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Quantitative elucidation of the structure–bitterness relationship of cynaropicrin and grosheimin derivatives

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Abstract

In previous work, cynaropicrin and grosheimin derivatives were submitted to a panel test for sensory evaluation. Bitterness variations seemed to be related to changes in molecular polarity. Reported incongruences have confused the understanding of the molecular mechanisms of bitterness variations. In this work, a classic QSAR analysis was applied to a training set of 15 sesquiterpene lactones and the structure–bitterness relationship investigated. The dipole moment, polarizability and hydrophobicity were studied and made it possible to conclude that bitterness of sesquiterpene lactones was not directly related to molecular polarity. The calculated molecular descriptors were used, in combination with classic QSAR, to define the relevant parameters responsible for the biological properties. The variable selection showed a satisfactory quantitative structure–activity relationship (QSAR) and a model was established.
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1. Introduction

Mammalian taste receptor cells (TRC) are small neuroepithelial cells, tightly packed into taste buds that are distributed in distinct regions of the tongue and palate epithelium. Mammals can taste a wide variety of chemosensory stimuli by two unrelated families of receptors (T1Rs and T2Rs). Receptor cells are innervated by afferent nerve endings that transmit information to the taste centres of the cortex through synapses in the brain stem and thalamus (Adler et al., 2000; Chandrashekar et al., 2000).

Most probably, taste receptors are formed by a few specific families of G protein-coupled receptor molecules (GPCRs), found in different locations on the plasma membrane of each TRC type. Bitterness is the most complex of the tastes: there are many more bitter compounds than there are found for the other tastes and also more receptors to which they can be bind. Bitter tastants are detected by a family of about 30 GPCRs (the T2Rs), most of which are co-expressed in the same subset of TRCs, suggesting that these cells are capable of responding to a broad array of bitter compounds (Cravotto, Nano, Binello, Spagliardi, & Seu, 2005; Zhang et al., 2003).

Frequently, bitterness is associated with toxicity and many bitter compounds are derived from plants, which have evolved to produce these substances as a chemical defence against several animals. In general, bitter foods are rejected; however a slight bitter taste is sometimes desired in human diet. Vegetable foods and drinks that contain such bitter compounds as, for example, isoflavones, polyphenols, catechins and flavonoids, may be found unpalatable by consumers. On the other hand, many bitter tastes have health benefits, such as the isothiocyanates from brocoli that can protect against cancer (Zhang, Talalay, Cho, & Psner, 1992).

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The range of compounds capable of producing a bitter taste is very large and diverse, including proteins, amino acids, fats, salts, glucosides, flavonoids and terpenes. In the last case, various sesquiterpene lactones show an intense bitterness, like caffeine and quinine. Food industries have tackled the problem by controlling bitter compounds in certain kinds of foods and beverages and by employing effective masking strategies to ensure that the product is acceptable to consumers. Fig. 1 shows cynaropicrin and grosheimin, compounds found in alcoholic extract of leaves from the artichoke (*Cynara scolymus* L., Compositae) and currently used for the production of bitter liqueurs (Cravotto et al., 2005).

Cravotto et al. (2005) isolated cynaropicrin and grosheimin from dry artichoke leaves and prepared a series of 14 derivatives. Twelve panellists were asked to score bitterness on a six-point scale ranging from 'like water' (0) to 'exceedingly bitter' (5). Analyzing the structure–bitterness relationships, it was concluded that the bitterness decreased when the polarity of the molecule increased, and was markedly reduced by either the loss of exomethylenes or the opening of the lactone ring.

In the current work, a classic quantitative structure–activity relationship (QSAR) analysis was applied to cynaropicrin and grosheimin and their sesquiterpene lactone derivatives reported by Cravotto et al. (2005), through a computer-aided investigation of the structure–bitterness relationship. Since molecular polarity was related to the bitter taste, we studied related properties, such as polarizability, hydrophobicity and dipole moment. Additionally, molecular descriptors, computed using DRAGON software, were used in this classical QSAR analysis.

2. Materials and methods

Molecular modelling computations (Cohen, 1996; Leach, 2001) were performed on SPARTAN for Windows v. 4.0 software. The molecules studied were cynaropicrin, grosheimin and their 13 sesquiterpene lactone derivatives (Cravotto et al., 2005), shown in Fig. 1, with their respective bitterness tastes (BA – biological activity) presented in Table 1. From the original literature, one compound, a grosheimin salt, was excluded from the analyses. This compound has a K^+ counterion that plays no significant role in the analysis.

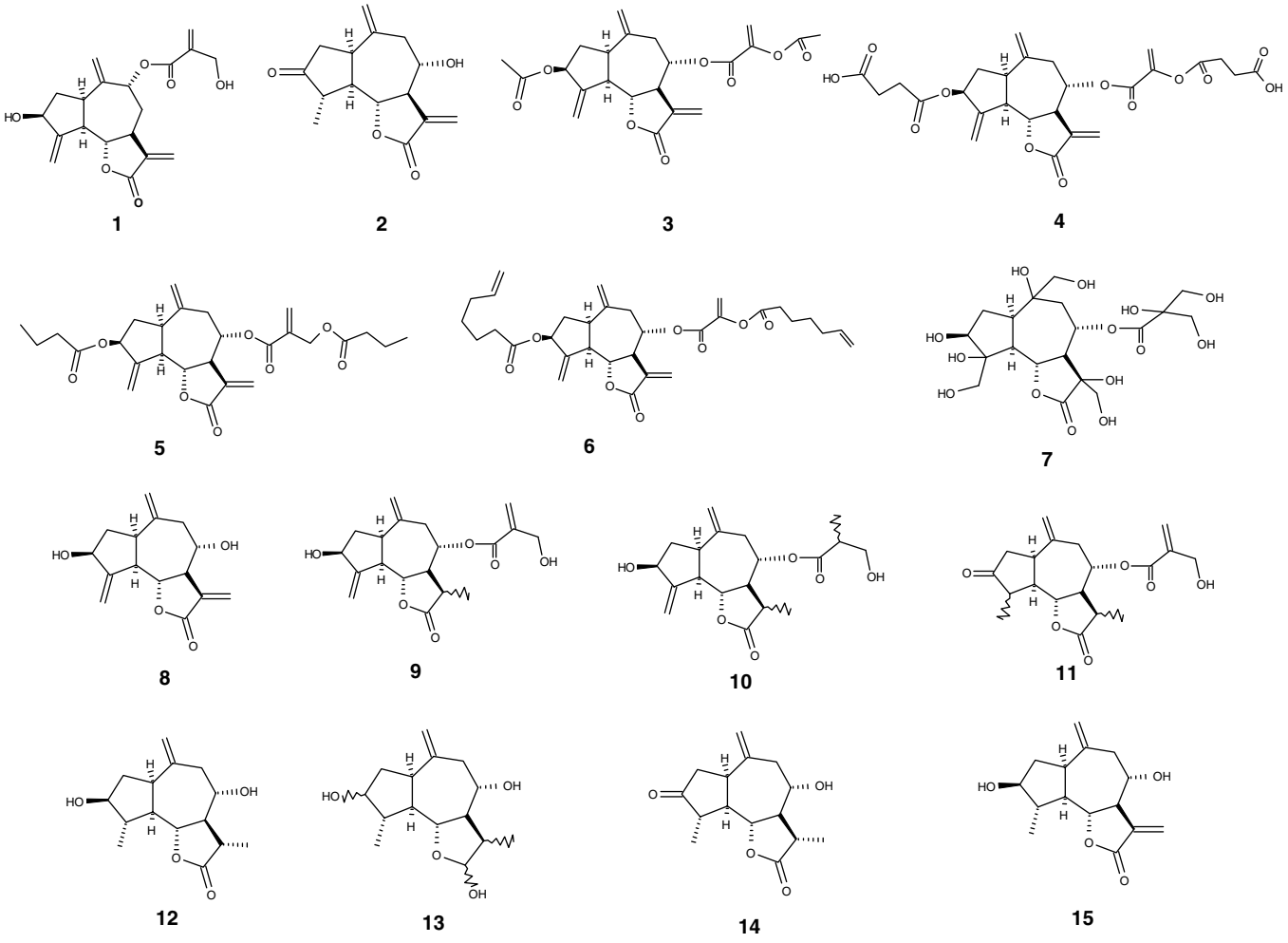

Fig. 1. Structures of cynaropicrin (1), grosheimin (2) and derivatives.

Table 1
Values of polarizability, dipole moment and lipofilicity calculated by AM1 semiempirical method and the sensorial activity date, selected from literature (Cravotto et al., 2005)

Compound	Polarizability (Å^3)	Dipole moment (D)	Lipophilicity	Sensorial activity (BA)
1	35.27	4.45	0.71	3.0 ± 0.2
2	27.04	6.02	1.25	3.3 ± 0.3
3	42.78	4.94	0.97	3.3 ± 0.5
4	51.57	5.86	0.56	1.0 ± 0.7
5	50.12	5.53	3.02	3.2 ± 0.3
6	60.75	5.91	4.97	3.0 ± 0.4
7	41.13	5.95	−5.22	0.0 ± 0.5
8	27.40	4.32	0.56	0.9 ± 0.4
9	35.46	3.44	0.87	0.8 ± 0.4
10	35.65	2.80	1.03	0.5 ± 0.4
11	35.10	6.13	1.56	4.2 ± 0.7
12	27.78	4.15	1.04	1.7 ± 0.5
13	28.33	1.83	1.00	1.3 ± 0.4
14	27.23	5.26	1.41	2.9 ± 0.4
15	27.59	4.12	0.88	0.7 ± 0.3

The molecules were submitted to geometry optimization and conformational analysis (systematic analysis with dihedral angle rotation at each 30°, from 0° to 360°) using the AM1 semiempirical quantum chemical method (Dewar, Zoebisch, Healy, & Stewart, 1985). The minimal energy conformer (gas phase) was submitted to single point calculation in order to obtain the polarizability, hydrophobicity (Ghose, Pritchett, & Crippen, 1988) and dipole moment values.

The correlation between computed theoretical parameters and bitterness taste, and the respective significance level, observed in these quantitative structure–activity relationships analyses, were calculated using the BILIN software (Kubinyi, 1993).

Additionally, the geometries of optimized molecules were used to generate other molecular descriptors, through the DRAGON plus v. 5.0 (Talete, 2005) programme. The model significance obtained in this work, with the exclusion of redundant and noisy information, was analyzed by MobyDigs v. 1.0 software that calculated the regression models by using genetic algorithms to perform variable selection (Talete, 2004).

In this work, we have used two approaches to obtain the QSAR models. In the first one, in order to verify the Cravotto suggestion, we have used only polarizability, hydrophobicity and dipole moment calculated values to mimic the molecular polarity.

In the second approach, many parameters generated by the DRAGON programme were used in QSAR analyses. The compounds were divided into two sets, a training set with 10 compounds and a test set with the remaining five compounds. To select the compounds for training and test sets, the whole range of bitter taste (BA) was considered. The test set was created to validate the predictive ability of the training set. The corresponding bitterness tastes, measured using identical assay conditions, were used as dependent variable to derive the QSAR models.

3. Results and discussion

The polarizability, hydrophobicity and dipole moment values, calculated with molecular modelling tools and the sensorial activity reported in Cravotto et al. (2005) manuscript, are listed in Table 1. Non-significant QSAR models were found among polarizability (P), dipole moment (DM) and lipophilicity (L) for the isolated or combined parameters, as can be seen in the Eqs. (1)–(7). These results may indicate that: (i) the parameters selected in this study are inadequate for these purposes or (ii), in opposition to hypothesis of Cravotto et al. (2005), the bitterness of these sesquiterpene lactones was not directly related to the polarity of these compounds. Table 2 shows no significant correlations established between theoretical parameters.

Eqs. (1)–(7) with:

- Three variables

$$\mathrm{BA} = -0.038(\pm 0.061)P + 0.637(\pm 0.470)\mathrm{DM} + 0.419(\pm 0.280)L - 0.020(\pm 2.340) \quad (1)$$
$$(n = 15;\ r = 0.787;\ s = 0.923;\ F = 5.979;\ Q^2 = 0.398;\ \text{s-PRESS} = 1.161)$$

- Two variables

$$\mathrm{BA} = -0.009(\pm 0.078)P + 0.529(\pm 0.630)\mathrm{DM} - 0.188(\pm 3.140) \quad (2)$$
$$(n = 15;\ r = 0.489;\ s = 1.249;\ F = 1.890;\ Q^2 = -0.119;\ \text{s-PRESS} = 1.516)$$
$$\mathrm{BA} = +0.498(\pm 0.430)\mathrm{DM} + 0.363(\pm 0.270)L - 0.715(\pm 2.110) \quad (3)$$
$$(n = 15;\ r = 0.745;\ s = 0.956;\ F = 7.473;\ Q^2 = 0.240;\ \text{s-PRESS} = 1.249)$$
$$\mathrm{BA} = +0.001(\pm 0.068)P + 0.362(\pm 0.350)L + 1.618(\pm 2.540) \quad (4)$$
$$(n = 15;\ r = 0.564;\ s = 1.183;\ F = 2.798;\ Q^2 = 0.025;\ \text{s-PRESS} = 1.415)$$

- One variable

Table 2
Linear cross-correlation (r) matrixes

	Polarizability	Dipole moment	Lipophilicity
Parameters shown in Eqs. (1)–(7)			
Polarizability	1.000		
Dipole moment	0.203	1.000	
Lipophilicity	0.081	0.000	1.000
	*n*OHp	*n*OHs	C-040
Descriptors shown in Eq. (8)			
*n*OHp	1.000		
*n*OHs	0.001	1.000	
C-040	0.0004	0.674	1.000

$$BA = +0.021(\pm 0.075)P + 1.223(\pm 2.850) \quad (5)$$
$$(n = 15;\ r = 0.164;\ s = 1.358;\ F = 0.359;\ Q^2 = -0.229;\ \text{s-PRESS} = 1.526)$$

$$BA = +0.497(\pm 0.540)DM - 0.358(\pm 2.620) \quad (6)$$
$$(n = 15;\ r = 0.486;\ s = 1.203;\ F = 4.012;\ Q^2 = -0.0233;\ \text{s-PRESS} = 1.393)$$

$$BA = +0.363(\pm 0.320)L + 1.633(\pm 0.710) \quad (7)$$
$$(n = 15;\ r = 0.564;\ s = 1.137;\ F = 6.063;\ Q^2 = 0.174;\ \text{s-PRESS} = 1.251)$$

In order to verify the influence of other parameters on the biological activity, new descriptors were generated. Dragon descriptors, for example, include different kinds of groups: constitutional descriptors (Todeschini & Consonni, 2000), topological indices (Todeschini & Consonni, 2000), molecular walk counts (Diudea, Topan, & Graovac, 1994), BCUT descriptors (Burden, 1997), Galvez topological charge indices (Galvez, Garcia, Salabert, & Soler, 1994), RDF descriptors, 3D-MoRSE descriptors (Schuur, Selzer, & Gasteiger, 1996), weighted holistic invariant molecular descriptor (WHIM) (Todeschini & Gramatica, 1997), empirical descriptors, GETAWAY (GEometry, Topology, and Atom-Weights AssemblY) (Consonni, Todeschini, & Pavan, 2002), functional groups (Todeschini & Consonni, 2000), atom-centred fragments (Ghose et al., 1988), and molecular properties (Todeschini & Consonni, 2000).

The descriptors employed in this study can be arranged in the following groups:

- descriptors 2D: 2D autocorrelations (96 descriptors);
- descriptors 3D: RDF (150 descriptors), 3D-MORSE (160 descriptors), GETAWAY (197 descriptors), WHIM (99 descriptors);
- others descriptors: functional groups (154 descriptors), atom-centred fragments (120 descriptors), molecular properties (29 descriptors).

Each descriptor set was separately submitted to variable selection, except the functional groups, atom-centred fragments and molecular properties, which were assembled, considering that all these blocks of descriptors are calculated based on fragments (Todeschini & Consonni, 2000).

Descriptor values obtained from the optimized structures of species were subsequently submitted to conformational analysis and the minimum energy conformer was selected. For compounds which are mixture of diastereoisomers (compounds **9**, **10**, **11** and **13**), the descriptor values were calculated, starting from the average values of the diastereoisomers.

For each block of descriptors, the constant variables were excluded, i.e. those that just presented one different value. For the remaining descriptors, pairwise correlation analysis was performed (Livingstone, 1995) and the descriptors that presented high correlations, $r > 0.99$, were excluded. Thus, the number of *DRAGON* descriptors used in our calculations was reduced to:

- descriptors 2D: 2D autocorrelations (59 descriptors);
- descriptors 3D: RDF (65 descriptors), 3D-MORSE (108 descriptors), GETAWAY (120 descriptors), WHIM (58 descriptors);
- other descriptors: functional groups + atom-centred fragments + molecular properties (41 descriptors).

Molecular descriptor information content depends on the kind of molecular representation that is used and on the defined algorithm employed for its calculation. There are simple molecular descriptors derived from counting some atom-types or structural fragments in the molecule, others derived from algorithms applied to a topological representation (molecular graph) and usually called topological or 2D-descriptors, and there are molecular descriptors derived from a geometrical representation that are called geometrical or 3D-descriptors.

The MobyDigs programme was used to calculate the regression models using genetic algorithms to select the variables. Search for the best models is done by using multiple linear regression (MLR) under the genetic algorithm (GA) approach, i.e. by the variable subset selection – genetic algorithm (VSS-GA) (Leardi, Boggia, & Terrile, 1992) method. In the GA terminology, a population is characterized by a set of candidate variables (the genetic heritage of the population) and is constituted by individuals, i.e. models made of one or more population variables (Leardi et al., 1992). The MobyDigs software provides many statistical indices useful for evaluating the performance of the developed regression models (Todeschini, Consonni, Mauri, & Pavan, 2004). The calculated indices are furnished as supplementary material.

The results obtained with DRAGON and MobyDigs softwares are shown in Eq. (8). The calculated versusobserved results are showed in Fig. 2. The selection variables showed: *n*OHp, *n*OHs, C-040, where *n*OHp and *n*OHs represent the number of primary and secondary alcohols, respectively, and the descriptor C-040 represents three kinds of carbon atoms: R–C(=X)–X, R–C≡X, X=C=X, where X represents any heteroatom (O, N, S and halogens).

The variable selection calculated is shown in Eq. (8) and Table 2.

$$BA = -0.451(\pm 0.386)n\text{OHp} - 2.465(\pm 1.281)n\text{OHs} - 0.878(\pm 0.547)C - 040 + 6.453(\pm 2.286) \quad (8)$$
$$(n = 010;\ r^2 = 0.817;\ s = 0.756;\ F = 8.929;\ Q^2_{cv} = 0.722;\ \text{s-PRESS} = 0.931,\ n_{ext} = 5;\ R^2_{ext} = 0.654;\ Q^2_{ext} = 0.628)$$

Analyzing Eq. (8), it is verified that all the variables contribute negatively to the obtained model. The coefficient of internal prediction (Q^2_{cv}) value and the F value are signifi-

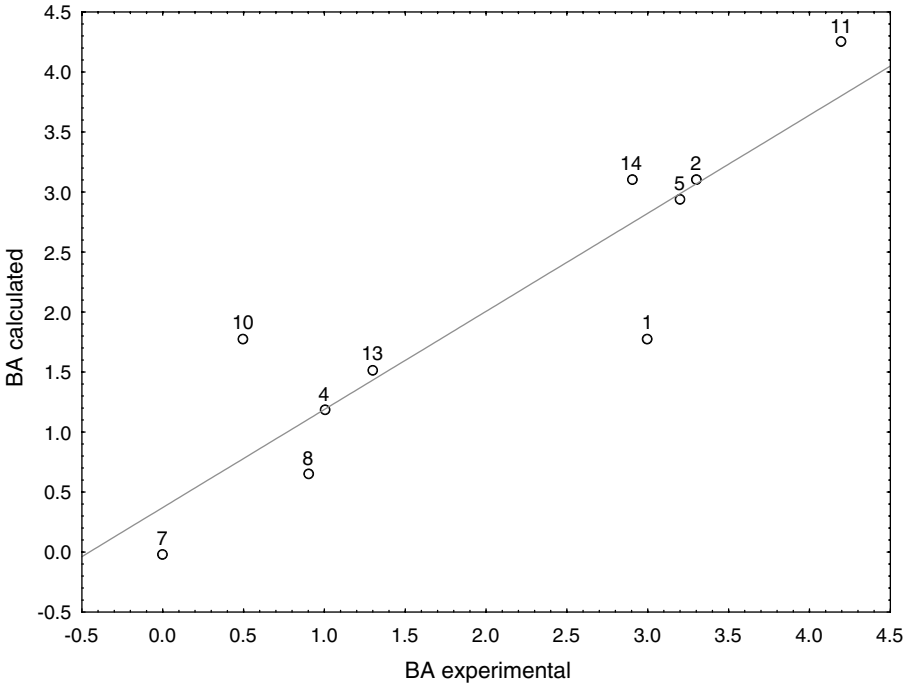

Fig. 2. Graph of experimental activity values versus calculated activity values, predicted by Eq. (8).

cant, this last one because considering 5% of trust, with 3 and 6 degrees of freedom, the minimum necessary F value is 4.76.

According to Table 2, the linear cross-correlation matrix between the descriptors present in Eq. (8) shows low values of correlation, which prevents collinearity (Livingstone, 1995), except between the descriptors *n*OHs and C-040, but is still not significant ($r^2 < 0.46$).

It is verified, through Eq. (8), that increasing the number of hydroxyl groups as well as the number of ester fragments in the molecule, will reduce the bitter taste. Cravotto et al. (2005) verified by SAR (structure activity relationship) studies that an increase of the bitter taste is related to the polarity. The Eqs. (1)–(7) show few significant relationships among the properties that are directly related to the polarity and the biological activity. The number of hydroxyl groups is not directly related to the polarity, considering that polarity is a physical property of compounds which is related to other physical properties, such as melting and boiling points, solubility, and intermolecular interactions between molecules. Additionally, symmetrically arranged polar bonds result in a low dipole moment.

Others descriptors, that depend on 2D as 3D molecular structure, did not generate statistically significant equations, mainly regarding the external test, which is important for the validation of a model (Livingstone, 1995).

Fig. 2, plotted using the data present in Table 3, shows the point adjustment regarding straight line used for the model calibration. This linear approach is validated analyzing Fig. 3, a graph of the residues between the experimental and predicted biological activity, which presents the random distribution of the points.

Table 3
Experimental and calculated or predicted values for sensorial activity, and the respective errors for training and test subsets

Compound	Sensorial activity experimental	Sensorial activity calculated	Error (calculated − experimental)
Training set			
01	3.00	1.78	1.22
02	3.30	3.11	0.19
04	1.00	1.18	−0.18
05	3.20	2.94	0.26
07	0.00	−0.02	0.02
08	0.90	0.65	0.25
10	0.50	1.78	−1.28
11	4.20	4.25	−0.05
13	1.30	1.52	−0.22
14	2.90	3.11	−0.21
		Sensorial activity predicted	Error (predicted − experimental)
Test set			
03	3.30	2.94	0.36
06	3.00	2.94	0.06
09	0.80	1.78	−0.98
12	1.70	0.65	1.05
15	0.70	0.65	0.05

Table 3 and Fig. 4 show a significant validation for the test set. A highly significant linear agreement was not observed, but the model was shown capable of differentiating the more active compounds from the others.

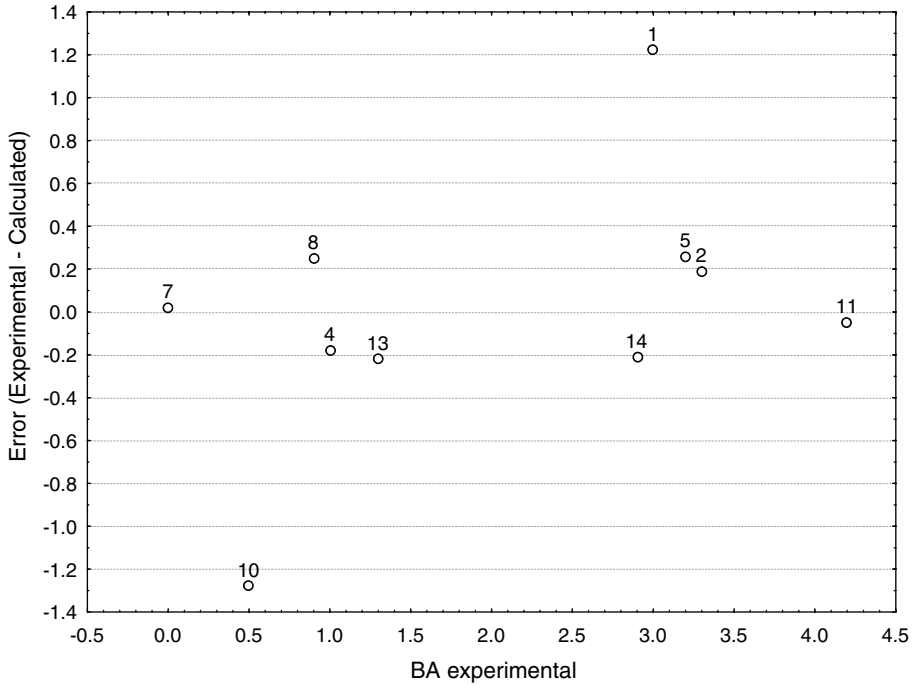

Fig. 3. Graph of experimental activity values versus respective errors.

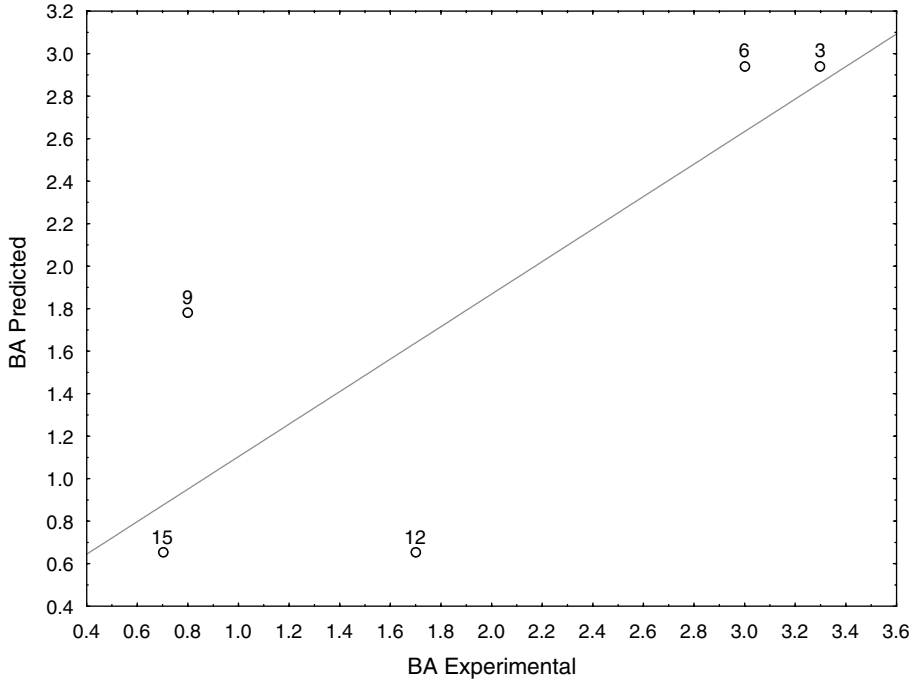

Fig. 4. Graph of experimental activity values, from test subset, versus predicted activity values obtained from Eq. (8).

4. Conclusions

Previous SAR study, using structural modifications of cynaropicrin and grosheimin, shed some light on structure–taste relationships, concluding that bitterness of these sesquiterpene lactones, appeared to be strongly dependent on the presence of oxygenated polar groups. The SAR study seems logical, but quantitative elucidation is necessary. Modifications of cynaropicrin and grosheimin were carried out to provide a library of compounds for our quantitative investigation.

In the first approach, using polarizability, dipole moment and lipophilicity parameters, a significant quantitative structure–bitterness relationship was not established

by the classic QSAR model. Chemometric modelling could be improved by including other molecular descriptors and using the MobyDigs software for the calculations of regression models through the genetic algorithms. From the variable selection shown, a satisfactory quantitative structure–activity relationship (QSAR) model was obtained with statistically significant models, with high internal validation coefficients and satisfactory external test. More significant classification of active bitter compounds was obtained from the results shown in this work.

The variable subset selection – genetic algorithm (VSS-GA) selected variables that are important for biological activity are: number of primary alcohols, number of secondary alcohols, and number of fragments R–C(=O)–O.

This work underlines the fact that the quantitative structure–bitterness relationship is complex and that it can not be directly related to molecular polarity. The results confirmed the importance of the hydroxyl groups and esters fragments for decreasing the bitter taste. However, it was not possible to identify which of the main derived molecular properties of these structures was directly related to the bitterness. This reflects the complexity of this particular biological activity and how little is known about the structures that cause bitterness. On the other hand, the employed method can be used for general classification of bitter sesquiterpene lactones in the development of new food products containing these compounds and relevant masking strategies that can be utilized to avoid the perception of the taste by the consumer.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2007.03.038.

References

Adler, E., Hoon, M. A., Mueller, K. L., Chandrashekar, J., Ryba, N. J. P., & Zuker, C. S. (2000). A novel family of mammalian taste receptors. *Cell, 100*(6), 693–702.

Burden, F. R. (1997). A chemically intuitive molecular index based on the eigenvalues of a modified adjacency matrix. *Quantitative Structure–Activity Relationships, 16*(4), 309–314.

Chandrashekar, J., Mueller, K. L., Hoon, M. A., Adler, E., Feng, L., Guo, W., et al. (2000). T2Rs function as bitter taste receptors. *Cell, 100*(6), 703–711, 200.

Cohen, N. C. (1996). *Guidebook on molecular modeling in drug design* (pp. 55–87). San Diego: Academic Press.

Consonni, V., Todeschini, R., & Pavan, M. (2002). Structure/response correlations and similarity/diversity analysis by GETAWAY descriptors. 1. Theory of the novel 3D molecular descriptors. *Journal of Chemical Information and Computer Sciences, 42*(3), 682–692.

Cravotto, G., Nano, G. M., Binello, A., Spagliardi, P., & Seu, G. (2005). Chemical and biological modification of cynaropicrin and grosheimin: a structure–bitterness relationship study. *Journal of the Science of Food and Agriculture, 85*(10), 1757–1764.

Dewar, M. J. S., Zoebisch, G., Healy, E. F., & Stewart, J. J. P. (1985). The Development and use of quantum-mechanical molecular models. 76. AM1: A new general purpose quantum mechanical molecular model. *Journal of the American Chemical Society, 107*(13), 3902–3909.

Diudea, M. V., Topan, M., & Graovac, A. (1994). Molecular topology. 17. Layer matrices of walk degrees. *Journal of Chemical Information and Computer Sciences, 34*(5), 1072–1078.

Galvez, J., Garcia, R., Salabert, M. T., & Soler, R. (1994). Charge indexes. New topological descriptors. *Journal of Chemical Information and Computer Sciences, 34*(3), 520–525.

Ghose, A. K., Pritchett, A., & Crippen, G. M. (1988). Atomic physicochemical parameters for 3-dimensional structure directed quantitative structure–activity relationships III: Modeling hydrophobic interactions. *Journal of Computational Chemistry, 9*(1), 80–90.

Kubinyi, H. (1993). *QSAR: Hansch analysis and related approaches* (pp. 91–100). Weinheim: VCH.

Leach, A. R. (2001). *Molecular modeling: principles and applications* (pp. 695–720). London: Prentice Hall.

Leardi, R., Boggia, R., & Terrile, M. (1992). Genetic algorithms as a strategy for feature-selection. *Journal of Chemometrics, 6*(5), 267–281.

Livingstone, D. (1995). *Data analysis for chemists* (pp. 61–135). New York: Oxford Science Publications.

Schuur, J. H., Selzer, P., & Gasteiger, J. (1996). The coding of the three-dimensional structure of molecules by molecular transforms and its application to structure-spectra correlations and studies of biological activity. *Journal of Chemical Information and Computer Sciences, 36*(2), 334–344.

Talete srl, Mobydigs Academic version – Version 1.0 – 2004. http://www.talete.mi.it.

Talete srl, DRAGON for Windows (software for molecular descriptor calculations). Version 5.3 – 2005. http://www.talete.mi.it.

Todeschini, R., & Consonni, V. (2000). *Handbook of molecular descriptors* (pp. 10–509). Weinheim.

Todeschini, R., Consonni, V., Mauri, A., & Pavan, M. (2004). Detecting "bad" regression models: multicriteria fitness functions in regression analysis. *Analytica Chimica Acta, 515*(1), 199–208.

Todeschini, R., & Gramatica, P. (1997). 3D-modelling and prediction by WHIM descriptors. 5. Theory development and chemical meaning of WHIM descriptors. *Quantitative Structure–Activity Relationships, 16*(2), 113–119.

Zhang, Y. F., Hoon, M. A., Chandrashekar, J., Mueller, K. L., Cook, B., Wu, D., et al. (2003). Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. *Cell, 112*(3), 293–301.

Zhang, Y. S., Talalay, P., Cho, C. G., & Psner, G. H. (1992). A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. *Proceedings of the National Academy of Sciences, 89*(6), 2399–2403.