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QSAR based on biological microcalorimetry
The interaction of *Saccharomyces cerivisiae* with hydrazides[1]

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Abstract

Calorimetry has been used as a rapid method for the determination of bioactivity. Some previous studies have been concerned with its applicability to derive structure activity relationships (SARs), which can in turn guide the synthetic chemisty through the synthesis of new chemical entities with improved antimicrobial potencies. However, earlier studies do not point out the use and importance of calorimetry in deriving *quantitative* structure activity relationships (QSARs), a field where it is possible not only to get information on SARs, but insight into modes of action can also be envisaged. In this paper we describe a QSAR based on biological microcalorimetry for a set of antimicrobial hydrazides acting against *Saccharomyces cerivisiae*. Results show that an extrathermodynamic relationship can be drawn based upon microcalorimetrically measured biopotencies and partitioning (log P_{TA}) using the same cell system.

Keywords: QSAR; Biological microcalorimetry; *Saccharomyces cerivisiae*

1. Introduction

QSAR studies may rely upon the correctness of *quantitative* measurement of drug potencies, that generally starts with in vitro screening [1]. One of the most widespread way of dealing with this is by the classical method of agar diffusion. However, there is a number of disadvantages [2,3] regarding the quantitative value since the activity process cannot be taken only as a mean of interaction with cells, but mainly with problems related to the diffusion process itself. In order to circumvent such problems we have developed a method of screening drugs using *biological microcalorimetry* [4–6] to derive quantitative biological potency values. In this method materials are taken in solution resembling the live biological cell system. Log $1/D_{50}$, where D_{50} is the dose to inhibit 50% of growth or cell metabolism, is taken as potency. The classical way of dealing with parameters for drug interaction with cellular systems is to derive a lipophilicity measurement (partition coefficient, P). Thus, we have also developed a technique, using the diffusion process of Taylor–Aris, in order to assess the partitioning, called here log P_{TA} (TA standing for Taylor–Aris), in the same cells used for the biological

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screening [7]. The great advantage of this technique is that the partition is between the cell systems and the medium containing set of drugs. This technique can be applied to different cell systems and extrathermodynamic equations can be drawn to successfully develop a mathematical (QSPR/QSAR) model directly from them, not from ''models'' that may mimic biological membranes [7].

In this paper we show an established QSAR between hydrazide potencies against *Saccharomyces cerivisiae* and log P_{TA}. This is, to our knowledge, the first time a QSAR is based upon microcalorimetyrically measured bioactivity.

2. Experimental

The study compounds were hydrazide antimicrobial agents (whose synthesis will be reported elsewhere [9]) and are shown below

ArCONHNHCOC$_6$H$_4$

where **Ar**=C$_6$H$_5$, *p*-Me–C$_6$H$_4$, *p*-Br–C$_6$H$_4$, *p*-*t*-But-C$_6$H$_4$, *p*-Pent-C$_6$H$_4$, *p*-C$_6$H$_5$–C$_6$H$_4$, *p*-MeO–C$_6$H$_4$, *p*-O$_2$N–C$_6$H$_4$.

Solutes were introduced at the beginning of a dispersion tubing (7.5 m length of stainless steel capillary, coiled in a 15 cm diameter helix and placed in a temperature controlled (±0.1 K) water bath. The temperature of the water bath was kept at 298.15±0.1 K) via a Rheodyne HPLC injector fitted with a 10 μl loop and were detected with a Diode-Array detector (Hewlett-Packard HP8455), working in the same way as normal HPLC systems. Measurements of retention time and peak width at half height of the dispersion curve were made at the computer. At least two replicates were made for each solute. Standard deviations (calculated from six replicates of some experiments) ranged from 1.5% to 2.0% [7]. *S. cerevisiae* was used as ''bioorganic solvents''. *S. cerevisiae* had previously been prepared [8] and was stored in liquid nitrogen. Ampoules of the cells were removed from the liquid nitrogen as required.

3. Results and discussion

First of all, it has to be pointed out that biological microcalorimetry can, in principle, deal with small differences in potencies of a related set of compounds. The requirement is that for a set of congeneric molecules which incorporate a small substructural modification with same pharmacophoric group it must be capable of disclosing any structure activity relationship [10]. Bearing this in mind we have next to deal with the conventional log (dose-response) curves that relate to a well-defined behavior within the set. This means that for such similar compounds the mode of action has to be demonstrated to be essentially the same; this is illustrated to be so as shown in Fig. 1 that represents a composite diagram for the eight studied hydrazides (whose structures are displayed in the legend to Table 1). Table 1 also shows their measured potencies and the related statistical parameters. Table 2 displays the Taylor–Aris partitioning parameters [7]. From Tables 1 and 2 and Fig. 1 Eq. (1) can be derived. Eq. (1) states that there is a linear relationship between log $1/D_{50}$ and log P_{TA} for *S. cerevisiae*. A negative slope for SARs involving *S. cerevisiae* has not been found before. Nevertheless, it discloses a hydrophilic interaction between hydrazides and the cells, instead of a hydrophobic interaction, that normally plays a role in the partitioning process [11,12].

Linear dependence of log $1/D_{50}$, for *S. cerevisiae*, versus log P_{TA}:

$$\log 1/D_{(50)\ S.\ c} = -1.223(\pm 0.67)\log P_{TA} + 2.673(\pm 0.49), \quad (n = 8;\ r = 0.878;\ s = 0.141;\ F = 20.147;\ r^2_{cv} = 0.532). \qquad (1)$$

Eq. (1) is a linear activity–lipophilicity relationship of the type log $1/D_{50}=a$ log$P_{TA}+b$, where log $1/D_{50}$ is the potency taken from Table 1 in such a way that larger potency values are found for more active compounds, and log P_{TA} is the dependence on log P, from the Taylor–Aris method, i.e. the logarithm of the experimentally determined partition coefficient for transfer of the drugs from a culture medium to the cellular system itself, not in models [7,9].

From the regression parameters displayed in Table 1, log $1/D_{50}$ can be calculated when the response is 50%, i.e. when cell respiration is diminished by 50%, i.e. a 50% ''cell kill''. The potencies so calculated allow a much better scaling and contribution term to elicit biological responses for a set of related compounds. In terms of quantitative measure-

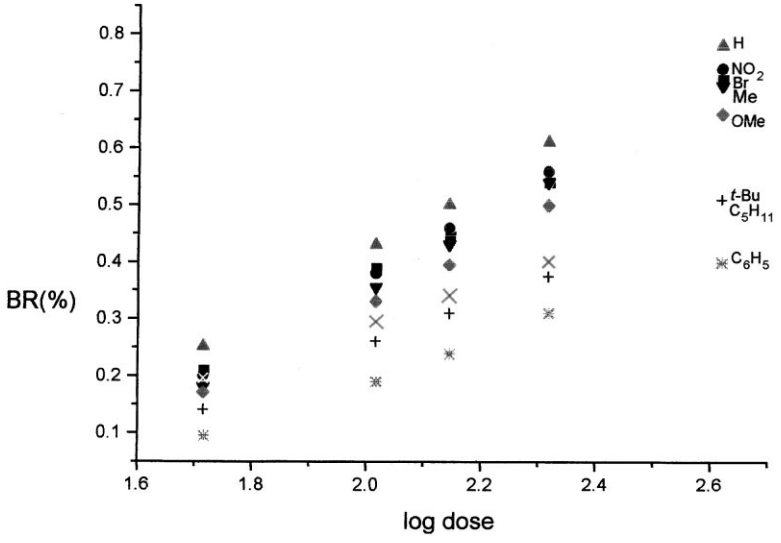


Fig. 1. Composite plot of biological response (BR) versus log dose for *S. cerevisiae*: the potency order for acylhydrazines against *S. cerevisiae* is: H>NO_2>Br>Me>OMe>*t*-Bu>C_5H_{11}>C_6H_5.

ments the contribution to biological activity of the varied constituent group of the active drug molecule can be calculated more accurately.

There is no novelty in a linear dependence upon log *P*. However, this dependence is not universally true. Nevertheless, the described model in Eq. (1) constitutes the first example of such a relationship in a cell system. The slope is almost 1, i.e. there is a simple linear relationship between drug potencies and partitioning.

The process by which chemicals enter into cells is by no means clearly understood.

There are three generally accepted pathways by which chemicals enter:

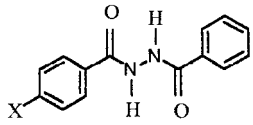
1. passive diffusion across the membrane (cell wall),
2. active transport by an energy-dependent system, and
3. diffusion through special channels.

The first, appears to be favored by increasing hydrophobicity in a set of congeners, and this is a common way of describing chemicals entrance to cells. The results shown here so far indicate that the negative slope of Eq. (1) either implies drug molecules being completely hydrated or deeply located into a hydrophilic cavity or groove of a biomacromolecule. The diffusion process could then take place through special channels, and a possible explanation for this may rely upon proteins that are commonly used for such events and they are known as porins [13]. However, this seems not plausible within this cell system since *S. cerevisiae* does not have porins on its biological membrane, but may have on mitochondrial porins [14,24].

On the other hand, one have to bear in mind that in order to gain access to the cytoplasm a small molecule would have to go through by endocytosis. Though it is not clear what it triggers, it can be argued that a compound can cross one membrane by endocytosis and a next membrane by another process. From the cytoplasm the drugs could then get into mitochondria via the porins. Nevertheless, in this case one may assume that the transport through the cell wall and plasma membrane to get to the cytoplasm is accomplished with no energy requirements. According to this assumption regarding Eq. (1), we may then suggest that potencies for the study hydrazides are dependent on their transportation through the ''pore'' porins towards the inner-mitochondria. Yet, in order to further this investigation in our laboratory we are developing new experiments, and the results shall be disclosed elsewhere.

Partitioning processes are usually studied through solvent models, e.g. octanol/water, or through more

Table 1
Statistical parameters for the interaction of hydrazides against *S. cerevisiae* and their microcalorimetric potencies



No.	X substituent	Dose (μmol/l)	BR (%)	Log D_{50}	Slope	Intercept	r	s	F	r^2_{cv}
1	*p*-Br	2.619	57	2.473	0.495	−0.724	1.000	0.005	4134.12	0.997
		2.318	42							
		2.145	34							
		2.017	28							
		1.716	12							
2	*p*-NO_2	2.619	74	2.216	0.598	−0.825	1.000	0.002	50 955.32	1.000
		2.318	56							
		2.145	46							
		2.017	38							
		1.716	20							
3	H	2.619	78	2.140	0.588	−0.758	1.000	0.004	10 560.00	0.999
		2.318	61							
		2.145	50							
		2.017	43							
		1.716	25							
4	*p*-Me	2.619	71	2.26	0.590	−0.833	1.000	0.004	10 608.50	0.999
		2.318	54							
		2.145	43							
		2.017	35.5							
		1.716	18							
5	*p*-OMe	2.619	66	2.326	0.545	−0.768	1.000	0.005	5954.23	0.999
		2.318	50							
		2.145	39.5							
		2.017	33							
		1.716	17							
6	*p*-*t*-Bu	2.619	45	2.753	0.389	−0.571	1.000	0.004	4984.30	0.999
		2.318	33							
		2.145	26							
		2.017	21							
		1.716	10							
7	*p*-C_5H_{11}	2.619	50	2.620	0.345	−0.403	1.000	0.005	6205.57	0.999
		2.318	40							
		2.145	34							
		2.017	29							
		1.716	19							
8	*p*-C_6H_4	2.619	40	2.900	0.342	−0.492	0.999	0.006	1458.95	0.952
		2.318	31							
		2.145	24							
		2.017	19.5							
		1.716	9.5							

r=Regression coefficient; s=standard error of estimation; F=the F-value; r^2_{cv} = squared correlation coefficient of predictions by ''leave-one-out'' procedure.

Table 2
Experimental Taylor–Aris data for *S. cerevisiae*

	X=H	X=Me	X=Me_3C	X=C_5H_{11}	X=C_6H_5	X=NO_2	X=MeO	X=Br
D_w	9.940	12.240	17.500	21.150	16.980	10.879	9.860	13.470
D_{obs}	8.814	10.505	13.420	15.500	13.129	9.768	8.719	11.200
f	0.113	0.142	0.233	0.267	0.227	0.102	0.116	0.168
P	0.127	0.165	0.304	0.364	0.293	0.114	0.131	0.202
Log P_{TA}	−0.894	−0.782	−0.516	−0.439	−0.532	−0.944	−0.833	−0.693

Adpated from [7]: D_w, observed diffusion for a buffered solution; D_{obs}, observed diffusion for a suspension of *S. cerevisiae*; f, fraction of organic molecules present in the particulate phase; *P*, partition coefficient for Taylor–Aris measurement.
For structures, see Table 1.

structured systems like micelles and liposomes [13–18]. The π_{oct} lipophilic constant for octanol/water system can be used to derive the importance of substructural contributions to the biopotencies of drugs [10]. Thus, we have attempted the description of some antimicrobial hydrazides through this physico-chemical descriptor and Eq. (2) shows a clear dependence on π_{oct}. Though Eq. (2) does not have the same statistical power of Eq. (1), there is a clear difference in the slopes, i.e. in the coefficient of log P_{TA} and in π_{oct}. but, nevertheless, both of those slopes are negative. The difference in the slopes does indicate the differing character of the non-polar phase in each case, i.e. it seems that the lipid phase in the cellular system is more hydrophilic than that in the octanol/water system.

Linear dependence of log $1/D_{50}$, for *S. cerevisiae*, versus π:

$$\log 1/D_{(50)\ S.\ c} = -0.210(\pm 0.13)\pi_{oct} + 3.745(\pm 0.18), \quad (n = 8;\ r = 0.853;\ s = 0.153;\ F = 16.073;\ r^2_{cv} = 0.382). \qquad (2)$$

One of the common problems in dealing with physico-chemical descriptors is related to the existence of a matrix correlation between them. The π_{oct} lipophilic constant is often collinear with molar refractivity, MR, (r^2=0.876) [9]. Eq. (3) shows that a linear relationship exists between log $1/D_{50}$ and MR. However, π_{oct} and MR cannot be separated and certainly they describe the same trend. Nevertheless, the slope is still negative and its meaning is that the less bulky is the substituent the more potent it is. Thus, Eq. (3) incorporates a further improvement to the model by including the substituent's size, i.e. substituents must not only have hydrophilic character, but must also be small.

Linear dependence of log $1/D_{50}$, for *S. cerevisiae*, versus π:

$$\log 1/D_{(50)\ S.\ c} = -0.273(\pm 0.11)(0.1)\mathrm{MR} + 3.883(\pm 0.17), \quad (n = 8;\ r = 0.926;\ s = 0.111;\ F = 36.66;\ r^2_{cv} = 0.712). \qquad (3)$$

In QSAR studies the determination of biological descriptors, i.e. quantitative drug potencies, can be crucial since it can be time-consuming and not truly *quantitative*. Thus it may be a ''bottle-neck'' which can, of course, obstruct model development. This seems not to be true for biological microcalorimetrically measured biopotencies, as can be seen from Figs. 2 and 3, where the experimental bioassay time required is only ca. 30 min! Moreover, the potential for automation and hence its application to high throughput screening (HTS) can also be envisaged. This is a significant challenge to pursue in this field [19,20]. Nonetheless, it has to be pointed out that in order to carry out an experiment in only 30 min runtime procedures must be available – particularly for instance the cell system. Of course, this is now not a problem since the development of frozen storage cells is well established [21–23]. However, it should be noted that what is needed in biological microcalorimetry is to expand the range of new cell targets that would be needed for *mass* screening.

Fig. 2 shows that the interaction of the study hydrazide antimicrobials can be ranked in potency according to substitution at the 4-position of the aryl ring. It reveals a relationship between biological activity and the Gibbs function of binding (or partitioning) in terms of ''extrathermodynamic relationships'' that can then be established through Eqs. (1)–(3), above. Fig. 3 shows, too, that these interactions resulted in a graded

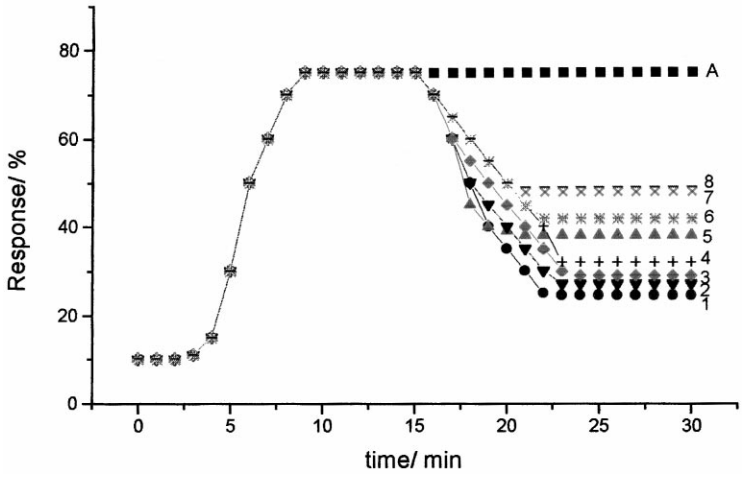


Fig. 2. Microcalorimetric outputs observed for interaction of all hydrazides with *S. cerevisiae*: A, control; dose (μmol/l): 1–8, 2.619. All compounds show the same trend towards the ''cell killing''. The total ''killing concentration'' is only achieved with higher doses, but just for the most hydrophilic drugs.

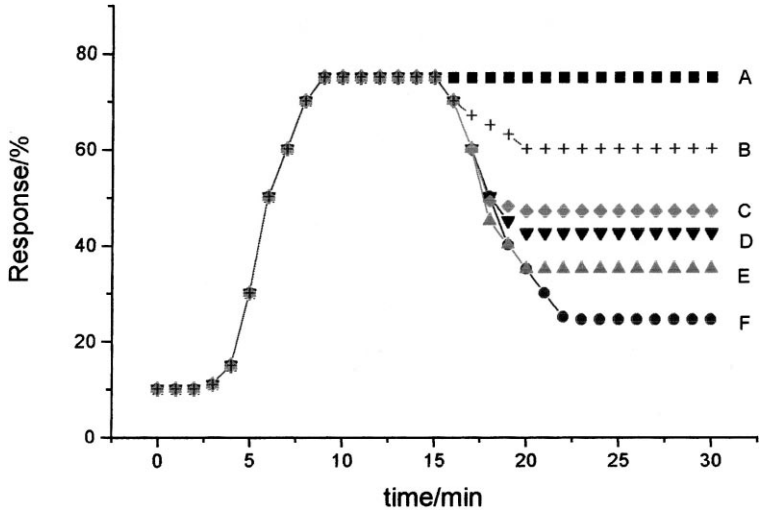


Fig. 3. Microcalorimetric outputs observed for interaction of hydrazide 3 with *S. cerevisiae*: A, control; doses (μmol/l): B, 1.716; C, 2.017; D, 2.145; E, 2.318; F, 2.619. Although the graded response is concentration dependent, none leads to total ''cell killing'' at the time of 30 min.

response according to drug concentrations. Though this is not a surprise because this dependence has already been found, it does indicate the concentration time-related response for such small subunit moiety alterations.

The success of QSAR analyses shown in Eqs. (1)–(3) constitute evidence for the additivity of group contributions to biological activity values.

4. Conclusions

For the first time we have shown that log P_{TA} and biological microcalorimetry can be used to derive a QSAR. This seems to be a good alternative to the octanol/water system largely because the cell suspension is more ''real'' – a better representation of a natural system, and microcalorimetry is a promising

tool for such QSAR studies. Moreover, another advantage of the Taylor–Aris approach is that the cells are not exposed to the harmful affects of bulk organic media, since this is a non-intrusive method [25].

The quantitative measurement of drug potencies through the QSAR in Eq. (1) does establish a guide to drug design through consideration of the detailed mechanism for such an interaction, hence allowing a better insight into the nature of the biological cell membrane as a solvent. Nevertheless, it is also important to note that the hydrophobicity between the lipoid phase of the cells is quite different from that represented by the octanol/water system.

Biological microcalorimetry is efficient, fast, and reproducible to better than 3%. It can be used instead of other techniques like agar diffusion or tube assays (serial dilution). In vitro screening can be performed in complex and defined medium using frozen cells. Calorimetric output can reveal biocide and biostatic compounds directly, and this is very important in order to control drug doses.

Overall, biological microcalorimetry can be applied to screening and for sensitive differentiation between related compounds, thereby generating a good quantitative biological descriptor. Log P can be used directly from the same biological systems through the Taylor–Aris diffusion technique, and microcalorimetry can also give an insight into the mode of action of antimicrobial drugs.

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