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International Journal of Pharmaceutics 282 (2004) 163–171
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Flow microcalorimetric studies of phenol and its chlorinated derivatives and a theoretical evaluation of their possible inhibition mode on *Chromobacterium violaceum* respiration

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Received 17 December 2003; received in revised form 9 June 2004; accepted 9 June 2004

Available online 31 July 2004

Abstract

The general belief that chemical structure determines the biological effect of drugs has led to several techniques to establish structure–activity relationships (SAR) that is useful in the development of more active compounds. Predicting toxic effects based on SAR, one can obtain toxicological data with a low cost–benefit ratio. Chlorophenols that represent a class of toxic agents frequently used in industrial processes are not satisfactorily described in the literature in relation to their toxicity. The main objective of this work is to relate the microbial activities of phenol, anisole and their chlorinated derivatives on *Chromobacterium violaceum* respiration with their physicochemical properties. Anisole and its chlorinated derivatives were used to evaluate the influence of phenol acidity on biological activity. The calculations were carried out at the semi-empirical AM1 and ab initio DFT levels employing the basis sets CEP-31G, CEP-31+Ge CEP-31G** that were parameterized using the continuum-solvation model COSMO for solvent contribution. Both empirical and theoretical properties were evaluated by chemometric analyses (hierarchical cluster analysis (HCA) and principal component analysis (PCA)), to correlate the physicochemical properties of the phenol, anisole and their chlorinated derivatives with their biological activities. The results obtained for the current work indicate that the biological activities of these compounds increase as the *n*-octanol/water (log *P*) partition coefficients, ionization energies (IP), melting points (mp) and dissociation constants increase and the solvent effects (SE), enthalpies of formation ($\Delta_f H^\circ$) and proton affinities (PA) decrease.

Keywords: Inhibition effect; Phenol, anisole and their chlorinated derivatives; *Chromobacterium violaceum* respiration; Flow microcalorimetry; Theoretical method; Chemometric analysis; SAR

1. Introduction

Chlorophenols (ClPs) are used world-wide as broad spectrum biocides and their residues and breakdown

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doi:10.1016/j.ijpharm.2004.06.014

products are ubiquitous in air, water, sediments and organisms (Devillers and Chambon, 1986; IPCS, 1989). All of these compounds are active antimicrobial agents to various degrees (Gonzalez and Hu, 1991).

Following our previous work and experience on biological flow and batch microcalorimetry (Beezer et al., 1986a, 1986b, 1986c; Volpe, 1997; O'Neill et al., 2004; Critter et al., 2004) we now report results of an in vitro study, in real time, of the antibacterial activity of phenol and anisole and their chlorinated derivatives (ClPs) and (ClAs): *p*-chlorophenol (4-MClP) and *p*-chloroanisole (4-MClA); 3,4-dichlorophenol (3,4-DClP) and 3,4-dichloroanisole (3,4-DClA); 2,4,6-trichlorophenol (2,4,6-TClP) and 2,4,6-trichloroanisole (2,4,6-TClA); 2,3,4,5-tetrachlorophenol (T4ClP) and 2,3,4,5-tetrachloroanisole (2,3,4,5-T4ClA); pentachlorophenol (PClP) and pentachloroanisole (PClA) on the respiration of *Chromobacterium violaceum* using a flow microcalorimetric technique.

C. violaceum is a Gram-negative, rod-shaped bacterium. It is a common saprophyte found in water and soil from tropical and subtropical regions of the world, and is generally considered to be non-pathogenic (Sneath, 1984), despite the fact that a few cases of fatal septicemia caused by *C. violaceum* have been reported (Kaufman et al., 1986; Ponte and Jenkins, 1992). In Brazil, large amounts of *C. violaceum* are found in the waters and in the soil of the banks of the Negro river, one of the largest affluent of the Amazon river (Caldas, 1977).

Flow microcalorimetry has been proposed as a useful tool for monitoring microorganism growth and respiration (Beezer, 1980). The heat output of the microcalorimeter, dq/dt (μW) versus time (min), a response–time curve, provides a continuous record of the progress of the metabolic process which, in contrast to many classical procedures, responds in real time to the metabolic activity of the only active microorganism present and thus displays details of the dynamic and complex process of respiration. The advantages cited are sensitivity, reproducibility, real time monitoring and an improvement in "time-per-test", when compared to usual bioassay procedures (Beezer, 1980). An advantage possessed by flow microcalorimetry is that there is no requirement for optically clear solutions. Indeed, suspensions of cells pass through the calorimetric measuring chamber without difficulty. This ability of flow microcalorimeters to follow the details of metabolism has, naturally, been extended to antibacterial activity (Volpe, 1997), drug–cell interactions (Beezer et al., 1986a), bioassay (Beezer et al., 1986b) and to many other pharmaceutical applications (Koenigbauer, 1994).

In this investigation, the main objective is to relate the inhibition effect of ClPs and CIAs on the respiration of *C. violaceum* with some physicochemical properties: solvent effect (SE), ionization energy (IE), enthalpy of formation ($\Delta_f H^\circ$), proton affinity (PA), melting point (mp), dissociation constant increment of phenol pK_a less the pK_a of the phenol derivative compounds (ΔpK) and the partition coefficient in *n*-octanol/water ($\log P$). The present study is based on earlier studies in which acidic effects were assumed to be the dominant factor in governing the microbial activities of the chlorophenol derivatives (Rutgers et al., 1998).

Anisole and its chlorinated derivatives, *p*-chloroanisole, 3,4-dichloroanisole, 2,4,6-trichloroanisole, 2,3,4,5-tetrachloroanisole and pentachloroanisole were investigated to verify the influence of phenol acidities on the biologic activity. The microcalorimetric data were complemented by semi-empirical and ab initio calculations. Both empirical and theoretical properties were evaluated through hierarchical cluster analysis (HCA) and principal component analysis (PCA) to correlate the physicochemical properties with biological activities.

2. Experimental

2.1. Reagents

All chemicals used such as glucose (Hoescht) and ammonium sulphate (Baker) were reagent grade. Phenol, anisole, the chlorophenols and the chloroanisoles were purchased from Acros and were used without purification.

2.2. Bacterial culture

C. violaceum CCT3496 was grown in a 1.50 dm^3 reaction flask (B. Braun Biotech., Biostat B2) containing a sterilized culture medium of composition: 3.0 g dm^{-3} yeast extract, 7.5 g dm^{-3} glucose and 7.5 g dm^{-3} bacteriologic peptone in distilled water in sterilized phosphate buffered solution (PBS) composed

of 8.0 g dm^{-3} NaCl, 0.20 g dm^{-3} KCl, 1.15 g dm^{-3} Na_2HPO_4 and 0.20 g dm^{-3} KH_2PO_4, with a final pH of 7.0. One flask was inoculated with 1.0 cm^3 of *C. violaceum* and incubated at 298 K on a rotary shaker (200 rpm; Gallenkamp, UK). After 14 h of incubation, the cells were separated from the culture medium by centrifugation (4000 rpm per 20 min). The cells were washed three times by suspending in PBS solution and then centrifuged. After the last centrifugation, the cells were suspended again in 100 cm^3 of PBS solution containing 10% DMSO and conditioned in 1.0 cm^3 cryogenic polypropylene ampoules (Corning).

The ampoules were inserted into a thin perforated Styrofoam plate which was placed 8 cm above the liquid nitrogen level in an aluminum container for liquid nitrogen. When the temperature in the control ampoule reached 193 K (measured with a solvent thermometer), the ampoules were immersed in liquid nitrogen and stored in a cryogenic cylinder (Kirsop and Snell, 1984). A viable count, which was performed periodically, gave 1.3×10^{10} cells cm^{-3}. The cells were stored for 6 months and recovered with 95% viability.

2.3. Calorimetry

The calorimetric response (CR) is defined by comparing the maximum height of the calorimetric power (dq/dt) versus time curve for each compound with the maximum height of a control curve (100% CR) (Beezer, 1980; Beezer et al., 1986c). The CR was determined by a Thermal Activity Monitor-2277 microcalorimeter (Thermometric AB, Sweden) at 298.15 ± 0.02 K, fitted with a flow-through cell. The media was pumped from a Biostat B2 bioreactor into the microcalorimeter (30 cm^3 h^{-1}) by a P-1 Pharmacia Biotech peristaltic pump. The microcalorimeter calibration was verified by determining the enthalpy of reaction of tris(hydroxymethylamino)methane in 0.1 mol dm^{-3} hydrochloric acid. The result (−29.69 ± 0.04 kJ mol^{-1}) is in agreement with the value recommended by IUPAC (−29.763 ± 0.003 kJ mol^{-1}) (Herrington, 1974).

The composition of the culture media used for the respiration of *C. violaceum* was: 1.80 g dm^{-3} glucose, 8.75 g dm^{-3} K_2HPO_4 and 1.88g dm^{-3} KH_2PO_4, at pH 7. Without the compound addition, this media was defined as a control preparation. The inoculation of microorganisms (900 μL of the cell suspension into the bioreactor) was always done immediately after thawing the ampoule for 3 min in a water bath at 310 K, followed by agitation of 20 s.

The flow microcalorimeter cannot be sterilized by conventional methods but can be decontaminated by successively pumping solutions. After each respiration experiment, the flow cell and Teflon tubes were rinsed with the following solutions for 40 min: sodium hydroxide (0.10 mol dm^{-3}), hydrochloric acid (0.10 mol dm^{-3}), water, sodium dodecyl sulphate (8.0 mmol dm^{-3}), and finally, sterile deionized water.

3. Theoretical

3.1. Theoretical calculations on the physicochemical properties

Physicochemical properties of phenol, anisole, the chlorophenols and the chloroanisoles are calculated in the gas phase using quantum chemical methods at ab initio level of computation at the Hartree–Fock and B3LYP levels, employing CEP-31G, CEP-31+G and CEP-31G** basis sets and modeling their atomic basis set conjunctions by Stevens–Basch–Krauss compact effective pseudopotential (CEP). Such procedures reduced considerably the computational demands when compared with calculations employing all electrons (Stevens et al., 1984).

Proton affinity (PA) and ionization potential (IP) were calculated as the differences in absolute enthalpies of reactions that characterize these processes:

$$\text{ionization energy}: \quad A_{(g)} \rightarrow A_{(g)}{}^{+} + e^{-};$$

$$IP = H(A^{+}) - H(A)$$

$$\text{proton affinity}: \quad HA_{(g)} \rightarrow A_{(g)}{}^{-} + H_{(g)}{}^{+};$$

$$\Delta H = H(H^{+} + A^{-}) - H(HA)$$

In the last expression: $H(X) = E_{electr} + E_{vib} + E_{rot} + E_{trans} + nRT$, where E_{eletr} is the electronic energy which was computed by the structural optimization for both neutral and anionic species via density functional theory (DFT) levels employing the CEP-31G and CEP-31+G basis set. E_{vib} is the vibrational energy, E_{rot} is the rotational energy and E_{trans} is the translational energy, all of which were computed at the Hartree–Fock level employing the CEP-31G basis.

The enthalpy of formation ($\Delta_f H^\circ$) was calculated directly by the quantum chemical method AM1. Solvent effect was obtained through the COSMO method (Klamt and Jonas, 1996), invoking quantum chemical methods at ab initio B3LYP level and employing CEP-31G** basis sets. The free energy of solvation ΔG can be calculated as: $\Delta G = (E + \Delta G_{ne}) - E^\circ$, where E° is the total energy of the molecule in a vacuum and E is the total energy of the molecule in the solvent. ΔG_{ne} is the non-electrostatic contribution from dispersion and cavity formation effects (Ben-Naim and Marcus, 1984). All the calculations were carried out with the Gaussian 98 program (Frisch et al., 1998). The *n*-octanol/water partition coefficients (log *P*), the dissociation constant increments relative to phenol (pK_a of phenol minus pK_a of phenol derivatives (ΔpK)) and the melting points (mp) were obtained from the literature (Saito et al., 1991; Devillers and Chambon, 1986; Mulller et al., 1986).

3.2. Chemometric methods

Structure–activity relationships (SAR) were determined by hierarchical cluster analysis and principal component analysis (Sharaf et al., 1986; Cronin and Schultz, 1997; de Souza et al., 1999).

3.2.1. Notation

Boldface letters represent matrices (upper case) and column vectors (lower case), such as $\boldsymbol{X}$ and $\boldsymbol{x}$. Scalars are represented by lowercase italic letters, such as *s* and *d*. Transposition of a matrix is symbolized by a superscripted "T", $\boldsymbol{X}^T$, and indices for matrix and vector dimensions are indicated by uppercase italic letters, such as *I* and *J*, are indicated by lowercase italic letters *i* and *j*.

3.2.2. Principal components analysis

Principal component analysis is a data compression method based on the correlation among variables (Beebe et al., 1998). Its aim is to group those correlated variables, replacing the original descriptors by a new set called principal components (PC) onto which the data are projected. These PC are completely uncorrelated and are built as a simple linear combination of the original variables. In mathematical forms, PCA is represented by Eq. (1), where the data matrix $\boldsymbol{X}(I \times J)$, corresponding to *I* molecules and *J* chemical properties, is decomposed into two matrices, $\boldsymbol{T}$ and $\boldsymbol{P}$, such that:

$$\boldsymbol{X} = \boldsymbol{T}\boldsymbol{P}^T \quad (1)$$

The matrix $\boldsymbol{T}$, known as the "score" matrix, represents the positions of the compounds in the new coordinate system where the PC is the axes. $\boldsymbol{P}$ is the "loading" matrix whose columns describe how the new axis, i.e., the PC is built from the old axes.

The PC contains most of the variability in the dataset, albeit in a much lower dimensional space. The first principal component, PC1, is defined in the direction of maximum variance of the whole dataset. PC2 is the direction that describes the maximum variance in the subspace orthogonal to PC1. The subsequent components are taken as orthogonal to those previously chosen and describe the maxima of the remaining variances. Once redundancy is removed, only the first few principal components are required to describe most of the information contained in the original dataset.

3.2.3. Hierarchical cluster analysis

Hierarchical cluster analysis has become, together with principal components PC, another important tool in multivariate data analysis (Beebe et al., 1998). Its primary purpose is to display the data in such a way as to emphasize its natural clusters and patterns. The results, which are qualitative in nature, are presented in the form of a dendogram allowing one to visualize the samples or variables in two-dimensional space. The distances between samples or variables are calculated and transformed into a similarity matrix $\boldsymbol{S}$ whose elements are the similarity indexes (Eq. (2)). For any two samples *k* and *l*, the similarity index is defined as:

$$S_{kl} = 1.0 - \frac{d_{kl}}{d_{max}} \quad (2)$$

where S_{kl} is an element of $\boldsymbol{S}$, d_{max} is the largest distance for any pair of samples in the dataset. d_{kl} is the Euclidean distance between samples *k* and *l*. The similarity scale ranges from zero to one. It is clear that the larger the index S_{kl}, the smaller the distance between *k* and *l*. Therefore, S_{kl} directly reflects their similarity.

An important part of this investigation was to determine the relationships between the inhibition effects of phenol, anisole and their chlorinated derivatives on *C. violaceum* respiration and the structural characteristics related to the physicochemical properties. For that,

PCA and HCA analysis were applied to the theoretical and experimental physicochemical data (PA, IP, $\Delta_f H^\circ$, SE, log P, ΔpK and mp).

4. Results and discussion

The calorimetric responses, CR, of *C. violaceum* respiration the addition of phenol, anisole and their chlorinated derivatives are listed in Table 1. The CR values show that inhibition effects increase with the degree of chlorination of the phenol ring, presumably as a result of increasing lipophilicity and acidity of these compounds. The experimental calorimetric heat output records are presented in Fig. 1, which shows, in real time, the effect of the increasing degree of chlorination of phenol, anisole and their chlorinated derivatives on the respiration process of *C. violaceum*. The power-time curves in Fig. 1 and the CR values in Table 1 gives the sequence for the phenolic compounds: PClP (6.2 μW) > 2,3,4,5-T4ClP (7.3 μW) > 2,4,6-TClP (16.4 μW) > 3,4-DClP (28.9 μW) > 4-MClP (67.8 μW) > phenol (72.5 μW). The CR values for anisole and chloroanisoles in Table 1 indicate that these compounds are less efficient for inhibition of *C. violaceum* respiration, but data indicate the same trends: 2,3,4,5-T4ClA (53.3 μW) > 2,4,6-TClA (66.8 μW) ≅ 3,4-DClA (67.7 μW) > 4-MClA (70.9 μW) > anisole (86.2 μW). The value of CR for PClA (69.9 μW) is an intermediate one. By comparing the CR values for anisole and phenol series, the highest acidic results for phenol derivatives gave a high toxicity effect, as shown through listed data in Table 1.

Table 1
Calorimetric response (CR) of phenol, anisole and chlorinated derivatives on the respiration of *C. violaceum* compared with control in which the maximum value of (CR) represents 100% of the microcalorimeter baseline deflection

Molecule	CR (μW)	Percentage
Control	92.2	100
Phenol	72.5	79
Anisole	86.2	93
4-MClP	67.8	74
4-MClA	70.9	77
3,4-DClP	28.9	31
3,4-DClA	67.7	74
2,4,6-TClP	16.4	18
2,4,6-TClA	66.8	73
2,3,4,5-T4ClP	7.3	8
2,3,4,5-T4ClA	53.3	58
PClP	6.2	7
PClA	69.6	76

The discrepancy in the value of CR of PClA led us to raise the hypothesis that since PClA is a very hydrophobic molecule, it could adsorb on the Teflon tubing of the microcalorimeter, thus decreasing its concentration in the *C. violaceum* culture that then reflects on the CR value. An experiment was then performed in the absence of cells to verify if anisole and its chlorinated

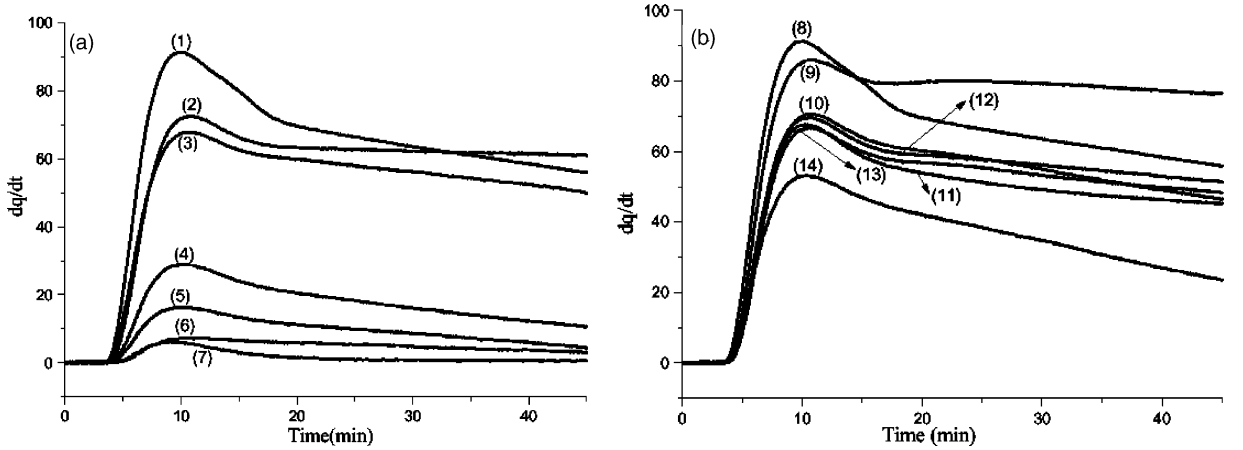

Fig. 1. Microcalorimetric response curves for (a): control (1), phenol (2), 4-MClP (3), 3,4-DClP (4), 2,4,6-TClP (5), 2,3,4,5-T4ClP (6), PClP (7); and (b): control (8), anisole (9), 4-MClA (10), 3,4-DClA (11), 2,4,6-TClA (12), 2,3,4,5-T4ClA (13), PClA (14) on the *C. violaceum* respiration. Concentration: 0.30 mmol dm^{-3}.

Table 2
Calculated and experimental values of physicochemical properties for phenol and chlorophenols: proton affinity (PA), ionization energy (IP), enthalpy of formation ($\Delta_f H^\circ$), solvent effect (SE), experimental pK_a values, experimental log P values, dissociation constant increment (ΔpK) relative to phenol is equal to 9.8 = pK_a for phenol

Compound	PA (kJ mol^{-1})		IP (kJ mol^{-1})		$\Delta_f H^\circ$ (kJ mol^{-1})	SE (kJ mol^{-1})	pK_a (experimental)[a]	log P[b]	ΔpK[c]	mp[b] (K)
	G	A	G	A						
Phenol	1408.2	1151.5	816	567	−93.1	−49.5	9.82	1.48	0.00	314
4-MClP	1373.8	1148.5	824	576	−122.6	−52.3	9.37	3.44	0.45	316
3,4-DClP	1340.6	1119.3	842	598	−145.0	−43.6	8.68	3.44	1.14	338
2,4,6-TClP	1311.3	1113.6	864	620	−160.3	−25.2	5.99	3.75	3.83	341
2,3,4,5-T4ClP	1286.3	1112.5	893	654	−181.1	−37.3	5.64	4.21	4.18	390
PClP	1274.3	1096.5	907	674	−187.2	5.1	4.74	5.07	5.07	463

G and A represent the gas and aqueous phases, respectively.
[a] Schüürmann (1998).
[b] Mulller et al. (1986).
[c] Devillers and Chambon (1986).

derivatives, especially PClA, could be adsorbed onto Teflon. However, over 50 min no change was observed on the absorbance intensity of the solutions at 327 nm.

The total of thirteen theoretical and empirical physicochemical properties of phenol, anisole and their chlorinated derivatives, which are the variables used in the PCA and HCA chemometric analysis, are shown in Tables 2 and 3. Before applying PCA and HCA methods, each one of the variables was autoscaled (Wold et al., 1987).

The results of this investigation for the phenolic species are shown in part 2 in Fig. 2. The first two PC describe 96.28% of the total variance: PC1 = 92.28% and PC2 = 3.80%. Score plots were examined and the most informative ones are shown in part 3 in Fig. 2, which shows that the first principal component against the second component (PC1 versus PC2) and also in part 4, which shows the loading vectors for PC1 and PC2.

The HCA dendrogram analysis in part 1 and PC1 analysis in part 3 in Fig. 2, discriminate among three groups: (A) low activity (phenol and 4-MClP); (B) intermediate activity (3,4-DClP, 2,4,6-TClP and 2,3,4,5-T4ClP); and (C) highly active (PClP). It can be noted from part 3 that PC1 alone is responsible for the separation among the high, intermediate and low activity compounds. Part 4 in Fig. 2 displays the loading vectors for these first two principal components, PC1 and PC2.The first principal component, PC1, has all variants with similar coefficients: PC1 = −0.38AP + 0.39IP − 0.38$\Delta_f H^\circ$ + 0.37SE + 0.39 log P + 0.38ΔpK + 0.36mp. For PC2, the total data variance also is represented by the all variants: PC2 = +0.27AP + 0.04IP

Table 3
Calculated and experimental values of physicochemical properties for anisole and chloroanisoles: ionization energy (IP), enthalpy of formation ($\Delta_f H^\circ$), solvent effect (SE). G and A represent the gas and aqueous phases, respectively

Compound	IP (kJ mol^{-1})		$\Delta_f H^\circ$ (kJ mol^{-1})	SE (kJ mol^{-1})
	G	A		
Anisole	776	581	−66.3	−32.0
4-MClA	788	591	−96.3	−31.7
3,4-DClA	808	610	−119.1	−24.4
2,4,6-TClA	818	621	−125.5	−10.6
2,3,4,5-T4ClA	829	636	−146.9	0.7
PClA	836	646	−152.6	7.5

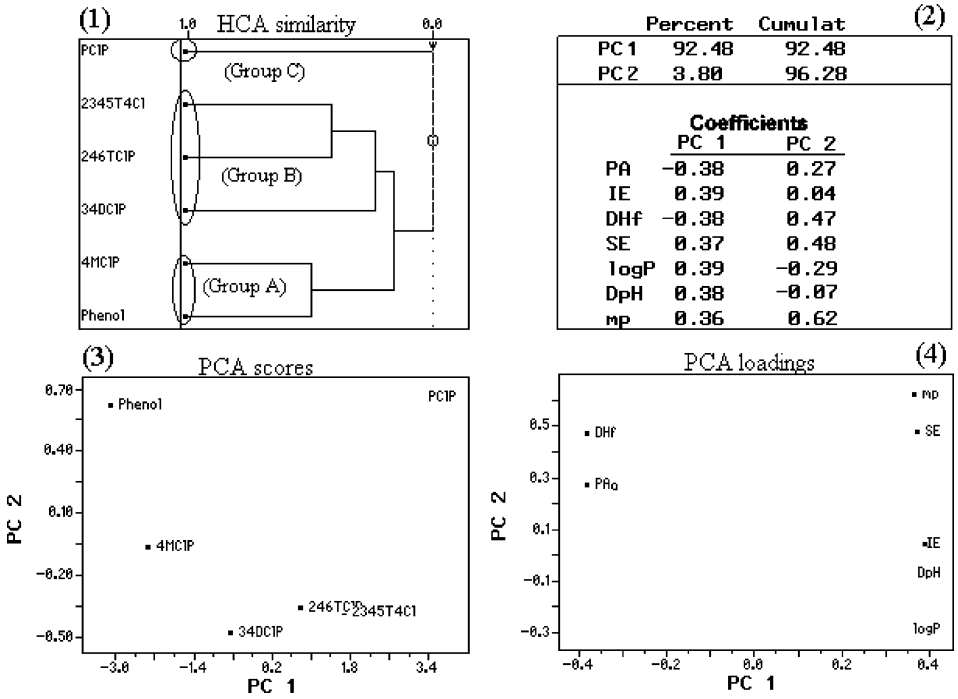

Fig. 2. HCA and PCA plots for phenols and chlorophenols: (1) dendrogram plot; (2) PC results; (3) scores plot; (4) loadings plot.

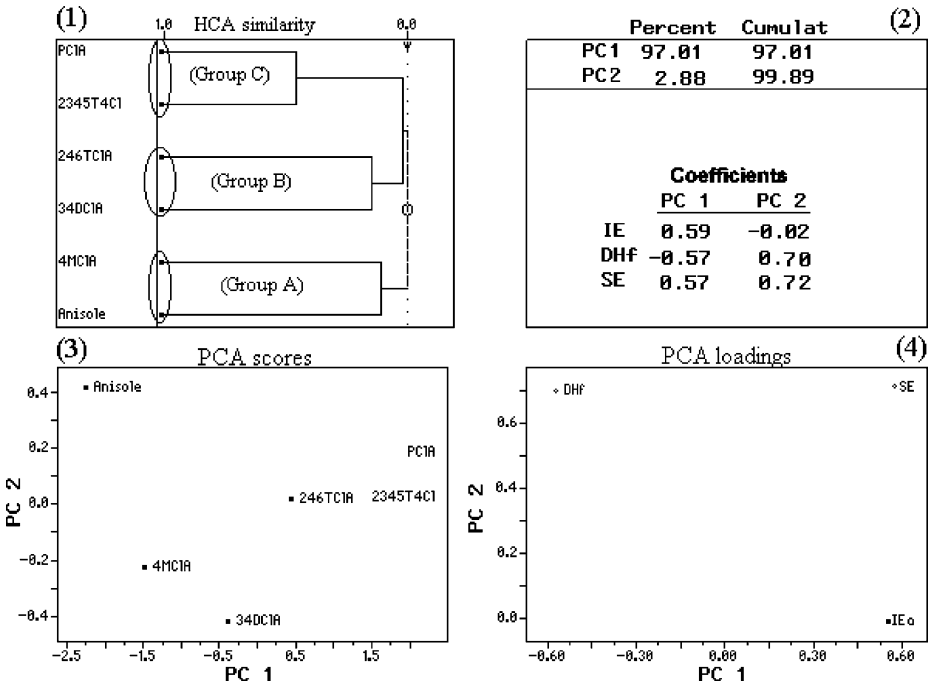

Fig. 3. HCA and PCA plots for anisole and chloroanisoles: (1) dendrogram plot; (2) scores plot; (3) loadings plot.

+ 0.47$\Delta_f H^\circ$ + 0.48SE − 0.29 log P − 0.07ΔpK + 0.62mp. However, it is dominated by the +0.62mp, the +0.48SE and the +0.47$\Delta_f H^\circ$, as shown in Fig. 2, parts 2 and 4.

Based on the results of the principal component as shown in Fig. 2, the results reveal in a convincing way how the highly active molecule (PClP) depends on all high values of log P, IE, mp, ΔpK and $\Delta_f H^\circ$ variables, which combine with small SE and PA values. These results indicate the same behavior as observed in the calorimetric response in Table 1, which divides these compounds into three distinct groups of bioactivity.

For anisole and its chlorinated derivatives, PC1 explain 97.01% of the total variance of the data and PC2 only 2.88% of the remaining variance, as shown in part 2 in Fig. 3. Analogously, the same behavior was found as for phenols, even using a small number of available variables, as shown in part 4 in Fig. 3. The PC1 scores are responsible for the separation of the high, intermediate and low activity compounds and this separation also can be seen clearly in the HCA dendrogram in part 1 in Fig. 3. On other hand, the PC1 loadings show that the variables responsible for the separation of the anisoles into three groups are IE, SE and $\Delta_f H^\circ$. The first principal component, PC1 is represented by all variants with similar coefficients: PC1 = +0.59IE − 0.57$\Delta_f H^\circ$ + 0.57SE, whereas PC2 is dominated by the +0.72SE + 0.70$\Delta_f H^\circ$. This classification, which is based on the variables of PCA and HCA, as shown in Fig. 3, reveals in a convincing way how the low activity compound (anisole) depends on small variable values for IE and SE, which were combined with a small $\Delta_f H^\circ$ value.

5. Conclusions

Microcalorimetric, theoretical, PCA and HCA methods were used to classify ClP and ClA compounds with regard to their inhibition effects on *C. violaceum* respiration into low, intermediate and highly active compounds. The acidity of phenols is one of the most important responses related to the inhibition mode on *C. violaceum*, but not a sufficient prerequisite for biological activity. Biologic activity is also determined by properties such as PA, IP, $\Delta_f H^\circ$, SE, log P and mp, which was clearly observed with the anisole compounds.

The calorimetric responses to phenol, anisole and their chlorinated derivatives showed that the inhibition effect on the respiration of *C. violaceum* increases with the degree of chlorination of the phenol and anisole rings, leading to changes in the phenol and anisole characteristics (log P, IP, $\Delta_f H^\circ$, ΔpK, mp, PA and SE). Through principal component analysis, the highly active compound PClP depends on high values of log P, IP, mp, ΔpK and $\Delta_f H^\circ$ variables, which combine with small SE and PA values. The PCA and HCA methods reveal in a convincing way how a low activity compound (anisole) depends on small values for IE and SE, which were combined with a small $\Delta_f H^\circ$ value. The behavior of the set of variables related to phenol, anisole and the respective chlorinated compounds can be useful to obtain new derivatives with high or low biologic activities, suggesting that bioactive molecules can be predicted from experimental and theoretical data.

Acknowledgments

The authors are indebted to FAPESP for financial support and a post-doctoral fellowship to MMB and to CNPq for fellowships to PLOV and CA.

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