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QSAR and conformational analysis of the antiinflammatory agent amfenac and analogues

Juan Ruiz*, Manuel López, Josefa Milà, Estrella Lozoya, Juan José Lozano and Ramon Pouplana

Unitat Fisicoquímica, Departament Farmàcia, Facultat de Farmàcia, Universitat de Barcelona, Avinguda Diagonal 643, E-08028 Barcelona, Spain

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SUMMARY

The new nonsteroidal antiinflammatory drug (NSAID) arylacetic amfenac (2-amino-3-benzoylphenylacetic acid) and 19 substituted derivatives were studied in order to correlate the biological activities with the structure-related parameters.

The geometry of amfenac in neutral and anionic form was totally optimized, starting from standard geometries and crystallographic data, using semiempirical AM1 and MNDO quantum-mechanical methods. Conformational analysis shows the existence of a rigid structure for rotations of the acetic acid chain ($\alpha°$) and the central carbonyl group ($\gamma°$) around the bonds with the phenylamine ring, whereas the carboxyl group ($\beta°$) and the phenyl ring of the benzoyl group ($\delta°$) can rotate almost freely.

Electrostatic potential maps were analyzed and showed that the electrostatic orientation effect seems to make an important contribution to the binding of the active compounds to prostaglandin synthase. An electrostatic orientation model of the binding site is proposed. The frontier orbital charge distribution was also described for each compound. On the other hand, steric, electronic and hydrophobic (log P) parameters were calculated and QSAR analysis showed that the most significant parameter for the antiinflammatory activity was the π-electron density of the HOMO orbital in the second aromatic ring. These results suggest a possible electronic charge transfer between the aromatic fragments and the receptor.

INTRODUCTION

Amfenac (2-amino-3-benzoylphenylacetic acid) is a new nonsteroidal analgesic antiinflammatory drug (NSAID) which was originally discovered as a metabolite of 7-benzoylindoline [1,2]. Amfenac is efficacious for the treatment of arthritis deformans, cervical syndrome, rheumatoid arthritis and other inflammation diseases [3–5]. In some kinds of biological test, amfenac shows excellent antiinflammatory and analgesic properties [6]. Recently, a new series of potential pro-

*To whom correspondence should be addressed.

drugs of amfenac have been synthesized and one compound, 2-amino-3-(4-chlorobenzoyl)phenyl acetamide, showed an interesting therapeutic index, which was greater than that of indomethacin [7].

Like other NSAIDs, amfenac inhibits prostaglandin biosynthesis by competitive interaction with the cyclooxygenase–arachidonic acid complex according to the mechanism described by Vane and Botting [8]. Gund and Shen proposed a hypothetical antiinflammatory receptor site for nonsteroidal agents like indomethacin. They [9] and Salvetti et al. [10] further assumed that the carboxyl group of NSAIDs binds to cyclooxygenase at the same site as the carboxyl group of the arachidonic acid substrate. Other models, such as those of Appleton and Brown [11] and Nicholson et al. [12], assume that the carboxyl groups of NSAIDs compete with the peroxy group in position 11 of the peroxy arachidonic acid. In this model the phenyl ring of NSAIDs occupies the π-system of carbons 13–15 in the arachidonic acid. Mehler and Gerhards [13] found an interesting correlation between the antiinflammatory activity of phenols, salicylic acids and benzoic acids with molecular orbital parameters such as the energies and charges in the frontier orbital HOMO (highest occupied π-orbital) and LUMO (lowest unoccupied π-orbital). This correlation suggests a charge transfer process from the receptor to the acidic function of the drug and from the aromatic ring of the drug to the receptor. Kulmacz [14] reported that interactions of many NSAIDs with the receptor site did not involve covalent complexes. Thus it seems that the aromatic moiety is the main binding point with the receptor site.

Recently, the prostaglandin endoperoxide synthase (PGH) has been the subject of considerable scientific interest in inflammation studies. This enzyme exhibits both cyclooxygenase and peroxidase activities in the same protein [15,16]. The human cyclooxygenase gene was cloned [17] and, interestingly, the enzyme was shown to contain an epidermal growth factor (EGF)-like domain [18]. The nature of prostaglandin and thromboxane biosynthesis has been reported by Smith et al. [19–21], who determined the amino acid sequence (the natural enzyme contains 576 amino acids) and proposed a model of the cyclooxygenase and peroxidase-active sites of prostaglandin synthase. In this model the heme group is placed at the peroxidase site via two histidines. The two-electron reduction mechanism of the peroxide to an alcohol leads to formation of an intermediate tyrosine radical. This intermediate radical is thought to abstract the 13-pro-S hydrogen atom from the arachidonate bound at the cyclooxygenase-active site.

On the basis of these studies, the heme group appears to be bound in PGH synthase but the substrate does not seem to interact with it, owing to the existence of an intermediate tyrosine radical.

In recent years, experiments carried out to elucidate the mechanism of competitive inhibition of the cyclooxygenase active site and a large number of QSAR studies [22–28] have appeared concerning the structure of arylacetic acids and other NSAID derivatives.

In the present paper, we describe a quantitative structure–activity study of amfenac and analogues in order to correlate different geometric, electronic and lipophilic parameters with biological activity in vivo, and thus explain the behaviour of drug–receptor interaction. We also describe possible ways of binding arylacetic acids to the cyclooxygenase active site, which leads to their antiinflammatory action. This set of compounds has an important electronic character. Therefore, the structure-related parameters for these compounds were obtained by semiempirical quantum-mechanical calculations.

MATERIALS AND METHODS

Compounds and pharmacological data

Initial studies of the synthesis and antiinflammatory activity of 2-amino-3-benzoylphenylacetic acid derivatives were performed by Walsh et al. [2], using the in vivo method of Evan's blue carrageenan-induced edema by pleural effusion. The data were reported as the change observed in the pleural volume of edema induced (ΔV).

Physicochemical properties of amfenac and 19 derivatives were studied. These derivatives were selected in order to obtain compounds which have different values of their physicochemical properties owing to the change of the substituents in the aromatic rings. Table 1 lists the antiinflammatory activities for 20 selected compounds. In general, addition of a substituent to ring A decreased the antiinflammatory activity, whereas the addition of a group in ring B had a pronounced effect on the tests in vivo and in vitro. The most potent compounds contain a halogen in the 4′-position, while derivatives with a substituent in the 3′-position were less active than amfenac in both tests [2].

Computational methods

Molecular models were built starting from standard geometries and crystallographic data [29,30], using the Cambridge Structural Database (CSD) [31].

Conformational analysis and all optimized geometries were determined using semiempirical MNDO [32] and AM1 [33] quantum-mechanical calculations which were carried out with a locally modified version [34] of the IBM/CMS MOPAC program [35]. These conformational results were verified by means of molecular-mechanic (MM) methods using the conjugate algorithm of the DISCOVER molecular simulation program [36]. The potential electrostatic maps were calculated from the semiempirical wave function [37,38] with a locally modified version [39] of the DIMA program [40]. Log P values were determined using the TRIBL program [41] and statistical calculations were performed using the ANOVA regression analysis option of the Statgraphics® program package [42].

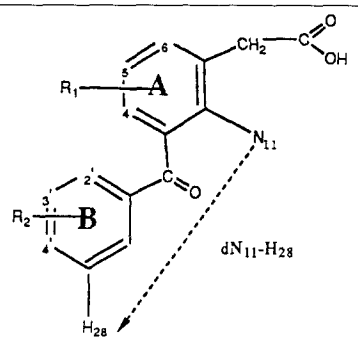
RESULTS AND DISCUSSION

Data analysis

Two general topics with respect to amfenac molecular geometry were considered. First, the existence of three coplanar regions in accordance with the crystallographic data of some arylacetic acids such as [4-(phenoxymetyl) phenyl] acetic acid [30]. The data shown in Fig. 1a correspond to the dihedral angles of the selected E amfenac minimum energetic conformation obtained by the AM1 semiempirical method. These three coplanar regions are defined by I, the carboxyl group; II, the coplanar arrangement of a nitrogen atom with aromatic ring A; and III, the phenyl group (ring B). Likewise, the dihedral angles between the planes (I/II) were 114.9° and 71.1° (II/III); thus the aromatic rings have an almost perpendicular disposition (70–72°).

Second, we considered the possible formation of two intramolecular hydrogen bonds between the carbonyl oxygens and the amino hydrogens.

TABLE I
QSAR PARAMETERS AND BIOLOGICAL ACTIVITIES



Compound	R_1	R_2	$p\Delta V^a$	$E_{HOMO}{}^b$	$E_{LUMO}{}^b$	$\pi_A{}^c$	$\pi_B{}^d$	μ^e	log P	$dN_{11}-H_{28}{}^f$	$p\Delta V^g$
1	H	H	1.415	−8.665	−0.473	0.673	0.633	3.179	1.23	6.688	1.165
2	4-CH_3	H	0.000	−8.662	−0.446	0.678	0.044	3.364	1.63	6.643	0.268
3	5-CH_3	H	1.041	−8.511	−0.456	0.668	0.579	3.366	1.73	6.682	1.083
4	6-CH_3	H	0.301	−8.650	−0.464	0.676	0.054	3.483	1.90	6.700	0.283
5	4-Cl	H	0.602	−8.860	−0.480	0.671	0.406	3.185	1.94	6.023	0.819
6	5-Cl	H	1.230	−8.692	−0.622	0.644	0.962	2.198	1.91	6.706	1.667
7	H	2′Cl	1.342	−8.643	−0.365	0.672	0.799	3.129	1.34	6.767	1.418
8	H	3′Cl	1.176	−8.745	−0.642	0.674	0.792	3.217	2.04	6.697	1.408
9	H	4′Cl	1.462	−8.765	−0.698	0.671	0.791	2.914	2.01	6.703	1.406
10	H	4′Br	1.633	−8.758	−0.740	0.674	0.718	2.935	2.23	6.702	1.295
11	H	2′,4′Cl_2	1.568	−8.705	−0.517	0.674	0.754	2.963	2.12	6.953	1.350
12	H	3′,4′Cl_2	1.000	−8.807	−0.844	0.673	0.723	3.852	2.82	6.702	1.302
13	H	4′CH_3	1.230	−8.641	−0.462	0.673	0.870	3.331	1.63	6.687	1.526
14	H	4′C_6H_5	1.170	−8.685	−0.703	0.672	0.450	2.821	2.79	6.687	0.886
15	5-F	4′Cl	1.568	−8.751	−0.843	0.655	0.791	1.985	2.44	6.671	1.406
16	5-F	4′Br	1.447	−8.768	−0.893	0.655	0.715	2.055	2.06	6.676	1.290
17	5-CH_3	4′Cl	1.505	−8.591	−0.673	0.668	0.791	3.093	2.51	6.685	1.406
18	5-OCH_3	4′Cl	1.415	−8.439	−0.713	0.640	0.792	3.634	2.10	6.642	1.408
19	5-OCH_3	4′Br	1.447	−8.461	−0.766	0.640	0.718	3.660	2.31	6.654	1.295
20	5-Cl	2′Cl,4′Br	1.398	−8.803	−0.699	0.644	0.720	2.878	3.01	6.734	1.298

[a] $p\Delta V = \log \Delta V$; where ΔV is the % reduction observed in volume of pleural fluid at a dose 4 mg/kg.
[b] In − eV.
[c] π-electron density in ring A (C_1-C_2-C_3-C_4-C_5-C_6).
[d] π-electron density in ring B (C_1'-C_2'-C_3'-C_4'-C_5'-C_6').
[e] Dipole moment in debyes.
[f] Distance in Å between N_{11} and H_{28}.
[g] Predicted pΔV value for the biological activity determined by the equation $p\Delta V = 1.524\ \pi_B + 0.201$, r = 0.855, s = 0.231 F = 49.02 and t-statistics = 7.0.

Conformational analysis

The conformational analysis was studied taking into account the possible free rotations represented in Fig. 1b. The arrangement between the two aromatic rings was determined by studying the rotation of torsion angles γ° (C_3,C_4,C_{13},C_{15}) and δ° (C_4,C_{13},C_{15},C_{16}).

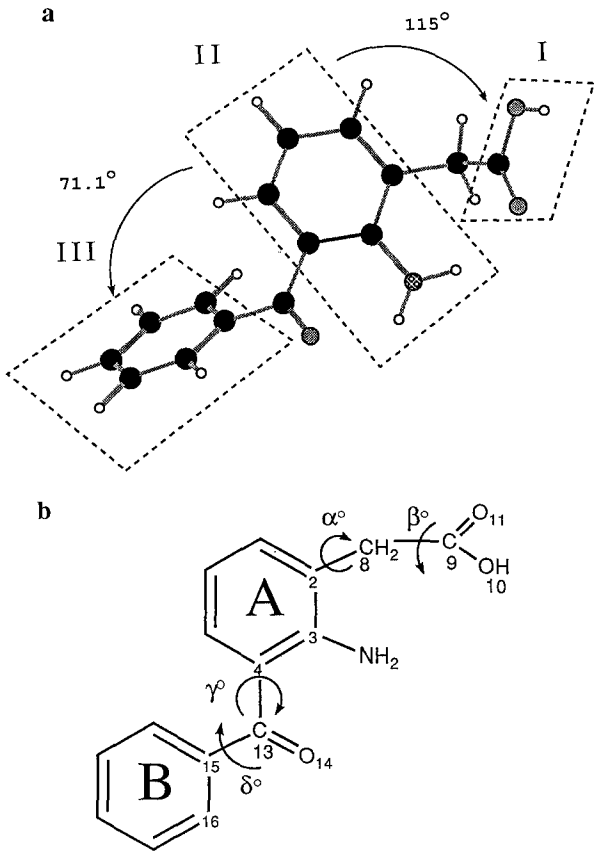


Fig. 1. (a) Perspective view of amfenac showing the planar disposition of the carboxyl group (I), phenylamine fragment (II), and benzoyl ring (III). The two aromatic rings are almost perpendicular. (b) Definition of A-B rings and the torsion angles used in conformational energy calculations $\alpha° = C_3,C_2,C_8,C_9$, $\beta° = C_2,C_8,C_9,O_{10}$, $\gamma° = C_3,C_4,C_{13},C_{15}$ and $\delta° = C_4,C_{13},C_{15},C_{16}$.

These bonds were rotated from 0° to 345° at intervals of 15°. Total optimization was carried out for each pair of γ° and δ° values using in every calculation [43] the semiempirical wave function AM1 and giving the conformational energy surface illustrated in Fig. 2a. In this map there are four γ° energetic minima values that correspond to two 'phenyl down' conformations with respect to the phenylamine central ring, A and B (H°f = −82.09 and −79.71 kcal/mol, respectively); the other two conformations are two 'phenyl up' conformations with respect to the phenylamine central ring, C and D (H°f = −79.54 and −81.63 kcal/mol, respectively). These four conformations are represented in Fig. 3a.

This conformational analysis indicates that A and D conformations have the phenyl ring oriented towards the other side of the amino group. These two conformations are more stable than B and C, with the phenyl ring oriented towards the adjacent amino group. This energetic difference of 2 kcal/mol could be due to hydrogen bonding between the benzoyl oxygen and the

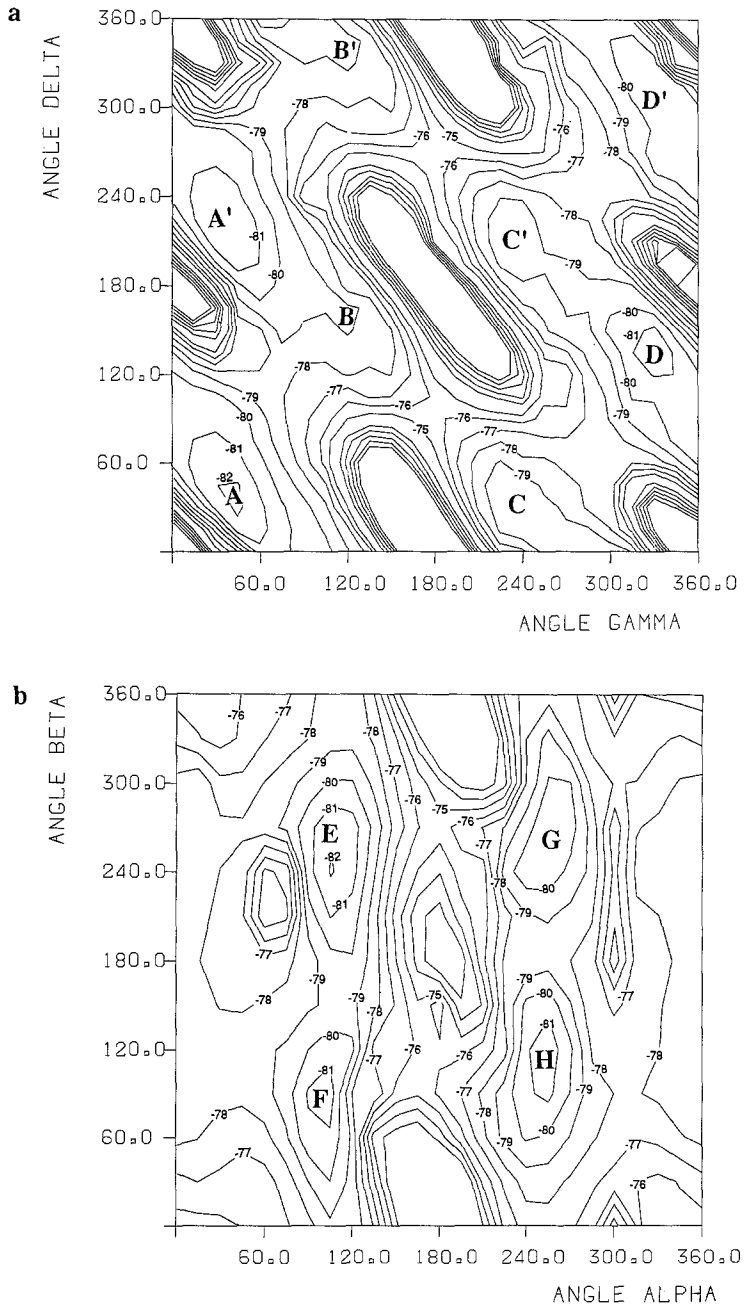


Fig. 2. Conformational energy contour maps of amfenac in neutral form calculated by the AM1 method for: (a) The free rotation of the benzoyl group ($\gamma°$ vs. $\delta°$). We can observe four energetic minima in the two maps. Curves are shown in increments of 1 kcal/mol. Minima on the surface are indicated by letters A–D and E–H. (b) The free rotation of the acetic acid chain ($\alpha°$ vs. $\beta°$).

nearest amino hydrogen in A and D conformations (this distance is 2.20 Å and 2.12 Å for A and D conformations, respectively), whereas this bonding is not possible in B and C conformations, due to the distant position of benzoyl oxygen to amino hydrogen (3.34 Å and 3.20 Å, respectively for B and C conformations).

MM methods gave identical results since the same four low-energy conformations were obtained. These conformations also have a difference of 2 kcal/mol according to the arrangement of the phenyl ring with respect to the adjacent amino group. For each (A,B,C,D) conformation there is an equivalent (A′,B′,C′,D′) conformation which corresponds to a 180° rotation of the δ° torsion angle. These minima can be observed in the map of Fig. 2a. Thus in the derivative compounds of amfenac with R_2 substituents in the second phenyl ring we find eight minimum energy conformations for the γ°/δ° rotations.

Amfenac shows a restricted rotation around the CO–phenyl A bond with an energy barrier of 7 kcal/mol. The preferred conformations have an angle γ° with values between 35° and 60° or between 315° and 340°, while the phenyl B group can rotate almost freely δ° (0–360°) with a barrier of 3 kcal/mol. This torsion angle can adopt conformational preferences in the range of δ° = 30–60° or 210–240° for γ° = 45° and δ° = 120–150° or 290–320° for γ° = 330°.

In order to evaluate the effect of the hydrogen bonding on the molecular geometry, we carried out a second rotational study for the neutral and anionic forms around the single bonds of the acetic acid chain defined by the rotation of torsion angles α° (C_3,C_2,C_8,C_9) and β° (C_2,C_8,C_9,O_{10}) (see Fig. 1b).

Each torsion angle, α° and β°, was varied by increments of 15° and 30° from 0° to 360°, respectively. The molecular geometry was optimized for each value of the two angles and the heat of formation (H°f) was calculated at each point by means of semiempirical AM1 and MNDO methods. A total of 1152 points on the surfaces were obtained in this way.

Rotation of dihedral angles α° and β° produces the conformational map illustrated in Fig. 2b. This map corresponds to an AM1-neutral form. Minima on the surface are indicated by the letters E–H. Comparison with the map for the anionic forms reveals no significant differences between the anionic and neutral forms. This is because four identical energy minima are observed, while comparison between the two semiempirical methods indicates a 10–15° displacement in the position of the minima, since the AM1 method quantifies the hydrogen-bond formation, while the MNDO method ignores hydrogen bonding [33]. The AM1 method was therefore chosen for the computational calculations with the amfenac derivatives.

Amfenac in neutral form shows four individual minima, which correspond to four minimum energy conformations: two 'carboxyl up' conformations (COOH above the phenylamine plane), E and F (H°f = −82.09 and −81.27 kcal/mol), and two 'carboxyl down' conformations (COOH below the phenylamine plane), G and H (H°f = −80.86 and −81.27 kcal/mol, see Fig. 3b).

The rotation around the Cα–phenyl A bond (α°) is somewhat hindered by an energy barrier of 7 kcal/mol, whereas the carboxyl group can rotate freely around the Cα–COOH bond (β°) with a very low energy barrier (< 3 kcal/mol) and can adopt preferred conformations with β° = 80–110° or 230–260°, and α° = 90–120° or 240–270°, since intramolecular hydrogen bonds between the carbonyl oxygens and the amine hydrogens can be formed in any of these four conformations. These conformational results are in agreement with MM methods and with other conformational studies of the acetic acid chain in arylacetic derivatives, which use CNDO quantum-mechanical methods [9,44] and ^{13}C, ^{1}H NMR measurements [45,46].

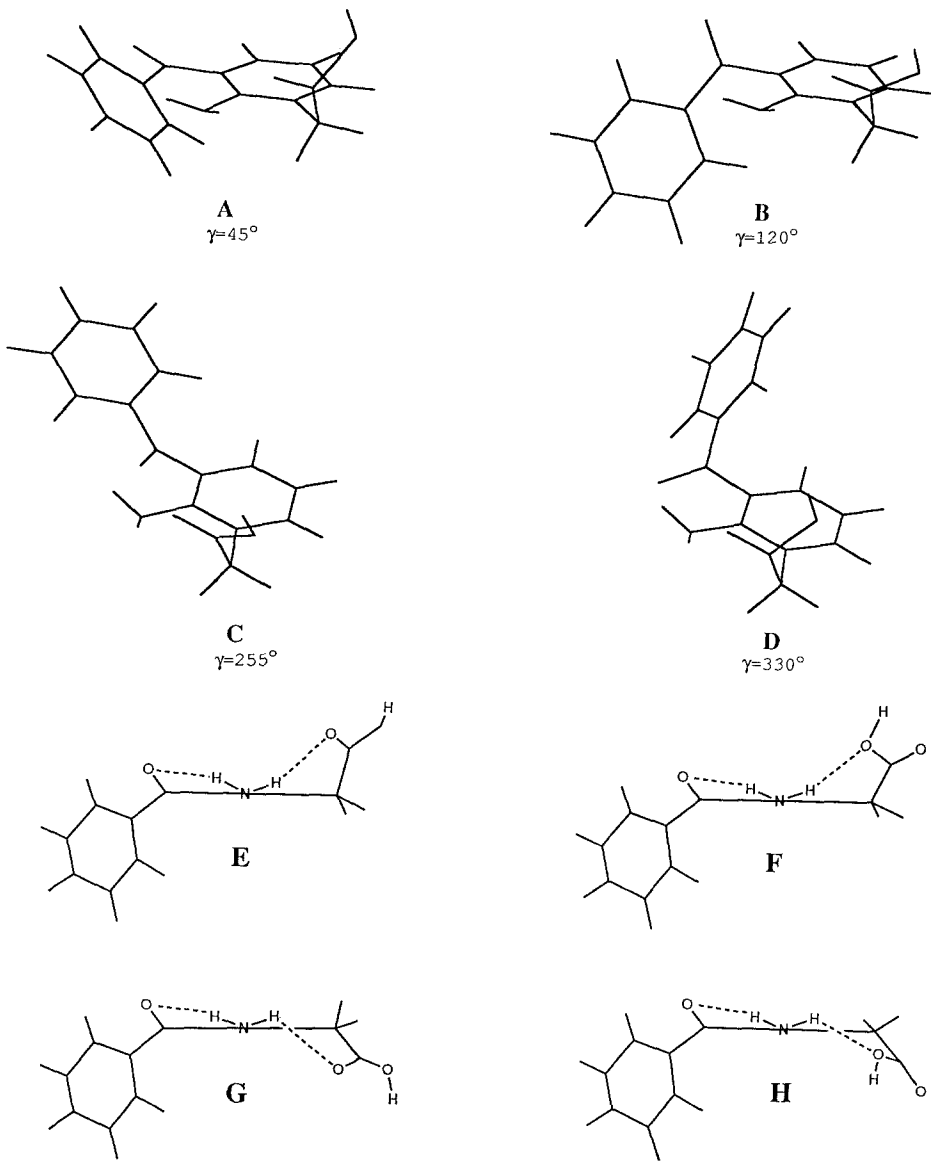


Fig. 3. Molecular models of the minimum-energy conformations of amfenac, as derived from the conformational analysis. A–D show the rotation of the benzoyl group; E–H show the rotation of the acetic acid chain. Intramolecular hydrogen bonds can be formed between carbonyl oxygens and amino hydrogens.

Selection of the bioactive conformation

The most likely bioactive conformation (E) was selected taking into account that only the two 'carboxyl up'-'phenyl down' conformations with $\gamma° = 45°$ (E and F conformations) are structurally similar to the arachidonic acid in its active form. These conformations give acceptable

fittings, in which the carboxyl group of amfenac competes with the 11-peroxy group or carboxyl group of the most stable conformer of arachidonic acid in accord with the two proposed models of Gund and Shen [9] and Appleton and Brown [11] for NSAID binding (see Fig. 4). E and F conformations do not present significant differences in the electronic orbital properties HOMO and LUMO or in the electrostatic potential maps. The only difference between E and F is the position of the OH group: the E conformation has the OH group on the outer side of the molecule, according to the propositions of Gund and Shen, who pointed out that the carboxyl group of NSAIDs binds to the enzyme through the outermost oxygen.

Quantitative structure–activity relationships

The molecular geometry for the selected amfenac derivatives was optimized, starting from the possible selected bioactive E conformation. We have examined a range of 20 substituted derivatives from the basic conformation of amfenac. The MO wave function and totally optimized geometries were determined for all compounds. The geometries of the different analogues show less variation (10–30°) from the amfenac minima values, mainly in the free rotation of the phenyl B group (δ°) due to the possible steric interactions between substituents and near hydrogens.

The main steric factors in this set of compounds were evaluated by studying the relation between the antiinflammatory activities and the geometric-related parameters. A steric parameter, the distance between the amino nitrogen (N_{11}) and the hydrogen in the *meta* position of the benzoyl group (H_{28}, Table 1) was chosen from the *fitting* between the selected amfenac bioactive conformation E and the arachidonic acid substrate [28].

This *fitting* was made between amfenac and an arachidonic acid-stable conformer taking into account the mechanism of prostaglandin endoperoxide formation. In this mechanism two arachidonic acid electronic centers are indicated: a carbon radical at C_{11} generated from the 13-pro-S hydrogen abstraction, and the hydroperoxyl radical from the oxygen attack at C_{15}. These reactive regions can be identified according to HOMO and *Next*-HOMO electron-density similarities in both molecules as shown in Fig. 5. For amfenac the main electronic centers correspond to the

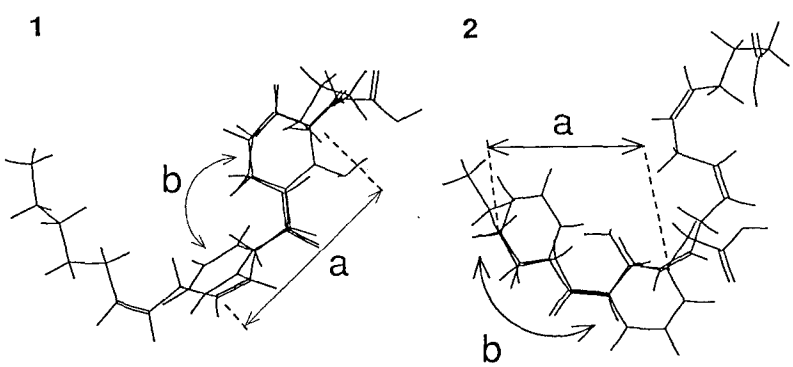


Fig. 4. Superimposition of the possible bioactive **E** conformation of amfenac with the stable conformer of arachidonic acid, the amfenac carboxyl group competes in (1) with the carboxyl group and in (2) with the 11-peroxy group of peroxy arachidonic acid, in agreement with the two models of inhibition of NSAIDs Gund and Shen [9] and Appleton and Brown [11], respectively. a is the distance between the two phenyl rings for amfenac (7.1 Å) in comparison with the reactive region of arachidonic acid (6.97 Å). b is the angle formed by two aromatic rings (71.1°) in amfenac in comparison with the reactive region of arachidonic acid (54.4°). The rms index is 0.176 for the two superimposition *fittings*.

amino (N_{11}) and *meta* (H_{28}) position of the phenyl ring (see later, minima B and E of amfenac molecular electrostatic potential).

The dN_{11}–H_{28} distance in active compounds was approximately 6.7 Å, similar to the reactive area of arachidonic acid (6.9 Å) [28], while some less active molecules show a significant difference, for example, compound **5** (4-Cl) 6.02 Å.

On the other hand, Walsh et al. [2] found that compounds with large bulky substituents near the 3-benzoyl position were less active, for example **2** (4-CH_3), and **5** (4-Cl). A similar reduction in the antiinflammatory potency was observed for derivatives that contain bulky substituents in the *meta* position like **8** (3′-Cl) and **12** (3′-4′Cl_2). These same steric properties were also present in other NSAIDs which contain a benzoyl group [27]. Thus, these aromatic rings seem to play a significant role in the interaction with the active site of cyclooxygenase.

The molecular electrostatic potential (MEP) map of all compounds in the selected bioactive E conformation was calculated from the semiempirical AM1 wave function. These maps are shown in Fig. 6. Curves are shown in increments of 10 kcal/mol at a plane 2.25 Å perpendicular to the phenylamine ring. The solid contours and the dotted contours correspond to negative and positive electrostatic potentials, respectively. There are five potential minima in the amfenac electrostatic potential map. A placed in the carboxyl region, B situated below the phenylamine fragment, C in the central carbonyl group, and electrostatic minima D and E around the benzoyl ring (Fig. 6a).

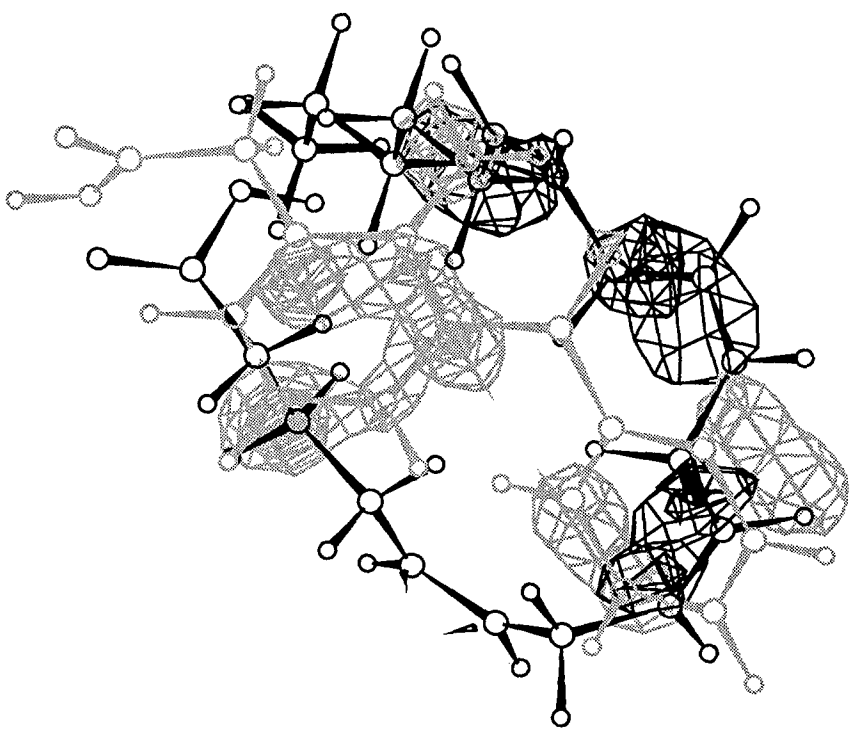


Fig. 5. *Fitting* of HOMO and NHOMO frontier orbitals for amfenac (in grey) and arachidonic acid (in black). Note the importance of electronic properties of the second amfenac aromatic ring with respect to an equivalent arachidonic acid electronic region.

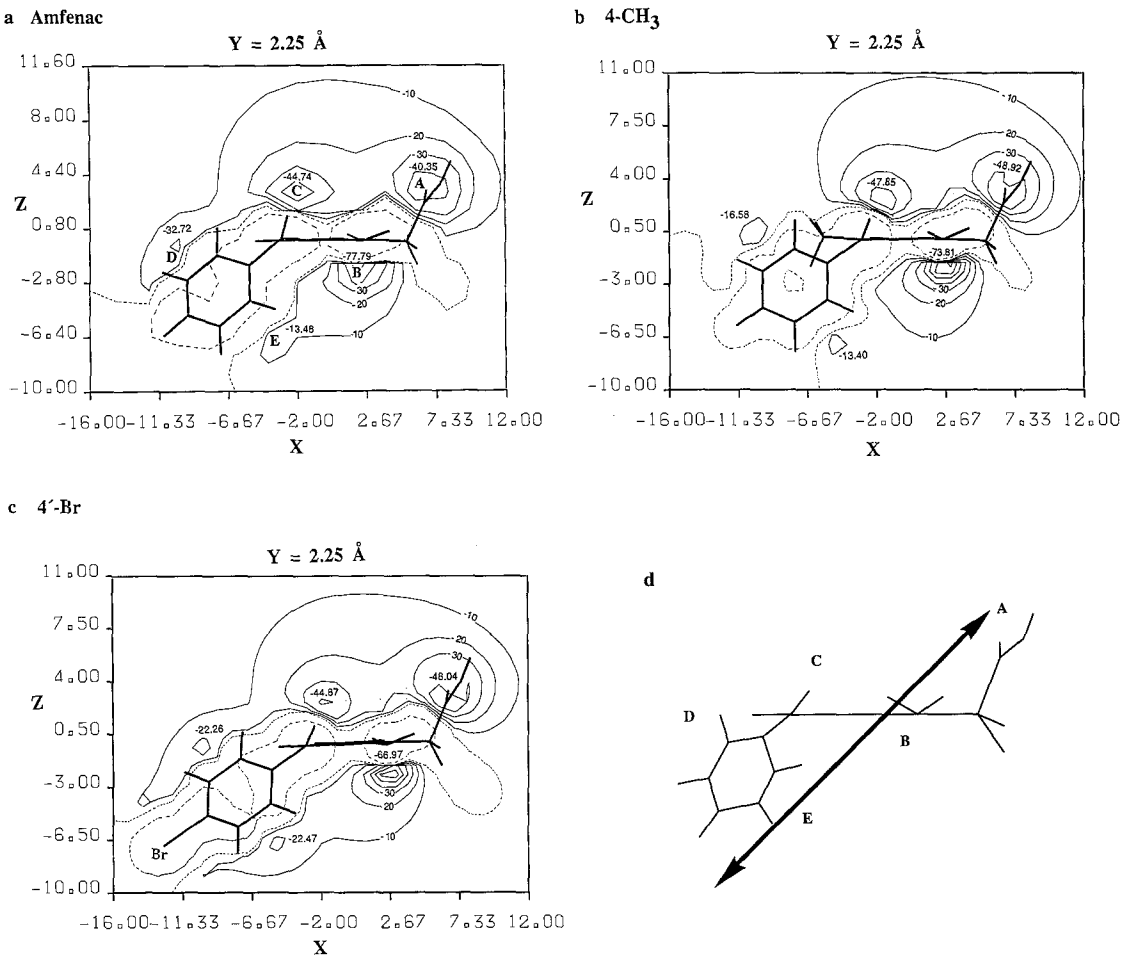


Fig. 6. Molecular electrostatic potential maps of (a) amfenac, (b) an inactive species 4-CH_3, (c) an active species 4'-Br and (d) orientation vector.

The distribution of the MEPs explains some of the differences observed in potency. The MEP maps were compared for all compounds and the most significant difference was the shift of the deep potential minima in negative regions around the benzoyl group (D and E minima). Inactive species decrease the value of the minima D and E, for example compound **2** (4-CH_3, IC_{50} > 1000 μM, ΔV = −1%, Fig. 6b), in contrast with some active molecules, such as amfenac and compound **10** (4'-Br, IC_{50} = 0.08 μM, ΔV = −43%, Fig. 6c), which show a high value for these minima.

The negative regions near the carboxyl and amine groups (A, B and C minima) were similar for all compounds and indicate a possible interaction with the enzyme, mainly by this area, according to a possible orientation vector represented in Fig. 6d. This vector corresponds to the molecular dipole moment that connects the negative regions placed in the benzoyl, phenylamine and carboxyl groups with the positive region of the benzoyl ring.

In this set of compounds, the different activity is due to the electronic density distribution.

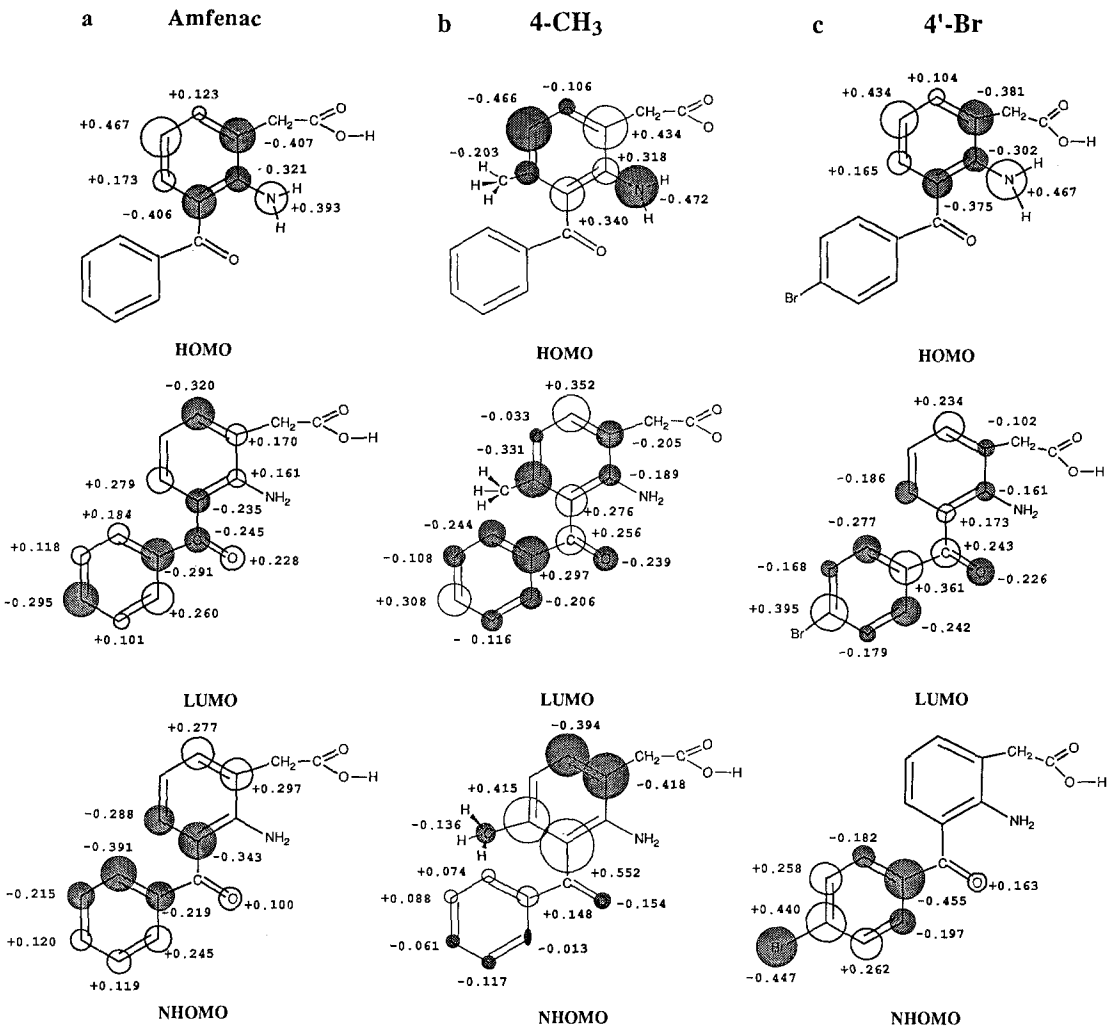


Fig. 7. HOMO, LUMO and NHOMO molecular orbital topology distribution for (a) amfenac, (b) 4-CH_3 and (c) 4'Br. Some active species like (a) and (c) increase the NHOMO-electronic population on ring B.

Therefore topological analysis was performed in the HOMO and LUMO frontier orbitals; these topological charge distributions for some compounds appear in Fig. 7. The HOMO frontier orbital is distributed in the phenylamine ring; the *Next*-HOMO orbital is mainly localized in ring B in the active compounds (such as amfenac and compound **10**) and in ring A in the most inactive compound (**2**); at the end, the LUMO orbital is distributed in an irregular way in the two rings.

A correlation study was performed between the frontier orbital density distribution and the biological activity in agreement with the cyclooxygenase model proposed by Gund and Shen, based on a charge transfer interaction between the enzyme and the inhibitor.

Calculation of the MO coefficients shows that the highest MO coefficients are mainly distribut-

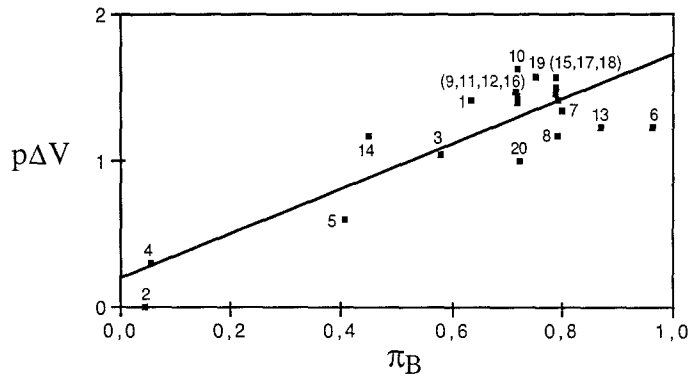


Fig. 8. Linear regression of biological activity pΔV with π-electron density in the aromatic ring B (π_B) for 20 substituted amfenac derivatives.

ed on the p atomic orbitals (p_x,p_y,p_z), while coefficients for s symmetry orbitals show a poor contribution.

This calculation suggests that the main electronic density distribution is due to the π-symmetry molecular orbital (HOPO). These results are in agreement with the π-charge transference model proposed by Mehler and Gerhards for benzoic and salicylic acids [13].

The calculated electronic parameters, for instance the energies and charges of the frontier orbitals, the magnitude (μ) of the dipole moment, and the biological activity, are reported in Table 1.

Hydrophobic–lipophilic properties were also considered. Log P values were calculated by the Hansch–Fujita method [47] and we used the TRIBL program [41]. The compounds do not show significant hydrophobic differences, whereas amfenac has a lower log P parameter (1.23) than other arylacetic derivatives, for example, fenclofenac 3.32, diclofenac 3.51, ibufenac 3.35, which appear more active than amfenac in tests in vivo. This difference shows the effect of lipophilicity on antiinflammatory activity as a consequence of hydrophobic binding and the transport through biological systems.

These hydrophobic (log P), electronic (π_B) and geometric (dN_{11}–H_{28}) parameters were correlated with the biological activities using statistical calculations and simple regression analysis.

The π-electron density in ring A (π_A) was approximately similar (0.64–0.67) in all compounds studied, whereas π_B was correlated with the antiinflammatory activity according to the following simple regression

$$p\Delta V = 1.524\,\pi_B + 0.201,$$
$$n = 20,\ r = 0.855,\ S = 0.231,\ F = 49.02 \text{ and } t = 7.0$$

This linear regression is shown in Fig. 8. The positive coefficient of the π_B term indicates that the high π-electron density in ring B enhances the antiinflammatory potency (Table 1). The correlations do not improve by addition of log P and dN_{11}–H_{28} terms.

The predictive ability of the proposed QSAR equation was tested for seven amfenac derivatives

and two compounds, 5-F,4′-F and 2′Cl-4′Br, and showed a deviation of 22% from the experimental data ΔV, whereas the other five compounds showed a correct adjustment between 1.5% and 10% of the ΔV.

CONCLUSIONS

The present set of amfenac derivatives show restricted rotations around the Cα–phenyl A and CO-phenyl A bonds. These rotations are hindered by a barrier of 7 kcal/mol, whereas rotations of the carboxyl and phenyl groups can rotate almost freely with a minimum energy barrier of 3 kcal/mol around the Cα–COOH and CO–phenyl B bonds. These rotations can adopt suitable positions in accordance with the formation of intramolecular hydrogen bonds, which are confirmed by using AM1 MO-semiempirical and MM methods.

All the derivatives studied can adopt the most probable biologically active conformation E, which is a stable 'carboxyl up' and 'phenyl down' conformation with respect to the phenylamine plane. This conformation has the phenyl ring oriented towards the other side of the amino group, in agreement with the structural disposition of the reactive area of arachidonic acid.

Distribution analysis of the molecular electrostatic potential for all compounds and arachidonic acid molecular fitting suggest the existence of a possible alignment of the molecule through the carboxyl, phenylamine and benzoyl groups in the interaction with the enzyme binding site.

In this orientation, the frontier orbitals of the inhibitor molecule may overlap with complementary orbitals of the enzyme, which leads to a charge transfer interaction between the two aromatic rings and the enzyme. These electronic interactions may contribute to stabilizing the inhibitor–enzyme complex. QSAR analysis shows that the antiinflammatory activity of these arylacetic derivatives is described by an electronic (π_B) parameter, while geometric and hydrophobic parameters have not the same relevance as electronic parameters, although these could be important properties in the drug–receptor recognition process.

In the amfenac analogues studied, there may be three possible electronic centers for interaction with the prostaglandin synthase complex: the first is represented by the carboxyl group, and the other two are defined by the aromatic rings. The results of the correlation analysis suggest that the electronic properties of the second ring play an important role in the antiinflammatory potency of these arylacetic derivatives.

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