Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Bioorganic & Medicinal Chemistry 12 (2004) 1377–1381
Bioorganic & Medicinal Chemistry

QSPR/QSAR in *N*-[(dimethylamine)methyl] benzamides substituents groups influence upon electronic distribution and local anesthetics activity

Leoberto Costa Tavares[a,*] and Antonia Tavares do Amaral[b]

[a]*Faculdade de Ciências Farmacêuticas, USP, Av. Prof. Lineu Prestes, 580, São Paulo, SP 05508-900, Brazil*
[b]*Instituto de Química, USP, Av. Prof. Lineu Prestes, 748, São Paulo, SP 05508-900, Brazil*

Received 24 July 2003; revised 6 January 2004; accepted 13 January 2004

Abstract—It was determined, with a systematic mode, the carbonyl group frequency in the region of the infrared of *N*-[(dimethylamine)methyl] benzamides 4-substituted (set A) and their hydrochlorides (set B), that had its local anesthetical activity evaluated. The application of the Hammett equation considering the values of the absorption frequency of carbonyl group, $\nu_{C=O,}$ using the electronic constants σ, σ_I, σ_R, $\Im$ and $\Re$ leads to meaningful correlation. The nature and the contribution of substituent group electronic effects on the polarity of the carbonyl group was also analyzed. The use of the $\nu_{C=O}$ as an experimental electronic parameter for QSPR studies was validated.

1. Introduction

The great majority of the local anesthetics currently used in medical practice have in common a lipophilic portion, generally an aromatic system, an intermediate aliphatic carbon chain and a hydrophilic portion, frequently the substituted amine group.[1,2] Although the mechanism of action at the molecular level is not fully cleared[1,3,4] it was considered that the balance between the lipophilic and hydrophilic portions influence significantly the biological activity, modulating its local anesthetical potency.[1,2,5]

On the other hand, some authors have considered that the electronic distribution of the carbonyl group, C=O, present in the majority of local anesthetics, has an important roll for the establishment of this activity.[1,2,6,7] Thus, it's proposed that substituents groups present in the aromatic ring affect the local anesthetical activity, by its effects hydrophobic and of polar nature. Once it's known that the inductive and resonance effects affect directly the electronic density on the carbonylic oxygen, as consequence it's proposed that the carbonyl group polarity can be, in principle, modulating the local anesthetic activity.

The biological activity of drugs, in particular local anesthetics, can be considered as the result of the interactions of these with the biophase.[1,2,8,9] The interactions drug-receptor depend, by its turn, of the physicochemical properties of the compound, determining and modulating the forces of chemical nature present in these interactions.

According to the approach and model proposed by Hansch and Fujita in 1964,[8,9] the biological activity of drugs can be expressed by physicochemical or structural properties relative to the compound. In this approach is searched to extend the concepts used at the studies of organic compounds for systems with more complexity. Thus, it's supplied subsidies to understand and/or to foresee the determinative mechanisms of the biological activity of chemical compounds in biochemical systems, either in vitro or in vivo.

The application of this approach, known as *Hansch Analysis*,[8,9] allows expressing the biological activity of drugs in function of physicochemical or structural parameters, such as lipophilic parameters, electronic/polar, steric and of dispersion relative to the compound.[1,2,8,9]

Keywords: Electronic substituent groups effects; Procainamide analogues; $\nu_{C=O}$ IR spectroscopy; Hammett equation; Electronic parameter; Local anesthetical activity; QSPR/QSAR.
* Corresponding author. Tel.: +55-3091-3693; fax: +55-3815-6386; e-mail: leoberto@usp.br

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.01.011

Despite of some limitations, the model proposed by Hansch and Fujita[8,9] still reveals sufficiently adequate for the study of the quantitative relations between the chemical structure and the activity of biological active compounds, QSAR.

The use of the Hansch's approach involves either the proposal of the mathematical model (simple linear, multiple linear or nonlinear) as the attainment of the structural parameters (experimental, calculated or taken from literature[9–11]) responsible for the activity, aiming the evaluation of the relative contributions of each parameter to the biological activity. The validity, limitations and the forecasting power of the proposed model are verified through the statistical parameters analysis gotten in the correlations. It's important to point out that the proposal of the mathematical model as much as its complexity degree depend of the precision that the drug–biophase interactions can be described, beyond the per se interactions. Therefore, the application of Hansch Analysis involves the rigorous and deep study of the physicochemical properties of the studied compounds, with the objective to describe and express, with success, the interactions established between the drug and the biophase.

Compounds containing the carbonyl group, C=O, present a strong absorption band in the infrared (IR) region, placed within 1928 and 1515 cm^{-1}, referring to the stretching vibration of the C=O bond. The terminal nature and the high value of the constant of force of this bond minimize couplings with adjacent groups, while its polarizability turns it susceptible to the effects coming from its sorroundings.[12,13] Therefore, the accurate position of the C=O group absorption band in the IR is result of the action of intermolecular and intramolecular factors, generally additive, such as the physical state of the sample and its concentration; of the solvent used and mainly of the electronic influence of substituent groups. So, systematic studies of variations in the absorption band position of the C=O in the IR region, $\nu_{C=O,}$ can be considered as being the result of the polar effects of the substituent groups expressing, therefore, variations in the electronic distribution in the carbonyl group.

These studies, although have already been described in literature,[5,12,13] must be validated for each set studied, by the application of the Hammett equation.[14] This validation becomes specially necessary when is intended the use of $\nu_{C=O}$ values as electronic parameter in Hansch Analysis. In this approach the parameter related to the electronic effect can be considered as being the result of the pure polar effect or polar associated to a distortion of the electronic density (polarizability) caused by the substituent group. However, this effect can be described by the molar refractivity, that can be also measuring effects related to the volume or to the lipophilicity, depending of the substituent set chosen. Thus, the attainment of validated experimental parameters becomes important, specially for representing the net result of all operative effects. With this purpose was verified in this paper the influence of the electronic effects of substituent groups upon the C=O group polarity, measured by the absorption frequency in the IR region, in set of compounds analogous to the procainamide and, after that, was searched to prove the validity of $\nu_{C=O}$ use as experimental electronic parameter in Hansch Analysis.

2. Material and methods

2.1. Compounds studied

In this work we used nine *N*-[(dimethylamine)methyl] benzamides 4-X-substituted, where X = NO_2, Br, Cl, F, I, H, CH_3, OCH_3 and $N(CH_3)_2$, s*et A*, (compounds **1a**–**9a**) and its respective hydrochlorides, *set B* (compounds **1b**–**9b**). The chemical structure proposed for each one of the studied compounds was confirmed by its IR spectra, of RMN-^{1}H and of RMN-^{13}C, while the purity degree of the compounds was determined by its melting point and elemental analysis of CHN.

2.2. Determination of the position of the absorption frequency of the carbonyl group in the IR region

Proceeding in accordance to the literature[5,12] was determinated, with a systematic mode, the position of the abortion frequency of the carbonyl group in the IR region of *N*-[(dimethylamine)methyl] benzamide 4-substituted, *set A*, and their respective hydrochlorides, *set B*. The spectra were obtained with $CHCl_3$ solution of the compounds in the concentrations 0.02, 0.04 and 0.08 M. The values of $\nu_{C=O}$ were determined for all compounds, except for the 4-NO_2 and the 4-$N(CH_3)_{2,}$ derivatives in the *set A* that presented solubility lower than 0.02 M. The spectra were executed in spectrometer IR 283, Perkin–Elmer.

2.3. Regression analysis

All the correlations were obtained using the Billin Program, 1998 version, which considers 95% of confidence level. Additionally, the F, Fisher's significance test, and r^2_{cv} and s_{PRESS} values were calculated.

2.4. Determination of the local anesthetical activity

The local anesthetical activity (LAA) was determined by the method of peripheral nervous blockade using the semi-quantitative technique, developed by TRUANT and modified by EICHBAUN & YASAKA.[15] It was used Wistar albino female rats, with weight varying between 200 and 220 g. The local anesthetical activity was evaluated for the *set B* compounds, except for the 4-CH_3 and 4-$N(CH_3)_2$, derivatives that did not show to be soluble in the concentration required for the test.

Aqueous solution 0.15 M of each compound was prepared and analyzed concerning its local anesthetic effects in groups of ten animals, using as positive and negative standard aqueous solution 0.15 M of procaine hydrocloride and 0.9% (w/v) sodium chloride. The solutions of the compounds were prepared immediately

before the execution of each essay. The pH of the solutions of the compounds studied, including the procaine hydrochloride, used as reference for the local anesthetical activity, remained within 4.5 and 5.0. The absence of injury of the blocked nerve was observed with the superficialization of the anesthesia, with consequent recovery of the normal movement of the treated members. The animals that suffered injury in the nerve were not considered.

2.5. Results and discussion

The effects of the substituents upon the carbonyl polarity of the compounds of s*ets A* and *B* were analyzed by the observation of the displacement of the absorption band position of the C=O in the IR region. In this study the systematic and methodology used turned the systematic erros constant and within the accepted experimental error range, as well as the interactions solut-solvent non-significant. In the concentration range used was not observed significant displacement of the $\nu_{C=O}$ value in function of the concentration. So, the average of the readings for each compound, in the three different concentrations analyzed, $\nu^{M}_{C=O}$, was considered as being the accurate value of the carbonyl absorption frequency. Thus, it was possible to attribute the observed displacements as being only due to the polar effects introduced and transmitted by the substituents.

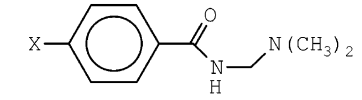
The importance of the experimental evaluation of the carbonyl polarity aiming the mapping of the electronic distribution in the studied compounds can be understood considering, initially, the limitations inherent to the electronic parameters found in literature,[5,8,9,11] defined from simple chemical reactions, what means, to simpler systems than the biological system. So, the polar interactions that occur in the interaction drug–biophase can be more complex than those present in the used systems to define the cited parameters, as for example, the σ parameter of Hammett, defined from substituted acids ionization.[15] Thus, in QSAR studies that focus the electronic property it is recommended, whenever possible, the use of parameters defined experimentally by adequate systematic according to the expected purpose. Alternatively it's recommended the utilization of constants and , of Swain and Lupton,[8,9,11] that relates with the field effect and resonance, that by definition, do not depend of the substituent position in the aromatic ring.

The validity of using $\nu^{M}_{C=O}$ values as describing parameter of the carbonyl electronic distribution, reflecting the intermolecular and intramolecular interactions present in the studied systems, was verified through the application of the simple equation[5,6,7,12] (eq 1) and expanded (eqs 2 and 3) of Hammett, to the frequency values, $\nu^{M}_{C=O}$, in function of different physicochemical parameters available in literature[9,11] related with the electronic property of the substituent group. The values of the substituent constants used in this paper are presented in Table 1.

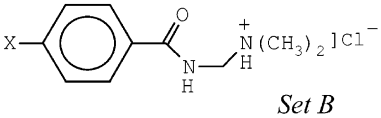
Table 1 presents the average values of carbonyl absorption frequencies (cm^{-1}), $\nu^{M}_{C=O}$,determined in the IR region for *N*-[(dimethylamine)methyl] benzamides 4-substituted, *set A*, and respective hydrochlorides, set B.

The application of the simple and expanded Hammett equation to the $\nu^{M}_{C=O}$ value, in function of electronic parameters of the substituent groups taken from literature, evidenced that the most significant correlations

Table 1. Carbonyl group absorption frequency values of *N*-[(dimethylamine)methyl] benzamides 4-X-substituted, Set A, and of the correspondent hydrochlorides, Set B



Set A



Set B

	Set A (cm^{-1})					Set B (cm^{-1})				
X	Compd	$\nu^{M}C=O$	Δ	$\nu_{C=O}$	$\lvert\Delta\nu^{M}\rvert$	Compd	$\nu^{M}C=O$	Δ	$\nu_{C=O}$	$\lvert\Delta\nu^{M}\rvert$
NO_2	**1a**	1675.5±0.1	8.6	1677.3	1.8	**1b**	—	—	(1684.4)	0.0
Br	**2a**	1669.5±0.1	2.6	1669.1	0.4	**2b**	1678.7±0.1	1.8	1678.8	0.1
Cl	**3a**	1670.1±0.3	3.2	1669.1	1.0	**3b**	1678.8±0.1	1.9	1678.8	0.0
I	**4a**	1669.1±0.2	2.2	1668.4	0.7	**4b**	1678.5±0.1	1.6	1678.3	0.2
F	**5a**	1669.1±0.3	2.2	1666.6	2.5	**5b**	1677.1±0.2	0.2	1677.1	0.0
H	**6a**	1666.9±0.1	0.0	1665.7	1.2	**6b**	1676.9±0.2	0.0	1676.5	0.4
CH_3	**7a**	1660.6±0.1	−6.3	1663.2	2.6	**7b**	1674.3±0.1	−2.6	1674.8	0.5
OCH_3	**8a**	1660.5±0.1	−6.4	1661.7	1.2	**8b**	1674.1±0.1	−2.8	1673.8	0.3
$N(CH_3)_2$	**9a**	1653.4±0.2	−13.5	1653.4	0.0	**9b**	—	—	(1668.2)	0.0

$\nu_{MC=O}$ is the average of carbonyl group absorption experimental frequency values obtained in $HCCl_3$ solution excepting the values in parenthesis valued by equation IIb; Δ is the $\nu^{M}_{C=O}$ difference between the X-substituted compound and the non-substituted compound; nu;$_{C=O}$ is the carbonyl absorption frequency (cm^{-1}) value calculated by equations IIa and IIb, for s*et A* and *set B* respectively; $\lvert\Delta\nu^{M}\rvert$is the difference in modulo of the experimental and calculated frequency values.

were obtained when the Hammett values for σ_p were used; eqs 2a and 2b, for sets A and B. In Table 1 are presented the difference values, in modulo, $|\Delta\nu^{M}|$, of the $\nu_{C=O}$ calculated by the eqs 2a and 2b, and the experimental values, $\nu^{M}_{C=O,}$ demonstrating the adjustment of the theoretical curves to the experimental values of the sets A and B.

The nature of the electronic effect transmitted by the substituent was verified by the application of the expanded equation of Hammett, using as electronic parameter the constants σ_I and $\sigma_{R,}$ and constants $\Im$ and $\Re$. The relative contributions of the inductive and resonance effects for the polar effect performed by the substituent were evaluated by the analysis of the coefficients of regression associated to the constants σ_I and σ_R (eqs 2a and 2b), as well as to $\Im$ and $\Re$ (eqs 3a and 3b). It's verified that there isn't significant predominance of one of the effects on the other for the studied sets.

$$\nu_{C=O} = \rho\sigma + \nu^{H}_{C=O} \quad (1)$$

$$\nu^{X}_{C=O} = 14.81\ (\pm 3.27)\sigma p + 1665.7\ (\pm 1.35) \quad (1a)$$

$$n = 9,\ r = 0.97,\ s = 1.71,\ F = 114.47,$$
$$r^2_{cv} = 0.90\ s_{PRESS} = 2.232$$

$$\nu^{X}_{C=O} = 10.03\ (\pm 1.74)\sigma p + 1676.5(\pm 0.32) \quad (1b)$$

$$n = 7,\ r = 0.99,\ s = 0.33\ F = 218.93\ r^2_{cv} = 0.95,$$
$$s_{PRESS} = 0.50$$

$$\nu^{X}_{C=O} = \rho_I\sigma_I + \rho_R\sigma_R + \nu^{H}_{C=O} \quad (2)$$

$$(\pm 8.87)\sigma_I + 16.23(\pm 9.49)\sigma_R + 1664.0(\pm 4.23) \quad (2a)$$

$$n = 9,\ r = 0.95,\ s = 2.49,\ F = 25.85,\ r^2_{cv} = 0.615$$
$$s_{PRESS} = 4.789$$

$$\nu^{X}_{C=O} = 10.25(\pm 2.80)\sigma_I + 10.94(\pm 3.78)\sigma_R + 1676.0(\pm 0.91) \quad (2b)$$

$$n = 7,\ r = 0.982,\ s = 0.458,\ F = 55.223,$$
$$r^2_{cv} = 0.86\ s_{PRESS} = 0.93$$

$$\nu^{X}_{C=O} = \rho\Im + \rho\Re + \nu^{H}_{C=O} \quad (3)$$

$$\nu^{X}_{C=O} = 16.10(\pm 7.15)\Im + 14.10(\pm 4.89)\Re + 1665.1 \times (\pm 3.29) \quad (3a)$$

$$n = 9,\ r = 0.97,\ s = 1.78,\ F = 53.36,\ r^2_{cv} = 0.84,$$
$$s_{PRESS} = 3.09$$

$$\nu^{C=O}_{X} = 9.99(\pm 2.05)\Im + 9.89(\pm 2.39)\Re + 1677.0(\pm 0.71) \quad (3b)$$

$$n = 7,\ r = 0.99,\ s = 0.33,\ F = 108.59,$$
$$r^2_{cv} = 0.89,\ s_{PRESS} = 0.81$$

Analyzing the ρ values, in modulo, it's observed that the carbonyl group polarity of the compounds studied is strongly influenced by the electronic effect of the substituents groups in *para* position in the benzenic ring.

The local anesthetical activity of the compounds of the set B was determined using the method of peripheral nervous blockade in vivo.[14] In this method the solution of the compound to be analyzed is injected in the sorroundings of the nerve to be blocked in a way that its biodisponibility can be considered, in principle, similar to the preparations in vitro.

In first analysis, fixing the dose and admitting that the dose–response curves of analogous compounds are parallel, it's accepted that the maximum response obtained is proportional to the potency. Thus, it's considered the maximum local anesthetical activity, LAA_{max}, of the analyzed compounds as being proportional to its potency. For these compounds the LAA_{max} occurred around 20 min counted from the administration, with posterior decline. In Table 3 are displayed altogether the values of LAA_{max} in relation to procaine for the series of *N*-[(dimethylamine)methyl] benzamides 4-substituted hydrochlorides studied.

Analyzing Table 2 it's observed that the *para*-nitro derivative (**1b**) is approximately three times less potent than the most active compound, the *para*-methoxi derivative (**8b**), that presents the same LAA_{max} as the procaine. It's also observed that substituent groups donors of electrons profit the local anaesthetic activity, and that opposite effect is observed for substituents receptor of electrons. These observations suggest that the carbonyl group polarity significantly influences the local anesthetic activity in the set of compounds studied.

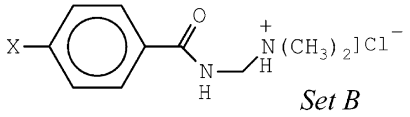
In this paper, the analysis of the relation between the chemical structure and local anesthetic activity is not

Table 2. Value of Hammett's substituent constants σ, σ_I e σ_R and Swain & Lupton's, $\Im$ and $\Re$

Substituent	$\sigma p^{(a)}$	$\sigma_I^{(b)}$	$\sigma_R^{(b)}$	$\Im^{(b)}$	$\Re^{(b)}$
NO_2	0.78	0.64	0.15	0.65	0.13
Br	0.23	0.44	−0.19	0.45	−0.22
Cl	0.23	0.47	−0.21	0.42	−0.19
I	0.18	0.39	−0.16	0.42	−0.24
F	0.06	0.52	−0.48	0.45	−0.39
H	0.00	0.00	0.00	0.00	0.00
CH_3	−0.17	−0.04	−0.14	0.01	−0.18
OCH_3	−0.27	0.27	−0.43	0.29	−0.56
$N(CH_3)_2$	−0.83	0.06	−0.53	0.15	−0.98

Sources: Hansch, C., Leo, A. J., Hoekman, D. Exploring QSAR: Hydrophobic, Electronic and Steric Constants. Washington, ACS, 1995, p. 348.

Table 3. Values of maximum local anesthetical activity relative to procaine, LAA_{max}, of hydroclorides of *N*-[(dimethylamine)methyl] benzamides 4-substituted



Set B

Compd	X	$LAA_{max.}$
1b	NO_2	0.3±0.1
2b	Br	0.9±0.1
3b	Cl	0.9±0.1
4b	I	0.7±0.1
5b	F	0.8±0.1
6b	H	0.9±0.1
8b	OCH_3	1.0±0.1
Procaine	—	1.0

adequate, considering that the effect introduced by the substituent groups is not only of electronic nature, having to be considered, among others, the effect upon the balance lipo-hydrophilic that influences significantly the local anesthetics activity. Also, this activity was evaluated in vivo, which means, in a very complex system; therefore, the study of the QSAR involving the proposition of mathematical models must to consider a more extensive analysis. Beyond the electronic property, other physicochemical properties, such as lipophilicity and structural conformation as well as information about the bioavailability and action mechanism[11] must be considered.

3. Conclusions

The results obtained validate the use of $\nu_{C=O}$ as experimental electronic parameter adequate for the application in QSAR studies. On the other hand, the proposition of mathematical models involving the study of the quantitative relations between the chemical structure and the local anesthetical activity is, with the results obtained till this moment, quite precocious, once the application of the *Hansch Analysis* requests accurate biological data, a higher number of compounds as well as the analysis and study of other factors involved in the interaction drug-biophase.

Acknowledgements

To Prof. Dr. H. Kubinyi, for the freeware use of the BILIN Program, to FAPESP and CNPq for the financial support.

References and notes

1. Wermuth, C. G. *The Practice of Medicinal Chemistry*; Academic Press: San Diego, 2000.
2. Foye, W. O.; Lemke, T. L.; Willians, D. A. eds. *Principles of Medicinal Chemistry*, 4th ed; Williams & Wilkins: Baltimore, 1995.
3. Caliendo, G. *Eur. J. Med. Chem.* **1995**, *30*, 603.
4. Caliendo, G. *Eur. J. Med. Chem.* **1996**, *31*, 99.
5. Amaral, A. T.; Oliveira, A. C.; Neidlein, R. *Eur. J. Med. Chem.* **1997**, *32*, 433.
6. Amaral, A. T.; Miyazaki, Y.; Capobianco, G. In *Trends in QSAR and Molecular Modeling*; Wermuth, C. G., (Ed.), Science Publishers B.V. 1993, Netherlands, p 558
7. Amaral, A. T.; Miyazaki, Y.; Tavares, L. C. In *QSAR and Molecular Modelling: Concepts, Computational Tools and Biological Applications*; Sanz, F.; Giraldo, J.; Manaut, F. (Ed.), J.R. Prous Science Publishers: Barcelona, 1995; p 134.
8. Hansch, C.; Leo, A. J. *In Exploring QSAR: Fundamentals and Applications in Chemistry and Biology*; ACS: Washington, 1995; p 557.
9. Kubinyi, H. *Methods and Principles in Medicinal Chemistry*; VCH: New York, 1993.
10. Hansch, C.; Fujita, T. *J. Am. Chem. Soc.* **1964**, *86*, 1616.
11. Hansch, C.; Leo, A. J.; Hoekman, D. *Exploring QSAR: Hydrophobic Electronic and Steric Constants*; ACS: Washington, 1995; p 348.
12. Amaral, A. T.; Amaral, L. *J. Org. Chem.* **1976**, *41*, 1623.
13. McLechlan, D.; Nyquist, R. A. *Spectrochim. Acta* **1964**, *20*, 1397.
14. Hammett, L. P. *J. Am. Chem. Soc.* **1937**, *59*, 96.
15. Eichbaum, F. W.; Yasaka, W. J. *Br. J. Anaesth* **1973**, *45*, 1022.