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SHORT COMMUNICATIONS

# Quantum Chemical Analysis of Structure-Activity Relationships in Nonsteroidal Anti-inflammatory Drugs

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SUMMARY

*Ab initio*, quantum chemical methods are being used to analyze and interpret structure-activity relationships in nonsteroidal anti-inflammatory drugs. The biological data for this study derive from full dose-response curves of the inhibitory potency of phenols, salicylates, and benzoates on prostaglandin production in mouse macrophages. To date, about 80 compounds have been assayed and from this group a sample of 30 has been selected for calculation. The results show a correlation between the potency of the active compounds and the orbital energy of the highest occupied molecular orbital (HOMO) with a correlation coefficient of $r \sim 0.8$. These results indicate that potency increases with decreasing binding strength of the $\pi$-HOMO electrons, suggesting that charge transfer may be important for interaction with specific or nonspecific binding sites.

Although the complex biological activity of NSAIDs[2] has been the subject of intense research, the mode(s) of action and structural requirements of these anti-inflammatory, non-narcotic analgesics continue to defy rationalization. A mechanism for explaining the enhanced concentration of acidic NSAIDs in the affected body compartments has been suggested (1), and a large number of studies have shown that the therapeutic and toxic effects of NSAIDs may be mediated through different sites of interaction (2). However, even the most plausible proposed molecular mechanism of their anti-inflammatory action, i.e., inhibition of prostaglandin biosynthesis (3), is still subject to controversy (4, 5).

Because of these difficulties, alternative approaches for relating the biological activity of NSAIDs to molecular structure and properties may be fruitful and yield new insight into their mode(s) of action. One such approach is to relate the observed activity to structural indices obtained from molecular quantum mechanics. In this communication we report some initial results from quantum chemical calculations carried out on a group of simple NSAIDs in which we examine correlations between biological activity and electronic structural parameters obtained from *ab initio* calculations.

An initial goal of these calculations is to obtain deeper insight into the NSAID's mode(s) of action by evaluating their dependence on the electronic structure of the biologically active species. Thus we are not interested in the first instance in finding predictive relationships, but instead we are looking for significant correlations between activity and *single* electronic parameters to help indicate which properties and possible interactions are the sources of the observed activity. A similar approach has been used with considerable success by Weinstein and collaborators (6) in their studies of the modes of action of the tryptamines on the lysergic acid diethylamide/serotonin receptor. A possible complicating factor in the present studies is that the existence of a specific receptor has not yet been firmly established.

Biological data for studies of this type are best derived from *in vitro* assays in order to constrain the degrees of freedom of the experimental system as much as possible. For the present work, biological activity is defined as the potency of the given compound to inhibit the 12-*O*-tetradecanoylphorbol-13-acetate-induced $PGE_2$ release from macrophages isolated from the peritoneum of young male NMRI mice. For methodological details, see ref. 7. Treatment of the cultures with various drug concentrations was carried out in serum-free medium (Dulbecco's modified Eagle's medium), and the potencies are reported as the molar $IC_{50}$ values.

About 80 compounds have been assayed to date, and from this group a sample of 30 molecules was selected for quantum chemical study. These molecules were chosen because they are the simplest structurally and thus

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[2] The abbreviations used are: NSAID, nonsteroidal anti-inflammatory drug; $PGE_2$, prostaglandin $E_2$; HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital; SAA, salicylic acid; BZA, benzoic acid.

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TABLE 1

*In vitro activity and π-HOMO orbital energies of calculated molecules*

$pIC_{50}$ values are the negative logarithms of the molar concentrations for 50% inhibition of prostaglandin release. Orbital energies were obtained from *ab initio* molecular orbital calculations. For definitions of scaled orbital energies see text. All molecules were calculated with standard geometries (ref. 8) and, with the exception of *p*-isopropyl BZA, are in the planar conformation.

| | Molecule | $pIC_{50}$ | $-\epsilon(\pi\text{-HOMO})$ | |
|---|---|---|---|---|
| | | | *kJ/mole* | |
| 1 | Phenol | 3.54 ± 0.16 | 937.6 | |
| 2 | 2-OH phenol | 5.34 ± 0.16 | 905.0 | |
| 3 | 3-OH phenol | 5.15 ± 0.11 | 927.6 | |
| 4 | 4-OH phenol | 4.43 ± 0.12 | 878.2 | |
| 5 | SAA | 3.33 ± 0.31 | 975.6 | |
| 6 | 3-OH SAA | 4.43 ± 0.19 | 933.1 | |
| 7 | 4-OH SAA | 3.02 ± 0.20 | 986.9 | |
| 8 | 5-OH SAA | 4.61 ± 0.61 | 912.6 | |
| 9 | 4-isopropyl BZA | 2.93 ± 0.14 | 1010.7 | |
| 10 | 2-F phenol | 3.57 ± 0.26 | 967.8 | |
| 11 | 4-F phenol | 3.98 ± 0.22 | 952.3 | |
| 12 | 5-F SAA | 3.82 ± 0.35 | 987.5 | |
| 13 | 3,5-OH BZA | 3.61 ± 0.08 | 966.2 | |
| | | | | Scaled |
| 14 | 2-Cl phenol | 4.62 ± 0.19 | 954.9 | 925.8 |
| 15 | 3-Cl phenol | 4.61 ± 0.14 | 973.0 | 943.8 |
| 16 | 4-Cl phenol | 4.86 ± 0.09 | 940.5 | 911.8 |
| 17 | 3-Cl SAA | 3.89 ± 0.30 | 981.2 | 951.3 |
| 18 | 4-Cl SAA | 3.31 ± 0.19 | 1014.5 | 983.6 |
| 19 | 5-Cl SAA | 4.06 ± 0.32 | 973.5 | 943.8 |
| 20 | BZA | | 1059.9 | |
| 21 | 3-OH BZA | | 984.0 | |
| 22 | 4-OH BZA | | 999.8 | |
| 23 | 4-Methyl BZA | | 1027.4 | |
| 24 | 4-Ethyl BZA | | 1023.2 | |
| 25 | 3-Cl BZA | | 1039.7 | |
| 26 | 4-Cl BZA | | 1052.0 | |
| 27 | 3-F BZA | | 1070.2 | |
| 28 | 4-F BZA | | 1080.9 | |
| 29 | 4-F SAA | | 1025.5 | |
| 30 | 3,4-OH BZA | | 962.8 | |

require the least computing time. The sample comprised phenol, benzoic acid, and salicylic acid and their hydroxy, fluoro, and chloro congeners.

All calculations were carried out using *ab initio* molecular orbital methods with programs developed in this laboratory. Details of the methods and programs used have been reported previously (8), and details of the present calculations will be given elsewhere. *Ab initio* methods are being used in order to have access to a large number of properties with a known level of reliability. This is required because the electronic properties which correlate with the biological activity of the NSAIDs have not yet been determined, so that it would be difficult to select an appropriate semi-empirical approach. Later it is hoped that it will be possible to use the computationally less demanding semi-empirical methods. For these initial calculations, standard geometries (9) and minimal basis sets (10) were used throughout.

The σ and π orbital energies of the HOMO and LUMO were analyzed for correlation with the potency of 18 of the 19 active molecules. The $pIC_{50}$ values ($-\log IC_{50}$) and π-HOMO orbital energies are listed in Table 1, and the results of the statistical analysis are reported in Table 2. As the correlation coefficients in Table 2A show, the π-HOMO orbital energies exhibit a significant correlation with the $pIC_{50}$ values, whereas the σ-HOMO and the LUMO orbital energies do not. These results are presented graphically in Fig. 1. The 4-OH phenol derivative was excluded from the analysis. It showed a much higher toxicity[3] in the assays than the other phenol derivatives [details will be reported later (11)] and, indeed, does not fit well into the correlation pattern observed for the π-HOMO energies. The correlation coefficient that includes this compound is also given in Table 2A.

The rather good correlation with the π-HOMO energies found in the present analysis is in contrast to the lack of correlation between potency and electronic structural indices found by Jones *et al.* (12) in their analysis using the Hückel molecular orbital method. Two reasons may be given for this difference in observed correlations. First, Jones *et al.* used a semi-empirical method for calculating electronic properties that is probably not of sufficient reliability to justify its use in their application. Second, Jones *et al.* used *in vivo* assays for determining drug potency. The *in vivo* system is of necessity much more complex than an *in vitro* system, so that it is virtually impossible to decompose the observed biological activity into its components and to ascertain to what extent each component contributes to the over-all activity. Such a decomposition would at least require *a priori* knowledge of the mode(s) of action of the drug, but this is just the information lacking for the NSAIDs. Correlations, however, can only be expected between similar molecular events in the biological systems and the electronic structural indices, but not between the *in vivo* response, composed of an unknown combination of molecular events, and the structural parameters obtained from molecular orbital theory. In connection with this it is of interest to note that seven of the compounds assayed here were also assayed by Hannah *et al.* (13), using an *in vivo* system. The correlation between the two assays for these molecules is not very high.[4]

Examination of the results reported in Table 1 and shown in Fig. 1 suggests that the first- and second-row substituents correlate similarly with the π-HOMO orbital energy, but that the second-row group is displaced from the first-row group. Separate analysis of the two groups yields correlation coefficients about 10% higher than that for the whole sample. It is noteworthy that for the other orbital energies the single group correlation coefficients can also be quite high, but the combined correlation is

[3] Compounds for which the cell population showed a viability of less than 90% in the dye exclusion assay were routinely rejected. In the case of 4-OH phenol the calculations were carried out before this assay was made.

[4] Hannah *et al.*(13) used carrageenan-induced foot edema to measure efficacy. The common compounds are SAA, 5-F SAA, 3-F SAA, 5-cyclohexyl SAA, 5-phenyl SAA, 3-phenyl SAA, and 4-phenyl SAA. 5-Cyclohexyl SAA yielded a $pIC_{50}$ of 5.9 in our *in vitro* assay but was inactive in the carrageenan assay. Correlation between the two assays for the remaining six compounds was $r = 0.35$, but when 4-phenyl SAA was excluded, $r = 0.76$.

TABLE 2

*Statistical analysis of in vitro activity with orbital energies*

Orbital energies are expressed in kilojoules per mole, $IC_{50}$ values in molarity; $\bar{\epsilon}$ is the mean orbital energy, $s$ is the standard deviation, $N$ is the sample size. All results were obtained with standard statistical methods.

| | A. Correlation coefficients | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $N$ | $\pi$-HOMO | $N$ | $\pi$-LUMO | $N$ | $\sigma$-HOMO | $N$ | $\sigma$-LUMO |
| Single sample | 18 | 0.78 | 18 | 0.46 | 17 | −0.07 | 17 | −0.05 |
| First row | 12 | 0.87 | 12 | 0.36 | 11 | −0.34 | 11 | 0.10 |
| Second row | 6 | 0.94 | 6 | 0.89 | 6 | 0.84 | 6 | 0.94 |
| Single sample (scaled) | 18 | 0.89 | | | | | | |
| Single sample | 19 | 0.72 | | | | | | |

| | B. Linear Regression: $pIC_{50}$ versus $\pi$-HOMO orbital energy |
|---|---|
| Single sample | $pIC_{50} = 0.01826\epsilon + 21.58$ |
| First row | $pIC_{50} = 0.02100\epsilon + 24.00$ |
| Second row | $pIC_{50} = 0.02160\epsilon + 25.24$ |

| | C. Means | |
|---|---|---|
| | $-\bar{\epsilon}$ | $s$ |
| Active group | 961.0 | 30.7 |
| Inactive group | 1029.6 | 36.4 |

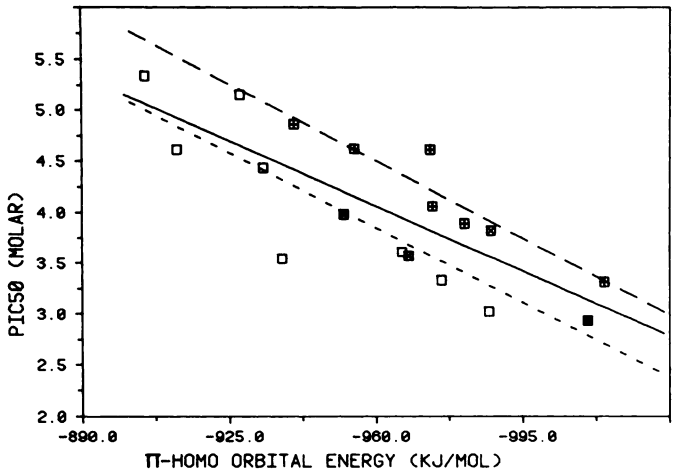


FIG. 1. *Linear regression for 18 calculated molecules of inhibitory potency as a function of the $\pi$-HOMO orbital energy*

Linear regression was applied to a sample treated as a single group and to a sample divided into two groups according to substituent type. ——, Single group regression; -----, all first-row substituents regression; - - - all second-row substituents regression. *Symbols* indicate inhibitory potency versus $\pi$-HOMO energy for individual molecules: □, hydroxy substituents; ⊠, fluorine substituents; ⊞, chlorine substituents; ▣, 4-isopropyl BZA.

small to completely random. The linear regression curves for the two groups treated separately as well as the curve for the whole sample are shown in Fig. 1. It is seen that the regressions for the two groups are essentially parallel and fit the data more reasonably than does the whole-sample regression. From the two regression curves (Table 2B) a scaling factor can be calculated by superimposing the two curves at the mean value of the orbital energy given in Table 2C. The scaling factor is 0.97 for the Cl substituents, and the scaled energies are given in Table 1. The correlation coefficient for the scaled group treated as a single sample is about 0.9, i.e., the same as for the two groups treated separately. Further calculations are being carried out to determine whether similar scaling factors can be applied to other properties.

The observed correlation shows that potency increases with increasing $\pi$-HOMO orbital energy, i.e., weaker binding of the $\pi$-HOMO electrons, which enhances the potential for interaction with specific or nonspecific interaction sites. Three models have been proposed for the cyclooxygenase receptor, where the NSAIDs carry out their purported inhibitory function (14–16). Although these models differ considerably in their details, all three include a $\pi$-electron interaction (acceptor) region. The present results support the need for such a region and suggest that enhanced ability to donate $\pi$-electrons helps to increase potency in inhibiting the release of $PGE_2$ in macrophages.

We conclude by reiterating that the analysis presented here cannot be used as a predictive tool thus far but rather as an indicator to aid in recognizing the types of interactions responsible for the observed biological activity. As is seen from Tables 1 and 2C there is some overlap of $\pi$-HOMO orbital energies between active and inactive compounds, suggesting that $\pi$-electron-donating ability is a necessary but not sufficient requirement for inhibition of $PGE_2$ release. Additional calculations are being carried out to substantiate these ideas, and it is hoped that the analysis can be made sufficiently complete to formulate reliable predictive algorithms. These results will be reported in forthcoming papers.

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