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Analysis of the interaction of substituted coumarins with the DPPH free radical by means of multivariate statistics

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Abstract

The interaction of some substituted coumarin derivatives with the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was analysed by means of multivariate statistics using a variety of molecular descriptors. The compounds contain a conjugated double bond system, which was considered to be an essential structural characteristic for the free-radical scavenging activity. Partial least-square analysis led to an adequate two-component model based on bulk descriptors and the electronic properties concerning atoms involved or next to the double-bond system.

Introduction

Free radicals and reactive oxygen species (ROS) have been suggested to play an important role in a variety of diseases and pathophysiological events, including inflammation, cancer, myocardial infarction, arthritis and neurodegenerative disorders (Bast et al 1991; Bulkley 1993; Halliwell 1994). Therefore, considerable research interest is focused on the investigation of the antioxidant potential of active compounds as a favourable biological property and several experimental protocols have been developed for this purpose (Ratty et al 1988; Tosaki et al 1993; Dapkevicious et al 2001). Among these protocols, the reducing ability against the stable 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) offers an easy and rapid way to measure the antioxidant properties of the compounds in-vitro. This popular test has been extensively used to assess the antioxidant activity of phenols and aromatic amines (Andreadou et al 1997; Bandoniene & Murkovic 2002). Reactivity against DPPH has been reported to be related to the inhibition of lipid peroxidation (Ancerewicz et al 1998) and a number of studies have investigated this interaction (Valgimigli et al 1995). Recently, quantitative structure–radical scavenging activity relationships have been reported using CoMFA (Benabadji et al 2004) or multiple regression analysis (Amić et al 2003). In the latter case the derived equation was mainly based on indicator variables.

In this study, multivariate data statistics based on molecular descriptors was applied to analyse the interaction with DPPH of a series of 4-substituted coumarins (Figure 1).

The compounds include at least one double bond in the side chain or within a ring so that a rigid conjugated system is created, stabilized via resonance with the styryl carbonyl moiety of the coumarinic skeleton. The presence of these conjugated double bonds was assumed to be essential for the antioxidant activity of the derivatives.

Materials and Methods

The data of the reducing ability (RA) of the substituted coumarins against DPPH were taken from Nicolaides et al (1998a, b) and converted to logit values according to equation 1.

$$\text{Logit (RA)} = \log (\%\text{RA}/(100 - \%\text{RA})) \quad (1)$$

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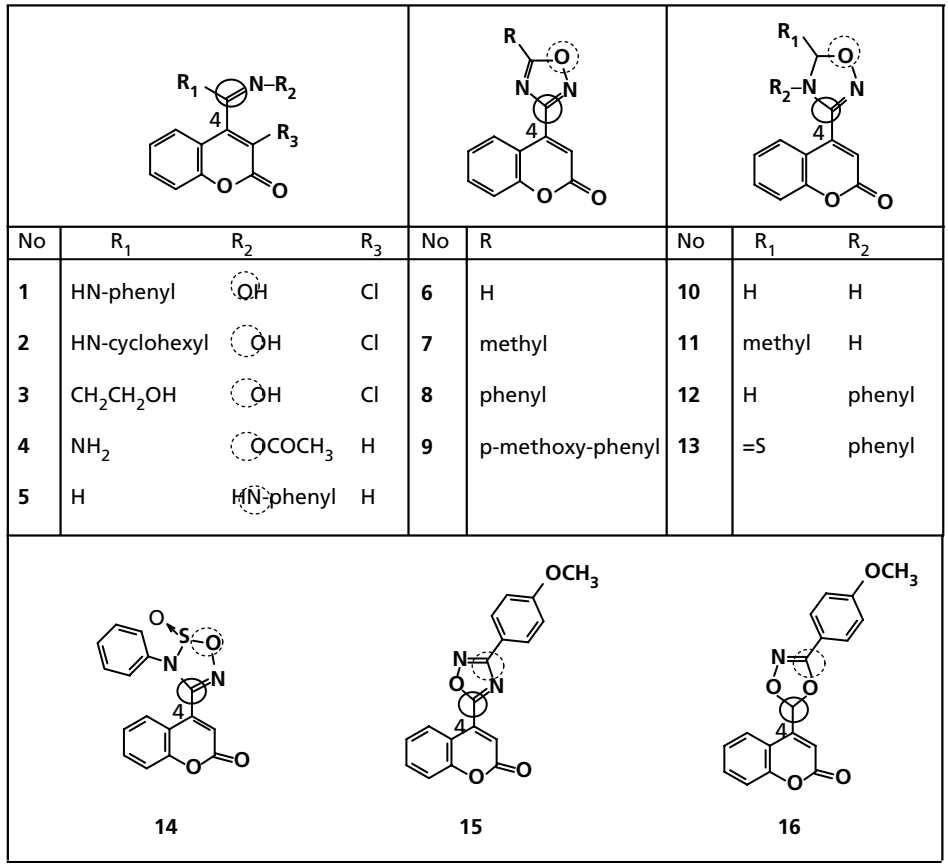

No	R_1	R_2	R_3
1	HN-phenyl	OH	Cl
2	HN-cyclohexyl	OH	Cl
3	CH_2CH_2OH	OH	Cl
4	NH_2	$OCOCH_3$	H
5	H	HN-phenyl	H

No	R
6	H
7	methyl
8	phenyl
9	p-methoxy-phenyl

No	R_1	R_2
10	H	H
11	methyl	H
12	H	phenyl
13	=S	phenyl

14 15 16

Figure 1 Chemical structures of the substituted coumarins. ○ position 1′, ◌ position 3′.

They correspond to the interaction of the substances with DPPH in equimolar concentrations. Compound **16**, lacking the conjugated double bond system, was inactive and no logit value could be calculated.

Molecular descriptors

Dipole moments (D) and partial atomic charges were calculated using Hyperchem v.5.0 (Hypercube, Gainesville, FL) after geometry optimization, as described by Karalis et al (2002).

Van der Waals surface area (SdW) and Van der Waals volume (VdW), solvent-accessible surface area (SASA) and solvent-accessible volume (SAVOL), polar surface area (PSA), polarizability (Pol) and molar refractivity (MR) were calculated for the optimized structures using Chemplus v.1.6 (Hypercube Inc). To calculate PSA, O, N and the attached H atoms were considered as polar regions. Non-polar surface area nPSA was obtained by subtracting PSA from SASA. The ratio SAVOL/SASA (Vol/Sur), as a measure of the wrinkled molecular surface, was also included as descriptor.

Electrotopological state indices (S_i) concerning the C4 atom of the coumarinic skeleton and the atoms constituting the substituents were calculated by Molconn-X.

Experimental octanol–water partition coefficients (logP) were taken from Vrakas et al (2003).

Statistical analysis

Multivariate data analysis, including principal component analysis (PCA) and partial least-square (PLS) analysis, was performed using SIMCA-P v.8.0 (Umetrics AB, Umea, Sweden) software package. The data matrix included the descriptors + the response variable, logit(RA). Data were centered and scaled to unit variance. The scores plot of the first two principal components served as a tool to explore the uniformity in the behaviour of the compounds. PLS was used to relate the information between the block of the variables X (descriptor matrix) and the response variable Y (Franke & Gruska 1995; Eriksson & Johansson 1996).

Results and Discussion

Principal component analysis (PCA) was initially applied to an X-data matrix containing all molecular descriptors and the logit values. Four principal components proved significant, leading to Model 1 with $r^2 = 0.888$ and

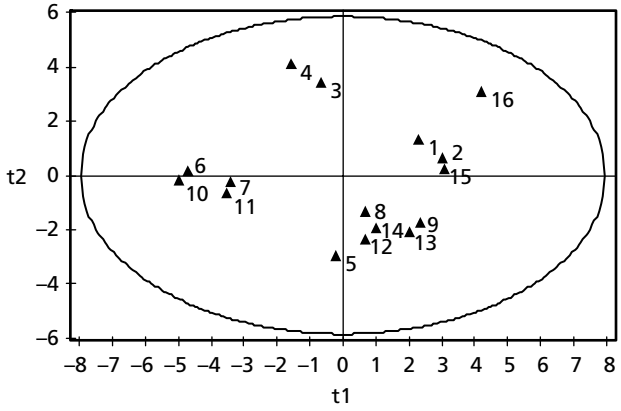

Figure 2 Plot of the scores, t1 and t2, of the first two principal components (PCA Model 1).

$q^2 = 0.568$. The scores plot of the first two principal components was used to explore the uniformity in the behaviour of the compounds. As shown in Figure 2, all compounds lie within the Hotelling T^2 ellipse. The bulky compounds, containing a phenyl or cyclohexyl group, are evenly distributed in the two right quartiles. Compounds **6**, **7**, **10** and **11**, which do not contain a bulk substituent, are clustered towards the left down quartile, while the two smaller, more polar derivatives **3** and **4** lie separately in the left upper quartile. Initial PLS analysis using the whole data set (except the inactive compound **16**) did not lead to any significant model. Considering the bulky compounds as a separate class, various models with $q^2 > 0.5$ were generated considering the variable influence on the predictions (VIP). The most adequate model was chosen according to the following criteria: firstly, the simplicity of the model concerning the number of components and the number of original variables implemented; secondly, the overfit of the model after 10 permutations of the response variable – the intercept of the regression line of the correlation coefficient between the original Y and the permuted Y variable versus the cumulative r^2 and q^2 should be close to zero. These criteria were best fulfilled by the two-component Model 2 with $r^2 = 0.783$ and $q^2 = 0.635$. Model 2 was derived using only 4 descriptors, the bulk parameters SvdW and VvdW, the partial charge (P1′) of the carbon atom at position 1′ of the substituent, involved in the conjugated double bond (position 1′ full line encircled in Figure 1) and the electrotopological state index (S3′) assigned to the oxygen (or nitrogen) of R_2 or the oxygen (or carbon atom) at position 3′ of the heterocyclic substituent, next to the conjugated double bond system (dotted line encircled in Figure 1). The position of the remaining compounds relative to Model 2 was then evaluated and one strong outlier, compound **6**, was detected. Apparently, compound 6 was responsible for the failure of PLS analysis for the whole data set. Since there is no obvious reason for the deviating behaviour of compound **6**, its experimental value should be considered with caution. The same PLS analysis was repeated for the entire set, excluding compound **6**. Model 3 with two components and $r^2 = 0.850$, $q^2 = 0.802$, practically did not differ from Model 2. Table 1 contains the observed and predicted logit(RA) values (RMSEE = 0.280), as well as the data used to derive Model 3. For compound **16**, a logit(RA) value equal to −0.841 was predicted, corresponding to a negligible %RA value. The histogram of the coefficients of the scaled and centered variables data is presented in Figure 3.

Conclusions

In the case of the investigated substituted coumarins, the electronic properties of the atoms participating or next to the conjugated double bond (P1, S3) were found to contribute significantly to the interaction with DPPH,

Table 1 Observed and predicted logit(RA) values and molecular properties used in the final PLS model and the regression equations

Compound	logit(RA) (observed)	logit(RA) (predicted)	Δ	SvdW	VvdW	S3′	P1′
1	−0.169	−0.018	−0.151	297.33	259.79	9.3498	0.054
2	−0.194	−0.327	0.133	317.39	275.90	9.4191	0.052
3	0.624	0.500	0.124	270.33	226.29	9.0334	0.052
4	0.352	0.600	−0.248	247.5	206.90	4.4382	0.087
5	0.366	0.181	0.185	275.26	239.64	2.9141	−0.181
6	−0.661	1.227	−1.888	200.44	174.44	1.2210	−0.063
7	0.561	0.857	−0.296	221.71	191.16	0.4559	−0.063
8	0.111	−0.153	0.264	278.91	248.78	0.4101	−0.06
9	−0.454	−0.666	0.212	313.98	273.65	0.3657	−0.072
10	1.609	1.078	0.531	209.81	180.00	0.7529	−0.069
11	0.419	0.727	−0.308	230.63	196.97	0.2501	−0.075
12	−0.613	−0.286	−0.327	286.89	255.22	0.3412	−0.054
13	−0.560	−0.598	0.038	305.53	267.11	0.2394	0.006
14	−0.583	−0.570	−0.013	298.77	261.72	−1.7669	−0.034
15	−0.857	−0.713	−0.144	310.09	273.47	0.4352	0.045
16	—	−0.841	—	314.8	274.59	0.3472	0.148

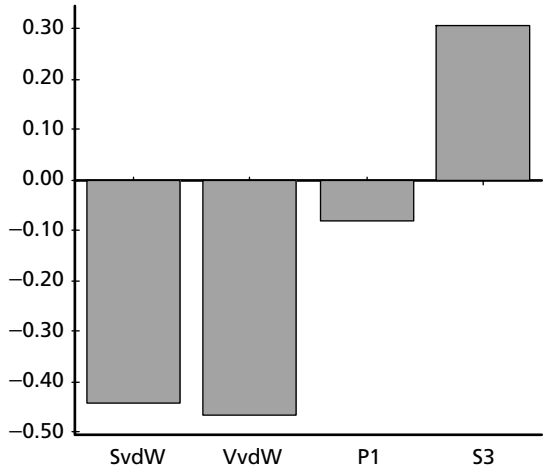

Figure 3 Histogram of coefficients for PLS Model 3.

confirming the initial assumption in the design of these molecules. Moreover, steric hindrance seems to be important for the interaction of the compounds with DPPH, since bulk properties showed a considerable negative contribution to the activity. To our knowledge, multivariate data analysis based on molecular descriptors was for the first time applied to derive a model for the interaction with DPPH. It revealed the structural features responsible for the activity, although the limited data set does not allow further speculation on the underlying mechanism.

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