International Journal of Pharmaceutics, 85 (1992) 199–202

IJP 02867

Bioactivity of a series of substituted *N*-(3-phenyl-2-propenylidene)benzeneamines: a microcalorimetric study

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(Received 24 February 1992)
(Accepted 31 March 1992)

Key words: Microcalorimetry; Bioactivity; Schiff base; Structure-activity relationship

Summary

The interaction of some Schiff bases with *Saccharomyces cerevisiae* has been studied by microcalorimetry. Flow microcalorimetry is shown to yield accurate and precise measurements of the bioactivities of the study compounds. In addition, it has allowed the establishment of a structure-activity relationship (SAR) for these non-homologous Schiff bases. The data show that biologically based SARs may be general and of potential in establishing modes of action.

Introduction

Schiff bases have long been known to have antimicrobial activity (for a review, see Montanari, 1991). They are active against a wide range of organisms, for example, *Trychophyton gypseum, Candida albicans, Mycobacteria, Erysiphe graminis* and *Plasmopora viticola*.

More recently, microcalorimetry has been shown to play a role in the study of the physicochemical properties of drug substances (Buckton and Beezer, 1991). Microcalorimetry has also been proposed as a technique for the establishment of biologically based quantitative structure activity relationships (QSARs; Beezer et al., 1986, 1987, 1988; Beezer, 1990; De Morais et al., 1990). The earlier work (Beezer et al., 1986, 1987, 1988) demonstrated that there was a QSAR for homologous series of alkoxyphenols and for hydroxybenzoates. These studies were succeeded by an investigation (De Morais et al., 1990) that surveyed the biological activity of a series of non-homologous cardanol derivatives. The intention in this latter work was to explore the possibility that microcalorimetry could be used to screen rapidly the bioactivity of derivatives of natural products. Schiff bases have been the subject of a previously reported limited qualitative microcalorimetric investigation (Perry et al., 1986). However, the particular derivatives studied had only limited bioactivity and the results were reported solely in a

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relative manner. This paper seeks to extend the screening enquiry into the study of Schiff base derivatives and, if possible, to derive mechanistic data for the interaction of the study compounds with a sensitive organism – in this case *Saccharomyces cerevisiae.* A general procedure applicable to a wide range of organisms and of compounds of both natural and synthetic origin would be of considerable advantage to the methods and techniques available to establish not only the *physical* but also the *biological* characteristics of newly discovered compounds. The applicability of such a technique would be enhanced further if the capacity to indicate modes of action and QSAR data were also available. It was to explore these possibilities that the work reported in this paper was undertaken.

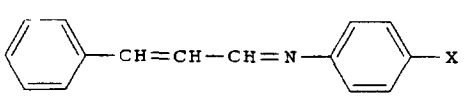
Materials and Methods

Compounds

The preparation, identification and purity of the studied Schiff bases (Fig. 1) have been described elsewhere (Montanari, 1991). The growth, preservation in liquid nitrogen, medium and handling of cultures and inocula were as previously reported (Perry et al., 1989).

Microcalorimetry

The instrument, its operation and all associated procedures were as previously described (Beezer et al., 1986, 1987, 1988). The Schiff bases were added to the microcalorimetric incubation mixture (total volume 50 cm^3) in 2 cm^3 of methanol. The effect of adding this quantity of methanol to the control incubation was taken into account in establishing the quantitative results.



X = H (i), MeO (ii), NO_2 (iii), Br (iv)

Fig. 1. Structures of the Schiff bases: X = H (compound i), MeO (ii), NO_2 (iii), Br (iv).

TABLE 1

Values of dose, response and associated standard deviation (SD) for the study compounds

Compound	Dose (μmol dm^{-3})	Response (%)	SD
(i)	966.18	35.58	1.45
	724.64	29.45	
	483.09	23.31	
	241.54	8.59	
(ii)	966.18	42.33	1.22
	724.64	37.42	
	483.09	28.83	
	241.54	16.58	
(iii)	966.18	38.65	1.55
	724.64	33.74	
	483.09	27.61	
	241.54	11.65	
(iv)	966.18	36.81	2.09
	724.64	31.90	
	483.09	24.54	
	241.54	10.42	

Results and Discussion

The quantitative results for the bioassay of the study compounds are presented in Table 1. In Table 2 the results are listed for the calculated (Hansch and Leo, 1979) values of the octan-1-ol/water partition coefficients (P) for the four compounds subject to quantitative experiments together with those of $\log(\text{dose})_{max}$ (defined as the maximum dose which can be added without eliciting a response – a quantity which is determined

TABLE 2

Values of log P (calculated, see text) and $\log(\text{dose})_{max}$ (for definition see text)

Compound	Log P	Log(dose)_{max}
(i)	4.03	2.02
(ii)	3.15	2.12
(iii)	2.84	2.15
(iv)	2.63	2.18

TABLE 3

Regression parameters for the log(dose)-response linear plots

Compound	Slope	Intercept	Correlation coefficient
(i)	44.2	−96.2	0.9970
(ii)	43.9	−88.68	0.9994
(iii)	45.6	−96.3	0.9950
(iv)	44.7	−96.2	0.9988

from the linear portion of the log(dose)-response curve). The regression parameters for the linear portion of the log(dose)-response line are shown in Table 3.

Fig. 2 shows typical experimental microcalorimetric output (power-time (*p-t*) curves). It will be noted that Fig. 2 displays two types of *p-t* curves which are indicative of differing modes of action of the test compounds upon interaction with *S. cerevisiae*. A type 'B' response was shown by compound (i) at all experimental concentrations and by compounds (ii) and (iii) at low concentration. This type of response implies a reduction in overall metabolic activity but not cell death – this conclusion was not experimentally tested in this work but has been found to be the case in all other microcalorimetric experiments where this outcome has been observed (Shafiq, 1991). The other type of response, 'C', which was exhibited by compound (iv) and by compounds (ii) and (iii) at high concentrations corresponds to a gradual decrease in cell numbers, i.e., to cell death. This latter type of response does indeed also represent the *kinetic* consequences of the interaction of the selected metabolic modifiers, in this case Schiff bases, with the sensitive interacting organism.

The linear portions of the log(dose)-response curves have been subjected to linear regression analysis the results of which are set out in Table 3. These data reveal that the intercept values of the lines are constant, except for compound (ii), and hence are relatively insensitive to the nature of the substituent. The slopes of these lines are also, within experimental error, equal. This means that the effective groups in the molecule are identical and only some other property not *directly* associated with the biological activity is affected when substituent groups are changed. Thus, a plot of $\log(\text{dose})_{max}$ vs σ, the Hammett sigma parameter which reflects the electron distribution in the aromatic ring, results in a scattergram. However, a plot of $\log(\text{dose})_{max}$ vs log P results in a linear trace (regression coefficients: $r = -0.9995$; slope $= -8.74$; intercept $= 21.65$). These data, therefore, appear to show that it is the variation in partitioning behaviour which is affected by change in substituent and not the bioactive groups in the molecules, since all the compounds retain the same incremental dependence of bioactivity upon concentration. Indeed, all these compounds are quite hydrophobic (cf. Table 2). The partition coefficients for partitioning into octan-1-ol are greater than those into hexane (data not shown). For example, the calculated value of log P in the hexane system for *N*-(3-phenyl-2-propenylidene)aniline is 2.38 whereas that for this same compound in an octan-1-ol system is 4.03, suggesting that hydrogen bonding between the solute (Schiff base) and the organic solvent (octan-1-ol) may assist passage of the solute from the aqueous phase into the organic solvent.

Interestingly, the differences in the observed *p-t* curves as a function of concentration for some of the study compounds do not, at least over the investigable range reported here, appear to inter-

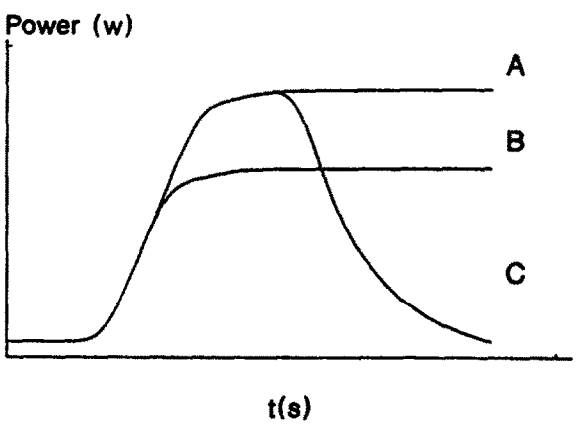


Fig. 2. Typical power-time traces obtained under respiratory conditions. A, normal respiration; B, reduced respiration upon interaction with a metabolic modifier; C, cell death caused by interaction with a metabolic modifier.

fere with the development of a linear log(dose)-response line.

It is encouraging to note that the existence of linear free energy relationships in directly determined biological responses has now been extended to another class of compounds. These results certainly appear to add weight to the view that such parameters, derived from the exploitation of a general reaction parameter, may themselves be general properties of biological systems upon interaction with metabolic modifiers.

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