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Quantitative structure–activity relationships: microcalorimetric determination of a group additivity scheme for biological response

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Summary

Flow microcalorimetric bioassay data for interaction of *m*-alkoxy phenols and for *p*-hydroxybenzoates have been obtained. Analysis of these data allows the identification of contributions toward the derived bioactivity from the parent structures (the molecule minus the n-CH_2 groups present in the side-chain) and the lipophilic groups, CH_2. These results are discussed with respect to drug quantitative structure–activity relationships.

Introduction

A review (Beezer et al., 1983a) has described the application of the flow microcalorimetric technique to the bioassay of drug substances. Most of the studies reported were concerned, in the main, with qualitative accounts of drug/microorganism interaction. However, a modest number of accounts of quantitative bioassays also exists. These reports have principally related to anti-fungal antibiotic bioassay. In an instance involving bacteria, the microcalorimetric bioassay of some *m*-alkoxy phenols was used as the measure of "biological response" in the description (Beezer et al., 1983b) of a drug structure–activity correlation (QASR).

The basis of QSAR is generally held to be the relationship between partition coefficients (P), for members of an homologous series and biological response (BR).

Much work has been devoted (Leo et al., 1971; Dearden, 1983) to the establishment of group contributions to the calculation of P values (Rogers and Davis, 1980; Dearden, 1983). Such group contributions, in principle, allow the prediction of P values for other members of the series and, by extension, of compounds outside the series. Thus group additivity is seen as an important feature in the practical application of QSAR data.

No such extensive data set exists for the identification of a structure–activity correlation involving BR. The simplest scheme proposed is the Free-Wilson technique (Free and Wilson, 1964). This scheme involves assigning a constant to every substituent and thus:

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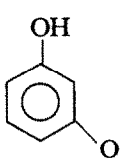
$$\text{Biological activity} = \sum(\text{substituent constants}) + X$$

where X is a constant denoting the overall average biological activity for the whole series. This model has formed the basis of several other models. Moreover, it is held that the major shortcoming of the additivity of 'de novo' models (substituent constants) when used alone is that the derived substituent constants have no direct physicochemical meaning and it is consequently extremely difficult to rationalize the requirement for better substituents. A further disadvantage of the Free-Wilson based models is their requirement for large data sets arising from the number of independent variables generated. BR as presently employed is regarded as being so imprecise in measurement that temperatures at which P values are measured, for use in QSAR are frequently not controlled. Attention has been drawn (Beezer et al., 1980, 1983c) to this disturbing feature of QSAR studies.

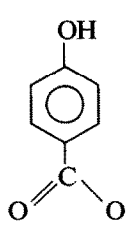
The reproducibilities, precision and accuracy attainable (Beezer et al., 1983a) in flow microcalorimetric investigations of drug/cell interactions make it possible to establish BR, for some systems, on a defined basis.

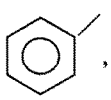
To examine this possibility we have investigated, via flow microcalorimetry, the bioassay of members of two homologous series (*m*-alkoxy phenols and *p*-hydroxybenzoates) on interaction with standardized (Beezer et al., 1976), liquid nitrogen stored cultures of *Escherichia coli* NCTC 10418.

For each drug considered here we may define a "parent" structure; for the *m*-alkoxy phenols this is:



and for the *p*-hydroxybenzoates it is:



The hydrocarbon side-chain is then composed of n-CH_2 groups in each case. Considerable simplification could be achieved in evaluation of BR if BR itself were factorizable into bioanalytically useful contributions from the lipophilic (CH_2 groups) and hydrophilic (parent structure) portions of the molecule. The designation of "parent" structure, lipophilic and hydrophilic portions of these molecules are identical to those used by us in a thermodynamic analysis of partitioning of these compounds between water and non-aqueous solvents and in a thermodynamic analysis of the Collander equation (Beezer et al., 1986a). Such an additivity principle would, we believe, be of considerable advantage in QSAR studies and to the pharmaceutical industry in that BR itself may be separated into the bioactivity effect of the parent structure and, for the compounds described here, the effect of adding successive methylene groups. Of course through a different choice of homologous series it would appear possible to identify other "group" effects e.g. for –OH, C = O, SH, NH_2,  , etc. such that a comprehensive group additivity scheme based upon such a bioassay scheme could be evaluated.

It is the purpose of this paper to initiate discussion of these possibilities.

Experimental

m-Alkoxy phenols were prepared and stored as described previously (Beezer et al., 1980); *p*-hydroxybenzoates were the generous gift of NIPA chemicals and were certified as 99.9% pure on delivery. They were used without further treatment.

Procedure

The preparation of solutions for all microcalorimetric incubations was as previously described (Beezer et al., 1983b).

Preparation of cells

Escherichia coli NCTC 10418 was grown in 250

ml flasks containing 40 ml of medium of composition ($g \cdot l^{-1}$): glucose, 4.0; $(NH_4)_2SO_4$, 2.0; $NaH_2PO_4 \cdot 2H_2O$, 7.8; K_2HPO_4, 8.4; $MgCl_2$, 0.13; $CaCO_3$ 0.003; $FeSO_4 \cdot 7H_2O$, 0.007; $MnCl_2 \cdot 4H_2O$, 0.001; $ZnSO_4$, 0.0005; $CaCl_2 \cdot 6H_2O$, 0.0002; $CuCl_2 \cdot 2H_2O$, 0.0003; H_3BO_3, 0.0001; $Na_2MoO_4 \cdot 2H_2O$, 0.0003; final pH 7.0. Forty flasks were inoculated with 0.8 ml of an overnight culture and incubated at 37°C on a rotary shaker (220 rpm: Gallenkamp, U.K.). Growth was followed by optical density (EEL colorimeter) and after 6 h incubation, at an optical density equivalent to 1.2 g dry weight of cells/litre, the cells were pooled, centrifuged, washed twice in 1/4 strength Ringer's solution containing 10% w/v of dimethylsulfoxide (DMSO) at 4 g dry weight of cells/litre. The maximum specific growth rate and the stationary phase organism concentration for the medium used were 0.7 h^{-1} and 2.0 dry weight of cells/litre, respectively. Cells in 1/4 strength Ringer's solution containing 10% DMSO were frozen in liquid nitrogen and recovered as described previously (Perry et al., 1980).

Results and Discussion

Tables 1 and 2 show the bioassay data derived from study of the named compounds together with the correlation coefficients, slopes and intercepts derived from plots of log(dose) vs response. Response was recorded as the peak height achieved in the power-time (PT) curve derived from treated incubations; the sensitivity employed was 10 μV.

It will be noted that excellent straight lines were obtained in all cases. From the limited data available such correlations are normal in flow microcalorimetric determination of bioactivity. The intercept value for each compound is designated $\log(dose)_{max}$ and is to be understood as the maximum dose of the drug which may be applied without eliciting a response.

A plot of $\log(dose)_{max}$ vs carbon number, n, in the side-chain yields, on omitting the point for the methyl substituent where it is not CH_2 added but CH_3, a straight line (correlation coefficient, 0.9999; slope, −0.5370; intercept, 2.6539). The value of the intercept in concentration terms, 450.7 mM, is, then, the concentration (hypothetical) of the parent structure above which a response will be elucidated. This, together with the slope of the line will therefore permit the evaluation of the relative bio-

TABLE 1

VALUES OF DOSE, log(DOSE), RESPONSE AND THE CORRELATION COEFFICIENTS, SLOPES AND INTERCEPTS DERIVED FROM PLOTS OF log(DOSE) VS RESPONSE FOR *m*-ALKOXY PHENOLS

Compound	Dose (mM)	log(dose)	Response (%)
m-methoxy	49.7	1.69	18
	43.6	1.64	27
	36.4	1.56	38
	26.1	1.41	58
	19.5	1.29	69
		corr. coeff.	0.9951
		intercept	1.844
		slope	−7.763
m-ethoxy	32.2	1.50	32
	26.6	1.42	30
	21.1	1.32	45
	13.7	1.13	79
		corr. coeff.	0.9995
		intercept	1.583
		slope	−5.712
m-propoxy	10.1	1.01	8
	7.5	0.88	24
	6.12	0.78	43
	4.77	0.67	64
	4.1	0.61	71
		corr. coeff.	0.9957
		intercept	1.043
		slope	−6.036
m-butoxy	2.42	0.38	15
	2.15	0.33	24
	1.57	0.19	45
	1.18	0.07	63
	0.95	−0.02	71
		corr. coeff.	0.9965
		intercept	0.4957
		slope	−6.973
m-pentoxy	0.74	−0.13	15
	0.55	−0.26	35
	0.42	−0.38	53
	0.33	−0.48	71
	0.26	−0.59	83
		corr. coeff.	0.9988
		intercept	−0.0247
		slope	−6.608

activities of the substituted compounds. The bioactivities are all related to the same, reproducible standard, i.e. complete inhibition of power output from a standard liquid nitrogen stored inoculum in a standard time. In this manner data equivalent to phenol coefficients could be evaluated. More interesting though, is the possibility that is offered to establish a group additivity scheme for evaluation of BR. It is apparent that this potential exists, since, as $\log(\text{dose})_{max}$ vs carbon number, n, is linear then so must $\log(\text{dose})_{max}$ vs Gibbs Energy of transfer of the drug from aqueous to lipoid phases (G_{trs}); $\log(\text{dose})_{max}$ vs log(partition coefficient) K_d (for the same transfer process), and $\log(\text{dose})_{max}$ vs aqueous solubility. These correlations thus may allow the evaluation of K_d, BR and solubility from a limited number of measurements. These data are of some significance, perhaps, to QSAR studies and to pharmaceutical research where drug structure is to be related to BR and to achievable solubility levels in the biophase. It should be noted that, whilst the form of the relationship derived here is similar to that of the Free-Wilson model, the parameters do indeed have apparent physical significance. However, some features of the limiting factors responsible for the existence of parabolic models are brought into focus. The maximum "concentration" of any homologue that can be applied without eliciting a response is shown in Table 3 along with some experimentally determined aqueous solubilities for the *m*-alkoxy phenols (Beezer et al., 1986b).

If these data are corroborated by other results as additivity schemes, in other laboratories, we look to extension of these studies to give a true

TABLE 2

VALUES OF DOSE, log(DOSE), RESPONSE AND THE CORRELATION COEFFICIENTS, SLOPES AND INTERCEPTS DERIVED FROM PLOTS OF log(DOSE) VS RESPONSE FOR *p*-HYDROXYBENZOATES

Compound	Dose (mM)	log(dose)	Response (%)
methyl	15.8	1.19	19
	13.7	1.14	30
	9.3	0.97	52
	7.0	0.85	70
	4.7	0.66	93
		corr. coeff.	0.9984
		intercept	1.344
		slope	−7.258
ethyl	8.4	0.92	24
	7.5	0.87	30
	6.6	0.82	43
	5.7	0.75	47
	4.7	0.67	62
	4.0	0.60	70
		corr. coeff.	0.9916
		intercept	1.084
		slope	−6.796
1-propyl	3.8	0.58	5.2
	3.1	0.49	15
	2.2	0.34	36
	1.9	0.28	38
	1.6	0.20	56
	0.87	−0.06	91
		corr. coeff.	0.9956
		intercept	0.5999
		slope	−7.338
1-butyl	1.45	0.16	14
	0.97	−0.01	28
	0.87	−0.06	30
	0.72	−0.14	44
	0.38	−0.41	85
		corr. coeff.	0.9831
		intercept	0.2115
		slope	−7.552

TABLE 2 (continued)

Compound	Dose (mM)	log(dose)	Response (%)
2-methylpropyl	1.44	0.16	10
	1.20	0.08	21
	0.96	−0.02	30
	0.77	−0.11	43
	0.57	−0.24	59
	0.39	−0.41	85
		corr. coeff.	0.9976
		intercept	−0.2272
		slope	−7.674
1-pentyl	0.81	−0.09	19
	0.63	−0.20	36
	0.48	−0.32	52
	0.38	−0.42	66
	0.29	−0.54	82
		corr. coeff.	0.9996
		intercept	0.05196
		slope	−7.176

TABLE 3

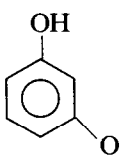
MAXIMUM "CONCENTRATION" OF *m*-ALKOXY PHENOLS THAT CAN BE APPLIED WITHOUT ELICITING A MICROCALORIMETRIC RESPONSE COMPARED TO THE EXPERIMENTAL SATURATING SOLUBILITY (mM)

Phenol	Maximum concentration (mM)	Saturating aqueous (mM)
parent structure	450.7	-
m-methoxy	447.3	311.0
m-ethoxy	443.9	100.3
m-propoxy	440.5	25.9
m-butoxy	437.1	8.24
m-pentoxy	433.7	2.13

"prediction" of BR in homologous series.

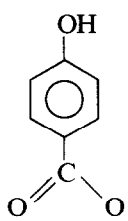
Finally, we recognize that a formal relationship between BR and structure is the basis of QSAR; here we attempt to demonstrate the nature of this relationship. Comparison of the data is shown in Table 3.

Structure *$(dose)_{max}$ (mM)*



450.7

CH_2 — for *m*-alkoxy phenols 3.4



50.3

CH_2 — for *p*-hydroxybenzoates 2.3

It is notable, but perhaps not surprising, that the parent structure of the phenol is less bioactive than is the parent structure of the *p*-hydroxybenzoate. Moreover, the contribution per CH_2 group to variation in bioactivity is different for the two homologous series. This finding presumably reflects the interaction of the hydrocarbon chain with the *m*-hydroxy group in one series and with a *p*-COOH group in the other series. Nonetheless the existence of such regularity in BR, and its evaluation, has, we think, not been noted previously. We do not illustrate here the correlation between $\log(dose)_{max}$ and other parameters such as log(partition coefficient) for transfer of these solutes between water and lipoid solvents as we have not a complete set of our own experimental data. We do, however, expect such a correlation to exist as exists for the *m*-alkoxy phenols (Beezer et al., 1980).

It is interesting to note that the 1-butyl and 2-methyl propyl substituents behave identically with respect to BR.

Finally we note again that this correlation is the formal basis of QSAR.

Acknowledgements

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