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Article

NMR Spectroscopy, Hammett Correlations and Biological Activity of Some Schiff Bases Derived from Piperonal

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Uma série de Bases de Schiff foi sintetizada através da reação de condensação do piperonal (3,4-metilenodioxibenzaldeido) com as correspondentes aminas primárias aromáticas. Seus espectros de RMN de ^{1}H e ^{13}C foram obtidos e estudou-se as correlações de Hammett envolvendo os deslocamentos químicos e as constantes dos substituíntes (σ_p, σ_R e σ_I). Observou-se correlações lineares e bilineares significativas para o carbono imínico (C-α) e C-1', indicando maior contribuição do efeito de ressonância do substituinte sobre os deslocamentos químicos. Os assinalamentos dos C-4' apresentaram-se bem alterados pelos efeitos dos substituíntes, especialmente pelos halogênios, na direção esperada. A série de Bases de Schiff foi ensaiada contra microorganismos, sendo que apresentou atividade significativa frente a fungos filamentosos, especialmente o *Epidermophyton floccosum*. Não foram observadas correlações significativas entre a atividade biológica e os efeitos eletrônicos.

A series of eleven Schiff Bases have been synthesized. They were obtained by condensation of piperonal (3,4-methylenedioxybenzaldehyde) with the corresponding aromatic primary amines. Their ^{1}H and ^{13}C-NMR spectra have been obtained and the Hammett correlations including chemical shifts and the subsitutent constants (σ_p, σ_R e σ_I) were studied. Linear and bilinear significant correlations were observed for iminic carbon (C-α) and C-1', showing a more significant ressonance effect on chemical shifts. The chemical shifts for C-4' were highly affected by substituent effects, especially for halogens in the expected direction. Their biological activity against microorganisms has also been measured and significant activity was showed against *Epidermophyton floccosum*. The biological activity did not give a reasonable relationship with electronic effects.

Keywords: *Hammett NMR correlations, piperonal*

Introduction

Carbonyl, nitrile and imidoyl groups are classed as heteropolar unsaturated functions. Much work has been carried out with carbonyl compounds, and the chemistry of nitrile compounds is also well explored. There are many different types of compounds which contain the imidoyl function and in total the body of work on this subject is quite substantial[1]. We have, for a long time, maintained interest in compounds containing >C=N-X functions and have included some comparisons of >C=O; >C=N and >C=N-X functions[2-8]. Later, we reported studies on NMR spectroscopy[9] and biological activity of some >C=N-X compounds[10,11]. We have also considered saturation of internal resonance effects in guanidines[12]. Studies of Schiff Bases have also included 4- and 4'-substituted-N-benzylidenanilines[9,10] and N-benzylidene-3,3-diphenyl-propylamines[13] and in both series, ^1H and ^{13}C-NMR spectra were analysed. Studies of infra-red and ultra-violet spectra for 3,3-diphenylpropylamine derivatives were also carried out together with preliminary tests of their biological activities.

The interesting results obtained with this series of Schiff Bases, led us to synthesize and study a series of eleven N-benzylidene-4'-substituted-anilines in which the benzylidene moiety was fixed as 3,4-methylenedioxybenzylidene. The choice of piperonal for the aldehyde moiety in these preparations stemmed from the fact that many compounds containing the 3,4-methylenedioxy group have some biological activity[14,15]. The ^{13}C-NMR spectroscopy and Hammett correlations were investigated for Schiff Bases derived from piperonal.

Materials and Methods

Preparation of the Schiff bases

The Schiff bases were prepared by condensation of piperonal with the corresponding amines following a general method of preparation[16]. The products were recrystallized from analytical grade ethanol to constant melting point. Their purity was confirmed by elemental analysis, and UV and IR spectroscopy. Details of their physical properties are given in Table 1.

NMR Data

The ^1H and ^{13}C-NMR spectra were determined at 30 °C on a Varian XL-100 spectrometer (^1H: 100 MHz and ^{13}C: 25.2 MHz), for *ca.* 0.3 M and saturated solutions in $CDCl_3$ or DMSO-d_6, respectively, with TMS as internal standard. The ^1H and ^{13}C-NMR spectral data used for Hammett correlations are shown in Table 2.

Biological assays

Assays with bacteria and yeasts were performed by the agar well diffusion method (cavity-plate), using trypticase soy agar and Sabourand-dextrose agar, respectively. The concentration of samples ranged from 2 to 4 mg/mL in pure DMSO for bacteria and DMSO/H_2O (1:1 v/v) for yeasts.

The bacteria used were: *Bacillus cereus, Staphylococcus epidermides* and *Escherischia coli*. The yeasts used were: *Candida albicans, Candida tropicalis, Cryptococcus neoformans* and *Torulopsis glabrata.* The cultures are place on the Micoteca of Departamento de Microbiologia do Instituto of Ciências Biomédicas of Universidade de São Paulo.

Assays with filamentous fungi involved the utilization of two-fold serial dilution in Sabourand-dextrose liquid medium. The concentration of samples ranged from 62.5 to 500 µg/mL. The test compounds were dissolved in DMSO (0.1 mL) and the solution was added to sterile Sabourand-dextrose agar solution. The maximum concentration of organic solvent was 2.5%.

The results are expressed in MIC (minimal inhibitory concentration). The fungi used were: *Microsporum canis, Microsporum gypseum, Trichophyton rubrum* and *Epidermophyton floccosum*. Solvent blanks were run against each test organism, in all assays. The results of the fungi assays are shown in Table 3.

Table 1. Physical and spectral data for Schiff Bases derived from piperonal and *p*-substituted anilines.

x	Yield (%)	M.p. (°C)	λ_{max}[a] (nm)	log ε_{max}	ν C=N[b] (cm^{-1})	Molecular Formula	Microanalyses (%), Found (required)		
							H	C	N
OCH_3	73.3	119-121	328;231;212	3.34;3.16;3.13	1625	$C_{15}H_{13}O_3N$	71.07 (70.59)	5.10 (5.10)	5.49 (5.27)
OC_2H_5	85.3	102-105	337;229;212	3.27;3.28;3.12	1630	$C_{16}H_{15}O_3N$	71.09 (71.37)	5.47 (5.57)	6.43 (6.20)
CH_3	99.2	98-99	328;230;212	3.28;3.21;3.13	1625	$C_{15}H_{13}O_2N$	73.78 (73.31)	5.40 (4.40)	5.78 (5.86)
C_2H_5	66.3	55-57	329;229;212	3.23;3.28;3.09	1620	$C_{16}H_{15}O_2N$	76.09 (75.89)	5.54 (5.93)	5.51 (5.54)
H	81.1	68-70	323;228;211	3.20;3.26;3.07	1625	$C_{14}H_{11}O_2N$	75.38 (74.67)	4.94 (4.89)	6.67 (6.23)
Cl	45.5	80-82	329;232;212	3.29;3.28;3.12	1620	$C_{14}H_{10}O_2NCl$	64.33 (64.74)	3.86 (3.85)	5.67 (5.39)
Br	68.6	108-110	330;233;212	3.29;3.30;3.13	1620	$C_{14}H_{10}O_2NBr$	55.49 (55.44)	3.40 (3.30)	4.51 (4.62)
I	70.9	129-131	330;233;212	3.20;3.30;3.13	1620	$C_{14}H_{10}O_2NI$	47.76 (47.86)	2.88 (2.85)	3.86 (3.98)
COOH	47.8	165-166	295;276;212	3.18;3.18;3.16	1645	$C_{15}H_{11}O_4N$	66.79 (66.90)	4.27 (4.13)	5.04 (5.20)
$SO_3^- Na^+$	53.2	250	330;238;208	4.18;4.17;4.20	1625	$C_{14}H_{10}O_5NSNa$	51.03 (51.06)	3.14 (3.04)	4.20 (4.25)
NO_2	83.3	198	350;218	4.11;3.95	1630	$C_{14}H_{10}O_4N_2$	62.98 (63.22)	3.83 (3.71)	10.05 (10.40)

[a] The UV spectra were determined in absolute ethanol at 25 °C in the range of concentration of (5-10) x 10^{-5} M.

[b] The values of $\nu_{C=N}$ were measured in cm^{-1} in KBr pellets.

Results and Discussion

Eleven 3,4-methylenedioxybenzylidene-4'-substituted-anilines have been studied in this work. For a discussion of the ^{1}H and ^{13}C-NMR spectra it is convenient to show the numbering used for these compounds, viz,

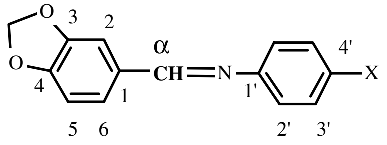

Table 1 shows the ^{1}H-NMR chemical shifts of the methine protons (joined to C-α), the ^{13}C-NMR chemical shifts of C-1, C-α, C-1' and C-4', the values of the Hammett substituent constants (σ_p), σ_R and σ_I values for resonance and inductive constants of the substituents, respectively.

A plot of the ^{1}H-NMR chemical shifts of the methine protons does not give a reasonable Hammett correlation, whereas there is such a correlation with the methine carbon. Thus, it appears that the ^{13}C-NMR shifts are essentially dependent on the perturbation of the aldimine π-bond. The extension of the effect to the methine protons is essentially a second order effect and we suggest that this accounts for the inadequate correlation.

Substituent effects (X) on ^{13}C-NMR: **(i) on C-1**: The values are quite independent of the 4'-substituent. The chemical shifts do not give a reasonable Hammett correlation. This situation is similar to that observed for the ^{1}H

Table 2. Chemical shifts[a] (δH, δC in ppm), Hammett substituent constants[b] (σ_p) and dual parameters[b] (σ_I and σ_R) for substituted Schiff bases derived from piperonal.

X	δH	δC-α	δC-1	δC-1'	δC-2'	δC-3'	δC-4'	σ_p	σ_I	σ_R
OCH_3	8.35	157.04	131.41	144.77	121.80	114.10	157.94	-0.27	0.26	-0.51
OC_2H_5	8.38	157.07	131.37	144.67	121.07	114.74	157.31	-0.24	0.22	-0.44
CH_3	8.31	158.22	131.16	149.22	120.90	128.44	135.23	-0.17	-0.04	-0.13
C_2H_5	8.33	158.32	131.41	148.19	119.40	127.48	141.67	-0.15	-0.05	-0.10
H	8.25	159.04	131.03	151.82	120.60	128.73	125.48	0.00	0.00	0.00
Cl	8.27	159.42	130.93	150.49	121.82	128.56	130.74	0.23	0.41	-0.15
Br	8.28	159.42	130.78	150.53	122.34	131.68	118.83	0.23	0.44	-0.17
I	8.27	159.42	130.53	151.23	123.13	138.27	89.71	0.18	0.40	-0.19
COOH	8.53	166.84	129.07	160.98	121.31	129.36	131.02	0.45	0.33	0.15
$SO_3^-Na^+$	8.45	162.34	129.54	158.77	120.16	128.54	143.81	0.09	0.15[c]	0.33[c]
NO_2	8.68	169.69	131.90	163.64	121.78	124.18	147.02	0.78	0.67	0.16

[a] Compounds with X=COOH, $SO_3^-Na^+$ and NO_2 were recorded in DMSO-d_6 and the others in $CDCl_3$; [b] Ref. 17; [c] Ref. 18.

Table 3. Minimal inhibitory concentration for the biological activity of the Schiff Bases with filamentous fungi.

X	*Microsporumcanis*		*Microsporumgypseum*		Trichophytonrubrum		Epidermophytonfloccosum	
	(μg/mL)	mM	(μg/mL)	mM	(μg/mL)	mM	(μg/mL)	mM
OCH_3	500	1.96	500	1.96	250	0.98	62.5	0.24
OCH_2H_5	NI	NI	NI	NI	NI	NI	250	0.93
CH_3	500	2.09	500	2.09	500	2.09	250	1.04
C_2H_5	500	1.97	500	1.97	250	0.97	62.5	0.25
H	250	1.11	500	2.22	500	2.22	250	1.11
Cl	500	1.93	500	1.93	250	0.96	62.5	0.24
Br	500	1.64	NI	NI	250	0.82	62.5	0.20
I	500	1.42	NI	NI	NI	NI	250	0.71
COOH	NI	NI	NI	NI	NI	NI	500	1.86
$SO_3^-Na^+$	NI	NI	NI	NI	NI	NI	NI	NI
NO_2	500	1.18	NI	NI	125	0.96	62.5	0.23

NI - no inhibition up to 500 μg/mL.

chemical shifts of the methine protons, which we have already discussed.

(ii) on C-α: There is an acceptable correlation with the ^{13}C-NMR chemical shifts of C-α. The plot of ^{13}C-NMR chemical shifts of C-α versus the Hammett constants gives an equation: $\delta C\text{-}\alpha = (159.43 \pm 0.58) + (11.57 \pm 1.82)\sigma_p$, where the value of the Hammett reaction constant $\rho = +11.57$ with a correlation coefficient of 0.9047 and standard deviation of 1.84 for n = 11. We suggest that the 4'-substituents perturb the aldimine π-bond. This is corroborated by the multiple linear regression which considers the inductive (σ_I) and resonance (σ_R) dual parameters, and the chemical shifts for ^{13}C-α, where a major contribution of the σ_R parameter is demonstrated.

$$\delta C\text{-}\alpha = (9.56 \pm 2.73)\,\sigma_I + (11.36 \pm 2.48)\,\sigma_R + (159.28 \pm 0.95)$$

n = 11
correlation coefficient (r) = 0.904
standard deviation (s) =1.953

The comparison of the relationships concerning δC-α with other results in the literature indicated similar chemical shifts considering the electronic effects. The substituted N-benzylidenanilines[9] showed an acceptable Hammett correlation with ρ = 4.06 (correlation coeficient = 0.922) and the ^{13}C-NMR shifts of C-α were more largely affected in the piperonal Schiff base series (ρ = 11.57), than those reported[9].

(iii) on C-1': The conventional effects of electron-withdrawing and electron-releasing groups were observed. There is a poor Hammett correlation with a correlation coefficient of 0.8670 for the equation

$\delta C\text{-}1' = (150.46 \pm 1.05) + (17.03 \pm 3.26)\sigma_p$ and n = 11; sd = 3.30. In this case the multiple linear regression considering σ_I and σ_R parameters, also showed the major dependence on the resonance effect and a better correlation.

$$\delta C\text{-}1' = (10.77 \pm 2.44)\,\sigma_I + (21.60 \pm 2.21)\,\sigma_R + (151.54 \pm 0.85)$$

n = 11
r = 0.969
sd = 1.745

As an example, for the compound without a 4'-substituent, the chemical shift is 151.82 ppm, whereas with the 4'-nitro group the chemical shift is 163.64 ppm and with a 4'-methoxyl group, the chemical shift is 144.77 ppm (other values are cited in Table 1).

(iv) on C-4': The results obtained are noteworthy. It is clear that groups which are linked by atoms which are obviously more electronegative produce chemical shifts in the expected direction, that is, values at low field are observed when compared with that with 4'-hydrogen (125.48 ppm). However, even the methyl group produces a considerable low field shift (135.23 ppm).

We associate this with the following structural feature. The imidoyl nitrogen is a strong electron-donor to the ring to which it is directly attached and this must *inter alia* increase markedly the electron-density on C-4'. This induces a perturbation towards the methyl group due to its polarizability, as compared with the hydrogen linked to C-4'.

The heavy halogens joined to C-4' are a special case. Their p-electrons cause a shielding in the order I > Br > Cl. This more than counteracts the electronegativity effect with I and Br and approximately cancels the electronegativity effect in the case of Cl. Thus, the C-4' chemical shifts are at high field for 4'-iodo (89.71 ppm) and 4'-bromo (118.83 ppm) and practically unchanged for the 4'-chloro compound (130.74 ppm). These values, δC-4', were compared with the corresponding substituted N-benzylidenanilines[9,19] showing small changes except for the 4-chloro derivative.

As shown in Table 3, the Schiff Bases were tested against the filamentous fungi, all dermatophytes. Most of the Schiff Bases were found to possess good activity, and especially against *Epidermophyton floccosum,* for which the most active compounds were 4'-Br, Cl, C_2H_5, NO_2 and OCH_3 with MCI values *ca.* 2 x 10^{-4} M. The assays with bacteria and yeasts showed that the Schiff Bases were inactive.

A good correlation was not obtained between the biological activity values (MCI, 1/MCI or log 1/MCI) and substituent constants (σ_p, σ_R and σ_I) indicating that electronic effects have no significant contribution in these results. This indicates that an evaluation of lipophilic parameters is important for a better understanding between bilological activity and chemical structure relationships.

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