, and c) Antipyretic.

"Non-selective" cyclooxygenase (COX) inhibitors, of the general arylalkanoic acid formula ArCRHCOOH (Ar=aryl or heteroaryl; R=H, CH3, alkyl) make up the largest group of NSAID´s, e.g. salicylates, indoles, propionic acids and fenamantes [5]. References detailing the historical and structural aspects of the development of the NSAIDs, including their mode of action and structure-activity relationships may be found in the literature [6].

Although there remain a number of other potential sites of action for anti-inflammatory agents, the mode of action of the NSAIDs is attributed primarily to the inhibition of prostaglandins (PGs) synthesis [7], and more specifically, to the inhibition of the COX enzyme system [8]. The COX-I and COX-II active sites are described as hydrophobic channels of amino acids [8a]. The known inhibitors of COX interact with the extreme amino acids of the channel and binds irreversibly [9] or reversibly [8a] with them. It is suggested that NSAIDs act at additional sites in the inflammatory cascade [10]. The carboxylate group of the NSAIDs play a key role for a successful interaction with the enzyme producing prostaglandins in the human body. This competes with the arachidonic acid as it was proposed by Vane for aspirin-like drugs [7b].

Theoretical studies of bioactive compounds are of interest in order to gain a deeper insight on their action and thus helping in the design of new drugs with therapeutic effects. The knowledge of physicochemical properties and sites of reaction of NSAIDs will provide a deeper insight of their probable action.

Molecular electrostatic potential (MEP) can be useful in understanding sites for electrophilic attack [11]. MEPs are well suited for analyzing processes based on the "recognition" of one molecule by another, as in drug-receptor, and enzyme-substrate interaction, because it is through their potentials that the two species first "see" each other. It is through this potential that a molecule is first "seen" or "felt" by another approaching chemical species, V(R)s have been used extensively for interpreting and predicting the reactive behavior of a wide variety of chemical systems in both electrophilic and nucleophilic reactions, the study of biological recognition processes [12] and hydrogen bonding interactions [13].

The V(R)s, created in the space around a molecule by its nuclei and electrons, have emerged as a useful analytical tool in the study of molecular reactivity [13]. Unlike many of the other quantities used at present and earlier as indices of reactivity, V(R), is a real physical property that can be determined experimentally by diffraction or by computational methods [14].

However, identification of reactivity patterns based on the MEP exhibits intrinsic drawbacks,

since the MEP is obtained through the classical electrostatic potential [15]. Then, it is not possible to determine sites for nucleophilic attack because the zones of positive potential are not necessarily expressing affinity for nucleophiles but the concentrated nature of the nuclear charges.

In this work we use procedures first proposed by Politzer et al. for using electrostatic potentials to predict and interpret nucleophilic processes [16]. A more general and rigorous approach is based in Bader´s Atoms in Molecules (AIM) theory [17] relying on electron density, $\rho(r)$, to reformulate chemical concepts such as atoms, bonds, electron pairs or reactivity. As well as the MEP, the electron density is a physically observable magnitude, independent of any arbitrary partition of the molecular orbital space [18]. This approach is in touch with the spirit of the density functional theory, which establishes that the total electronic density is the fundamental magnitude in many-electron systems [19].

In an effort to elucidate a deeper insight on the physicochemical properties of Niflumic [2-3((3-trifluoromethyl)phenylamino)-3-pyridinecarboxylic acid], Diclofenac {[2-(2,6-dichlorophenyl) amino]-benzeneacetic acid} and Indometacine [1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid] acids we carried out ab-initio calculations performed on the NSAIDs. The conformational space of those compounds has been scanned using molecular dynamics (MD) calculations and further density calculations were performed to optimize the geometry of the lowest-energy conformers of each species obtained in the simulations. The MEPs are obtained and analyzed and the topological analysis was performed in the frame of the Bader's Theory [17a].

The topological properties of the three molecules show that the C-C, C-N and C-H ring bonds in all the structures are typical of covalent interactions. The C=O and O-H bonds in the carboxylate groups are strong shared interactions. Two hydrogen bonds are localized in the Niflumic acid while no hydrogen bonds were found in the other molecules.

The oxygen atoms of the carboxylic groups are sites that exhibit the highest concentration of charge. Therefore, it is assumed that from all the possible sites, the oxygen atoms will be the preferred ones for an electrophilic attack.

2 METHODS AND COMPUTATIONAL DETAILS

2.1 Atoms-in-Molecules Theory: an overview.

AIM theory permits to follow the Lewis standpoint of a chemical reaction, to determine the electrophilic and nucleophilic zones of a molecule from the topology and topography of the Laplacian of the charge density, $\nabla^2\rho$ [17-19]. It is based upon the critical points (CPs) of the

molecular charge density, $\rho(r)$. At these points, the gradient of the electronic density, $\nabla\rho(r)$, is null and it is characterized by means of the three eigenvalues, λi (i = 1, 2, 3), of the $\rho(r)$ Hessian matrix CPs are named and classified as (r,s) according to their rank, r (number of nonzero eigenvalues), and signature, s (the three eigenvalues algebraic sum). In molecules there are four types of CPs having special interest: (3,-3), (3,-1), (3,+1) and (3,+3).

A (3,-3) critical point corresponds to a maximum in $\rho(r)$, characterized by $\nabla^2\rho(r) < 0$ and occurs usually at the nuclear positions. A (3,+3) critical point relates to a decreasing of the electronic charge and it is characterized by $\nabla^2\rho(r) > 0$. This point is also known as cage critical point. (3,+1) points or ring CPs, are saddle points. Finally, a (3,-1) point or bond CP, is located frequently between two neighboring nuclei, denoting the existence of a chemical bond between them.

Several properties evaluated at the bond CP make up powerful tools to classify a given chemical structure [20]. Briefly, two negative eigenvalues of the Hessian matrix (λ_1 and λ_2, respectively) measure the degree of contraction of $\rho(r)$ at a normal direction to the bond towards the CP, while a positive eigenvalue (i.e., λ_3) gives a quantitative indication of the contraction degree parallel to the bond and from the CP towards each of the neighboring nuclei. When the negative eigenvalues dominate, the electronic charge is locally concentrated in the region of the CP leading to an interaction attributable to covalent or polarized bonds. They are characterized by large $\rho(r)$ values, $\nabla^2\rho(r) < 0$, and $|\lambda_1|/\lambda_3 > 1$. When the positive eigenvalue is dominant, the electronic density is locally concentrated at each atomic basin. The interaction is classified as closed shell and it is typical of highly ionic bonds, hydrogen bonds, and van der Waals interactions. This particular interaction is described by relatively low $\rho(r)$ values, $\nabla^2\rho(r) > 0$ and $|\lambda_1|/\lambda_3<1$.

AIM theory permits the identification of reactive sites by means of the Laplacian of the charge density, $\nabla^2\rho$. AIM defines the valence-shell charge concentration (VSCC) as the outer molecular zone where $\nabla^2\rho < 0$. This zone is the one which, upon chemical combination, is distorted to yield non-bonded critical points (NBCP), which are minima in $\nabla^2\rho$ (maxima of charge concentration), corresponding in number and position to the electron pairs defined by the Lewis and related models [15, 17]. NBCP correspond to zones where an electrophilic attack can occur.

2.2 Calculation details

The conformational space for the molecules of the Niflumic [2-3((3-trifluoromethyl)phenylamino)-3-pyridinecarboxylic acid], Diclofenac {[2-(2,6-dichlorophenyl) amino]-benzeneacetic acid} and Indometacine [1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid] acids was studied using the molecular dynamics (MD) module of the HyperChem package [21]. Several simulations were accomplished with the aid of the MM+ force field also available in that package. The starting geometries were those characterized by the gauche,

cis- and trans-conformations around the NH or C=O atoms between two rings.

The starting geometries were heated from 0 to 600 K in 0.1 ps. Then, the temperature was kept constant by coupling the system to a simulated thermal bath with a bath relaxation time of 0.5 ps. The simulation time step was 0.5 fs. After an equilibration period of 1 ps a 500 ps-long simulation was run saving the coordinates every 1 ps. Those geometries were then optimized to an energy gradient less than 0.001 kcal mol^{-1} $Å^{-1}$ using the MM+ force field.

The lowest energy conformers of the molecules, obtained according to the above methodology were further studied using the density functional theory as implemented in the Gaussian 98 package [22]. Geometry optimizations were performed using the Becke's three parameter hybrid functional [23] with the Lee-Yang-Parr correlation functional [24], a combination that gives rise to the well known B3LYP method. The 6-31G** basis set is used for all the atoms. The fully optimized molecular geometries were characterized as minima in the potential energy surface by the absence of imaginary vibrational frequencies. Calculations were carried out with Gaussian 98 package [22] using the density functional theory (DFT) and the same basis set as above. The MEPs were obtained after the Gaussian calculations and visualized with the Molekel program [25].

Topological analysis and the local properties evaluation were made with the PROAIM software [26] using the wave functions calculated at the B3LYP level and the 6-311++G** basis set implemented in the Gaussian 98 computer program [22]. The graphs of structures and the contour maps of the charge density Laplacian were obtained with the help of the PROAIM program [26].

3 RESULTS AND DISCUSSION

3.1 Structural data and topological analysis

The optimized geometries of Niflumic [2-3((3-trifluoromethyl)phenylamino)-3-pyridinecarboxylic acid], **1**, Diclofenac {[2-(2,6-dichlorophenyl) amino]-benzeneacetic acid}, **2**, and Indometacine [1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid], **3**, acids used to carry out the study proposed in the present work are shown in Figure 1. Detailed information about the geometric parameters of the optimized structures of the species **1**, **2** and **3** is deposited as supplementary material.

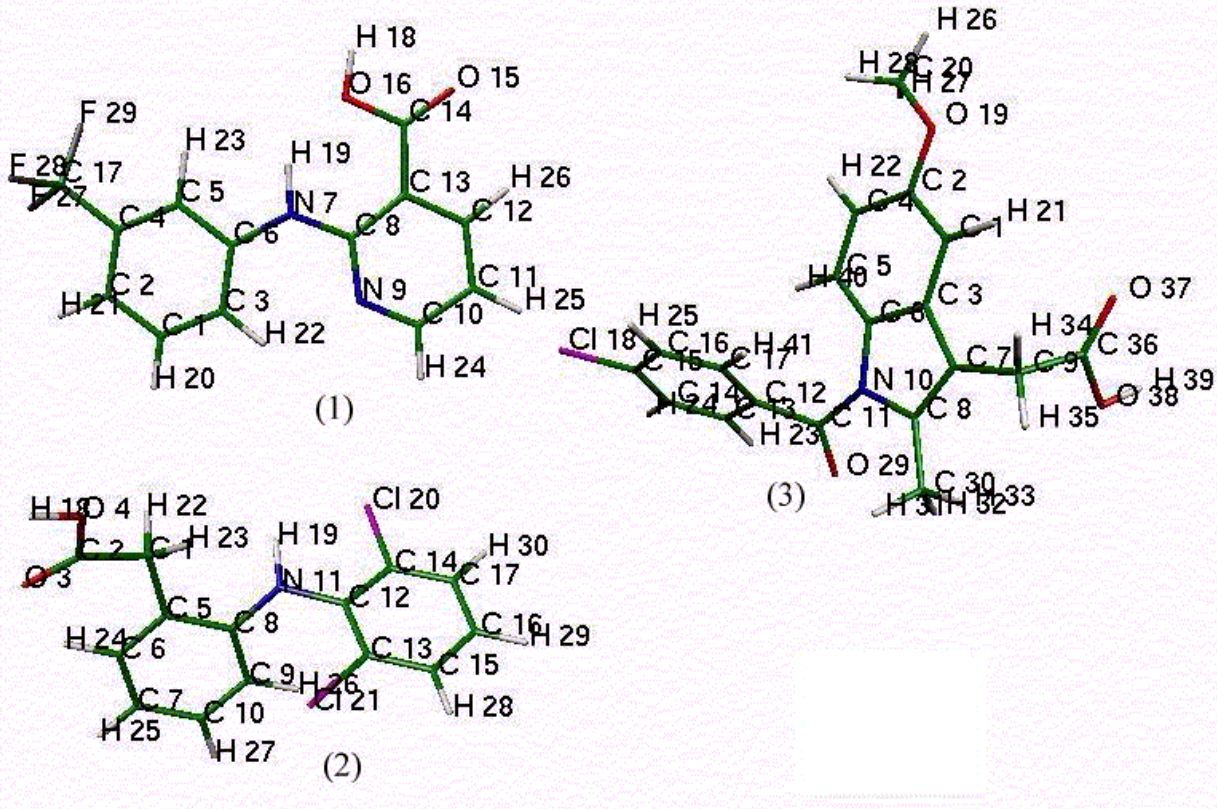

Figure 1. Optimized geometries of structures under study in the present work.

Table 1 shows the properties of the electronic charge density in some selected BCP´s of the studied species. The values of ρ_b and $\nabla\rho_b$ for the C-C and C-N ring bonds in all structures are typical of a shared or covalent interaction, one that is dominated by a contraction of ρ towards the bond path leading to its accumulation in the internuclear region. The ρ_b values are within the range 0.2861-0.3121 au and $\nabla\rho_b$ are within the range -0.7336 and -0.8822 au for C-C interactions and 0.2908-0.3402 au and -0.8283 and -0.9512 au for C-N interactions, respectively

ρ_b and $\nabla\rho_b$ for the C-F and C-Cl bonds are intermediate between those for a shared interaction as found in C-H, C-C and C-N bonds and in a closed-shell (or ionic interaction), for which ρ_b is less than 0.1 au and $\nabla\rho_b > 0$ 30 au. ρ_b is ~ 0.2697 au and $\nabla\rho_b$ is -0.2849 au for the C-F bond. For the C-Cl bonds these vary by 0.005 from the ρ_b = 0.1884 au and by 0.009 from the $\nabla\rho_b$ = 0.2529 au. Thus, we conclude that C-F and C-Cl bonds are relatively weakly shared interactions in all these cases.

In the carboxylic group the values of ρ_b and $\nabla\rho_b$ lie within the range: 0.4125-0.4144 au and -0.2774 and -0.2183 au, for the C=O bonds; 0.2868-0.2974 au and -0.4950 and -0.5038 au for the C-O bonds and 0.3544-0.3560 au and -2.4744, -2.4856 au correspond to O-H bonds. In all cases $\nabla\rho_b$ values are large and negative pointing to strong shared interactions.

Table 1. Topologic properties of charge density[a] calculated at different BCP´s for structures **1**-**3**.

Acid	Bond	ρ_b	$\nabla\rho_b$	λ_1	λ_2	λ_3	E_b
Niflumic (**1**)	C_{17}-F_{29}	0.2697	-0.2849	-0.6050	-0.5394	0.8595	-0.3688
	C_{17}-C_4	0.2672	-0.6954	-0.5413	-0.5173	0.3632	-0.2361
	C_5-H_{23}	0.2809	-0.9596	-0.7487	-0.7324	0.5216	-0.2786
	C_4-C_5	0.3086	-0.8460	-0.6450	-0.5207	0.3198	-0.3158
	C_6-N_7	0.2848	-0.7678	-0.5676	-0.5245	0.3242	-0.3678
	N_7-H_{19}	0.3418	-1.7579	-1.3375	-1.2737	0.8534	-0.4916
	C_8-N_9	0.3414	-1.0619	-0.7547	-0.6671	0.3599	-0.4537
	C_{14}-0_{15}	0.4125	-0.2774	-1.0810	-0.9906	1.7943	-0.6995
	C_{14}-O_{16}	0.2868	-0.4950	-0.6038	-0.5919	0.7007	-0.4039
	0_{16}-H_{18}	0.3544	-2.4856	-1.7729	-1.7405	1.0278	-0.6858
	H_{19}-O_{16}	0.0298	0.1246	-0.0430	-0.0401	0.2079	0.0028
	N_9-H_{22}	0.0196	0.0696	-0.0203	-0.0192	0.1091	0.0025
Diclofenac (**2**)	C_{17}-H_{30}	0.2822	-0.9745	-0.7606	-0.7447	0.5307	-0.2803
	C_{17}-C_{14}	0.3095	-0.8579	-0.6515	-0.5239	0.3176	-0.3195
	C_{14}-Cl_{20}	0.1884	-0.2529	-0.3019	-0.2829	0.3318	-0.1269
	N_{11}-H_{19}	0.0565	-1.5983	-1.2784	-1.2100	0.8900	-0.4568
	N_{11}-C_8	0.2744	-0.7476	-0.5449	-0.5054	0.3028	-0.3303
	C_8-C_9	0.3064	-0.8423	-0.6456	-0.5230	0.3263	-0.3108
	C_2-O_3	0.4141	-0.2249	-1.0904	-1.0089	1.8744	-0.7029
	C_2-O_4	0.2967	-0.5126	-0.6393	-0.6232	0.7499	-0.4262
Indometacin (**3**)	C_9-C_{36}	0.2512	-0.6020	-0.4936	-0.4715	0.3630	-0.2101
	C_9-H_{34}	0.2770	-0.9304	-0.7292	-0.7242	0.5230	-0.2721
	C_7-C_8	0.3233	-0.8924	-0.6963	-0.5069	0.3077	-0.3477
	C_8-N_{10}	0.2789	-0.7015	-0.5386	-0.4807	0.3178	-0.3576
	C_1-C_3	0.3040	-0.8332	-0.6299	-0.5223	0.3200	-0.2412
	C_2-O_{19}	0.2811	-0.3734	-0.5572	-0.5480	0.7318	-0.3956
	C_{11}-O_{29}	0.4046	-0.2443	-1.0482	-0.9547	1.7585	-0.6790
	C_{12}-C_{17}	0.3035	-0.8268	-0.6293	-0.5254	0.3280	-0.3045
	C_{15}-Cl_{18}	0.1936	-0.6020	-0.3139	-0.2968	0.3373	-0.1330
	C_{36}-O_{38}	0.2974	-0.5038	-0.6429	-0.6248	0.7639	-0.4281
	C_{36}-O_{37}	0.4144	-0.2183	-1.0920	-1.0071	1.8808	-0.7036
	O_{38}-H_{39}	0.3560	-2.4744	-1.7577	-1.7277	1.0110	-0.6849
	O_4-H_{18}	0.3559	-2.4729	-1.7567	-1.7266	1.0102	-0.6844

[a] ρ_b, $\nabla\rho_b$, λ_I and E_b in au.

Two hydrogen bonds are localized in Niflumic acid: between N_9-H_{22} and between O_{16}-H_{19}: The ρ_b values are 0.0196 and 0.0298 au respectively. The gradients, $\nabla\rho_b$, are of similar magnitude and are positive in each case (0.0696 and 0.1246 au, respectively). The $|\lambda_1|/\lambda_3$ relationship in a covalent

bond is > 1. In the N-H hydrogen bond the ratio $|\lambda_1|/\lambda_3$ value is 0.1858 au and in the O-H hydrogen bond this value is of 0.2068 au (whereas these are e.g. 1.5673 and 1.7249 au for the N-H and O-H covalent bonds in Niflumic acid). The associated bond paths in the hydrogen bonds are slightly curved and, consequently, the bond path lengths (R_b) for N_9-H_{22} and O_{16}-H_{19} exceed their geometric lengths (R_e). For the first case R_b = 4.2937 au and R_e = 4.2144 au and in the second the values are 3.5667 and 3.5380 au, respectively.

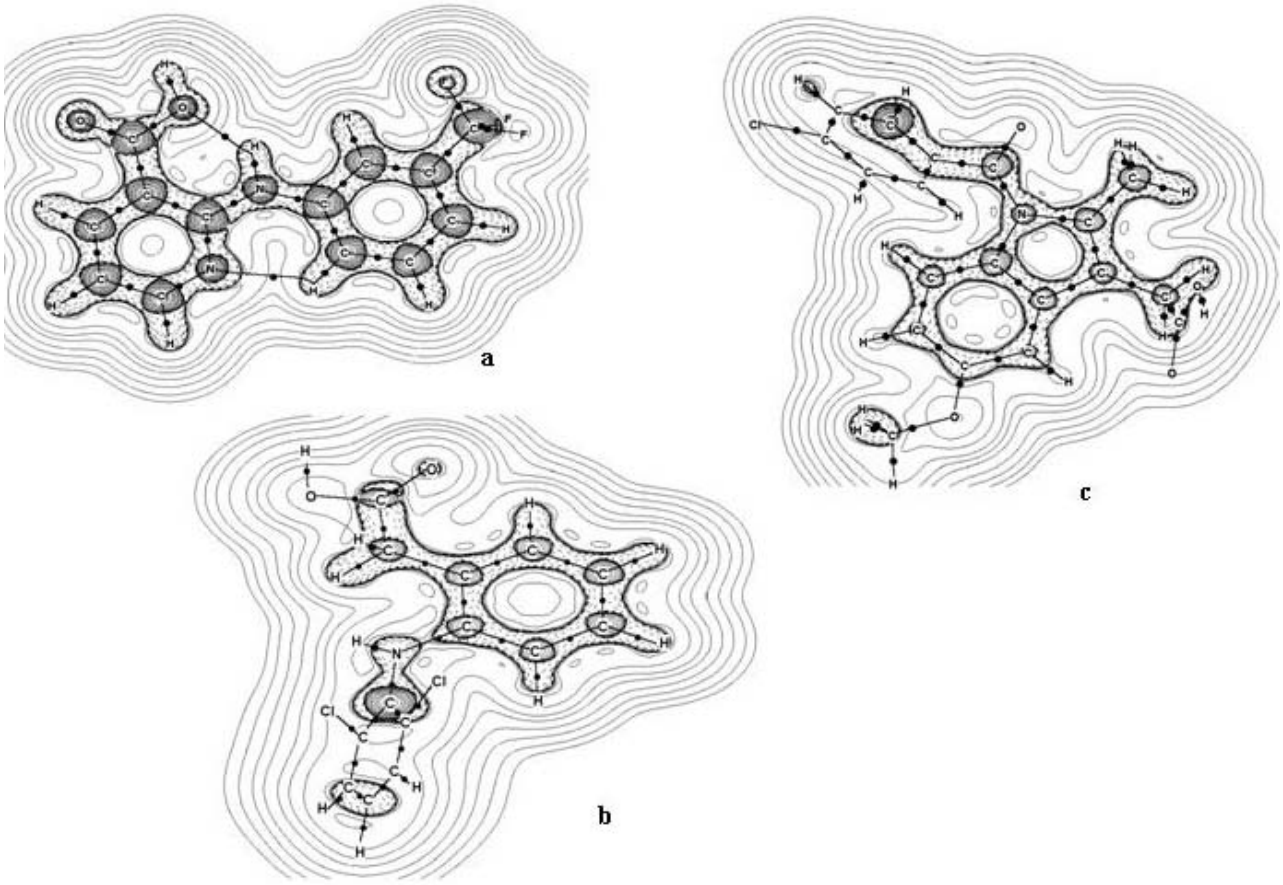

Figure 2. Laplacian of the electronic charge density of (a) Niflumic, (b) Diclofenac and (c) Indometacin Acids. Solid lines represent regions of electronic charge concentration and broken lines denote regions of electronic charge depletion. Bond CP are indicated with circles. The molecular graph is also indicated. The contours of the Laplacian of the electronic charge density increase and decrease from a zero contour in steps of ±2 x 10^n, ±4 x 10^n, and ±8 x 10^n, with *n* beginning at -3 and increasing by unity.

The plots of the Laplacian distribution for the Niflumic, Diclofenac and Indometacine acids, Figures 2 (a), 2 (b), 2 (c); show the sharing of the charge concentration between the atoms. As it is found in the Niflumic acid for C-H, C-C, C-N bonds, the regions of charge concentration for N_9-H_{22} and O_{16}-H_{19} interactions are largely localized within the basins of the hydrogen atoms as expected for typical hydrogen bond interaction.

3.2 Molecular Electrostatic Potential Maps

As we have mentioned earlier, the electrostatic potential has been used primarily for predicting sites and relative reactivities towards electrophilic attack, and in studies of biological recognition and hydrogen bonding interactions [11-14]. The emphasis of these studies has been on negative regions of V(r). Here, the molecular electrostatic potentials of Niflumic, Diclofenac and Indometacin acids are depicted in Figure 3 (a), (b) and (c). Each of these molecules has several possible sites for electrophilic attack in which V(r) calculations have provided insights into the order of preference.

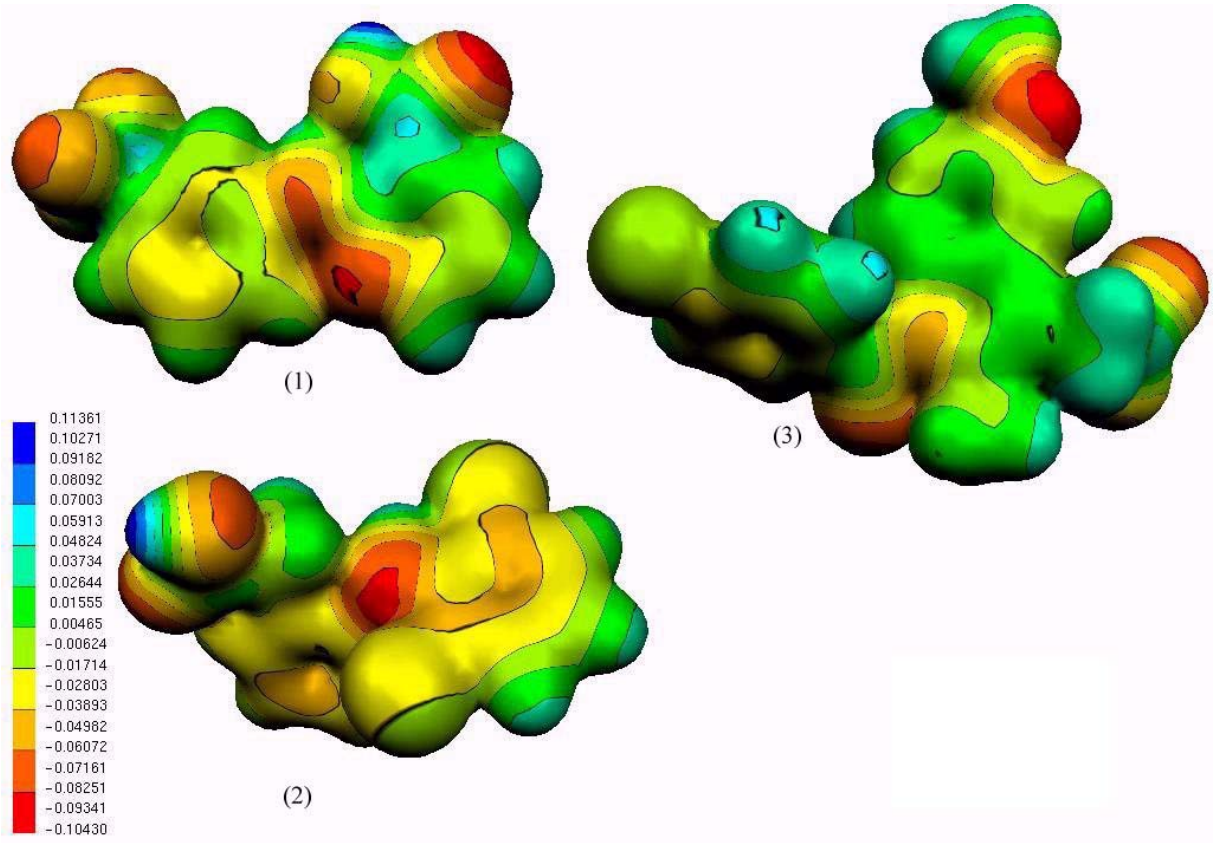

Figure 3: Calculated 3D electrostatic potential contour map of (**1**) Niflumic [2-3((3-trifluoromethyl)phenylamino)-3-pyridinecarboxylic acid], (**2**) Diclofenac {[2-(2,6-dichlorophenyl) amino]-benzeneacetic acid} and (**3**) Indometacine [1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid] acids in [au].

Figure 3 (a) shows the calculated 3D electrostatic potential contour map of Niflumic acid in [au]. Negative regions are associated with N_9, N_7, O_{15} and $F_{27\text{-}29}$. The most negative V(r) values are associated with N_9 and O_{15} with a value around -0.0819 au while the N_7 and $F_{27\text{-}29}$ values are about -0.0619 au. A value of Vmin -0.012 au is found above and below the C_1-C_6 aromatic ring and extended through the N_9-C_8-N_7-C_6-C_3 region. Thus, it would be predicted that an electrophile will preferentially attack Niflumic acid at the O_{15} position. Alternatively, we found a maximum value of 0.117 au on the H_{18} atom on the positive regions of V(r) indicating that this site is probably

involved in nucleophilic processes

Figure 3 (b) shows the calculated 3D electrostatic potential contour map of Diclofenac in [au]. Negative regions are associated with N_{11}, O_4 and O_3 with three local minima. These Vmin are near N_{11}, O_4 and O_3 with values of -0.057, -0.077 and -0.087 au, respectively. Thus, we infer that an electrophile species will preferentially attack Diclofenac at the O_3, O_4 and N_{11} positions and that O_3, O_4 and N_{11} will be the favored sites for protonation. A much weaker Vmin= -0.036 is found above and below C_{12} and C_{14} of the C_{12}-C_{17} ring and above and below the center of the C_5-C_{10} aromatic ring. When focusing on the positive regions of V(r) we found a maximum value of 0.116 au on the H_{18} atom indicating that this site is probably involved in nucleophilic processes.

The calculated 3D electrostatic potential contour map of Indometacine in [au] is depicted in Figure 3 (c). Negative regions are associated with O_{37}, O_{38}, O_{19}, O_{29} and N_{10} with five local minima. These Vmin are near O_{37}, O_{38}, O_{19}, O29 and N_{10} with values ranging between -0.104 and -0.0718 au. A much weaker Vmin= -0.049 au is found above and below the C_{12}-C_{17} aromatic ring. Then, for this case an electrophile will preferentially attach Indometacine at the O_{37}, O_{38}, O_{19} and N_{10} positions. A maximum value of V(r) at 0.116 au on the H_{39} atom suggests that this site is probably involved in nucleophilic processes.

3.3 Sites of electrophilic attack

NBCPs have been determined on the oxygen and nitrogen atoms with the highest Vmin value for the three studied NSAIDs.

Results are collected in Table 2. A single NBCP is found at the pyridine nitrogen in Niflumic Acid (N_9). This NBCP is coplanar with the aromatic ring. On the other hand, two NBCP are found for the amine nitrogen in Niflumic (N_7) and Diclofenac (N_{11}) Acids. The first of these NBCP is located at the apex of the pyramidic nitrogen (the place where the lone electron pair is usually represented). The second NBCP appears pointing towards the base of the pyramid. In Nifllumic Acid the value of $\nabla^2\rho$ in both NBCP are similar and in Diclofenac Acid the value of $\nabla^2\rho$ for the first NBCP is larger than in the second one. The existence of these two NBCP can be explained in the light of the conjugation between the amine group and the aromatic ring. In absence of conjugation we can expect a single local maximum of charge concentration, a NBCP, corresponding to the lone electron pair placed in a hybrid orbital pointing to the apex of the pyramid. However, when the amine group is conjugated with the aromatic ring, the electron pair is more similar to a two lobed *p* orbitals than to a hybrid orbital with a single lobe. Since there are not a pure *p* orbital in both cases, the two lobes are not equivalent. In Indometacine Acid, two NBCP are found in N_{10} with a larger value of $\nabla^2\rho$ for the first NBCP than for the second.

Table 2: Values of Laplacian of the charge density, $\nabla^2\rho$ [au], at the Non-Bonded Critical Points (NBCP) of the selected atoms in structures **1**-**3**.

Atom	Niflumic (**1**)	Atom	Diclofenac (**2**)	Atom	Indometacin (**3**)	Position of NBCP
O15	-4.7511 -4.8711	O3	-4.8271 -4.9525	O37	-4.8676[a] -4.9481[a]	In plane
O16	-4.7122 -4.7139	O4	-4.7616 -4.7966	O38	-4.7464 -47915	Top and down of plane
N9	-2.7630		- - -	- - -		In plane
N7	-1.5582 -1.5579	N11	-2.2926 -1.3236	N10	-1.6178 -1.4635	Top and down of plane
				O29	-5.1206 -5.1563	In plane
				O19	-5.0995 -5.1261	Top and down of plane

[a] Those NBCP are slightly out of plane.

When the value of $\nabla^2\rho$ is compared at the different non-bonded critical points on N atoms of the three species, the NBCP at the pyridine nitrogen exhibits the highest concentration of charge (see Table 2).

On the carbonyl O atom of the carboxylate group two NBCP are found, compatible with two lone electron pairs corresponding to an oxygen atom with sp^2 hybridization. At the same time, on the O atom bound to the H atom in the carboxylic group are found two NBCP localized at the upper and lower positions of the plane, compatible with lone electron pairs placed in two lobed *p* orbitals of the OH group.

In Indometacine acid the oxygen atom of the C=O group (O_{29}) has two NBCP localized in a plane, and this situation can be interpreted as two lone electron pairs placed in two hybrid orbitals. On the other hand, in the O_{19} atom the two NBCP are placed at the upper and lower position regarding a plane, compatible with two lone electron pairs localized into *p* orbitals.

In the three studied cases the oxygen atoms of the carboxylic groups have $\nabla^2\rho$ values of the NBCP larger than those of the nitrogen atoms. However, Indometacine acid is the molecule that presents the highest values of the Laplacian in NBCP over the oxygen atoms of the carbonyl and ether groups.

Provided that $\nabla^2\rho$ can be considered as an indicator of the proton affinity, the center with the highest $\nabla^2\rho$ value seems to correspond to the preferred protonation site [11b]. Nevertheless, in situations where a nitrogen or an oxygen atom are involved in hydrogen bonding, some electrophiles have been observed to react with another site with less negative V(r) [12c]. So, in Niflumic acid the proposed order of preference for an eletrophilic attack is $O_{15} > N_7$.

In Diclofenac as well as in Indometacine acid, it is assumed that from the possible sites, the oxygen atoms will be the preferred sites for an electrophilic attack. Although it can not be deduced that the oxygen atoms are the only protonation sites, the higher negative value of $\nabla^2\rho$ implies that

the approach, e. g. proton, will directed to this zone rather than to the nitrogen atoms.

A remarkable result of this work, merging from this analysis is that, besides that the O_{19} and O_{29} atoms in Indometacine acid, the oxygen atoms of the carboxylic groups are sites that exhibit the highest concentration of electronic charge. So, the carboxylate group of the NSAIDs studied play a important role for interaction with the enzyme producing prostaglandins in the human body, as it was proposed by Vane [7c].

4 CONCLUSIONS

We have scanned the conformational space of the NSAIDs: Niflumic [2-3((3-trifluoromethyl)phenylamino)-3-pyridinecarboxylic acid], Diclofenac {[2-(2,6-dichlorophenyl) amino]-benzeneacetic acid} and Indometacine [1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid] acids drugs using molecular dynamics. We completed these analysis with density calculations optimizing the lowest-energy conformer's geometry for each simulated species.

Through the analysis of the topological properties of the three molecules it was shown that the C-C, C-N and C-H ring bonds in all the structures are typical of covalent interactions and that the C-F and C-Cl bonds are of intermediate character. The C=O and O-H bonds in the carboxylate groups are strong shared interactions. Two hydrogen bonds are localized in the Niflumic acid between N_9-H_{22} and O_{16}-H_{19} while the N-H and O-H bonds in this acid are of the covalent type.

Plot analyses for the Laplacian distribution in the Niflumic, Diclofenac and Indometacine acids show that, unlike the charge sharing concentration between the atoms as found in C-H, C-C, C-N bonds, the regions for the N_9-H_{22} and O_{16}-H_{19} interactions are largely localized within the hydrogen basins pointing to a probably molecular planar structure.

A remarkable result of this work, merging from the MEPs and NBCP analysis, is that the carboxylate group of the NSAIDs studied here play a key role for a successful interaction with the enzyme producing prostaglandins in the human body.

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Supplementary Material

Detailed information about the geometric parameters of the optimized structures of the species Niflumic [2-3((3-trifluoromethyl)phenylamino)-3-pyridinecarboxylic acid], **1**, Diclofenac {[2-(2,6-dichlorophenyl) amino]-benzeneacetic acid}, **2**, and Indometacin [1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid], **3**, acids is deposited as supplementary material.

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Biographies

First Author is Professor of Inorganic Chemistry at the Universidad Nacional del Nordeste, Argentina, and has obtained a Ph.D. degree in engineer from the Universidad Nacional de La Plata. The major research project is in the modeling molecular area.

Second Author is Professor of General Chemistry at the Engineering Faculty, Universidad Nacional de La Plata and has obtained a PhD in Chemistry from the Universidad Nacional del Sur. Her major research project is in Quantum Chemistry and Vibrational and Electronic Spectroscopy.