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Quantitative structure-activity relationships studies of antioxidant hexahydropyridoindoles and flavonoid derivatives

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Abstract

In order to predict the antioxidant activity of 22 pinoline derivatives (1,2,3,4-tetrahydro-β-carbolines), two dimensional quantitative-structure activity relationships (2D-QSAR) analysis of 19 hexahydropiridoindoles and 12 flavonoids was realized. Five statistically significant models were obtained from randomly constituted training sets (21 compounds) and subsquently validated with the corresponding test sets (10 compounds). Antioxidant activity (pIC_{50}) was correlated with 5 molecular descriptors calculated with the software DRAGON. The best predictive model (n = 21, $q^2 = 0.794$, $N = 2$, $r^2 = 0.888$, s = 0.157) could offer structural insights into the features conferring a strong antioxidant activity to compounds built from a pinoline scaffold prior to their synthesis.

Keywords: *pinoline, β-carbolines, melatonin, hexahydropyridoindoles, flavonoids, 2D-QSAR, antioxidant*

Introduction

N-acetyl-5-methoxytryptamine (melatonin) is a neurohormone synthesized in the pineal gland during the dark period in all species [1]. Besides its effects on circadian and seasonal rhythms [2] it has been found to be one of the most efficient free radical scavenger for hydroxyl radical [3,4] and peroxyl radical. In addition, several *in vivo* studies report that melatonin protects cells against oxidative agents such as safrole [5,6], paraquat [7] and kainic acid [8]. The antioxidant properties of melatonin are now well documented [9], and recent studies suggest that structurally related indole-based derivatives and their cyclic analogues such as β-carbolines may have a similar antioxidant capacity [10,11]. Because of the protective effects of melatonin against lipid peroxidation, several melatonin structural analogues have been synthesized, screened and a QSAR analysis with the low-density lipoprotein oxidation model was made in the laboratory [12,13]. Among the structural analogues of melatonin studied, some derivatives of a minor melatonin metabolite called pinoline (6-methoxy-1,2,3,4-tetrahydro-β-carboline) have been studied for identifying more efficient antioxidant compounds [14,15,16]. Pinoline has been isolated from the nervous system of mammals [17,18] and can be performed under physiological conditions from 5-methoxytryptamine [19] or as a minor metabolite of melatonin [20]. Pähkla et al. first reported the antioxidant activity of pinoline and showed that this compound is five fold less effective than melatonin in scavenging hydroxyl radical. However, considering that the hydroxyl radical scavenging ability of melatonin is much higher than that of well-known antioxidants such as glutathione and mannitol, pinoline can still be considered as a good hydroxyl radical scavenger [21]. Other studies showed that melatonin is slightly more potent than pinoline in inhibiting lipid

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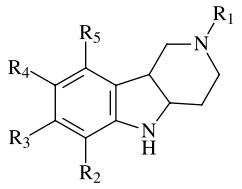
Table I. Structures and antioxidant activities (pIC_{50}) of hexahydropyridoindoles derivatives



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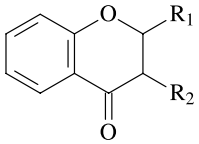
Compounds	R_1	R_2	R_3	R_4	R_5	pIC_{50}
1	CH_3	H	H	CH_3	H	4.60
2	CH_3	NO_2	H	CH_3	H	4.58
3	CH_3	Br	H	CH_3	H	4.80
4	CH_3	NH_2	H	CH_3	H	4.90
5	CH_3	$N(CH_3)_2$	H	CH_3	H	4.66
6	CH_3	H	H	OH	H	4.68
7	CH_3	H	H	OCH_3	H	4.48
8	CO-O-*i*-Bu	H	H	OCH_3	H	5.12
9	CO-O-CH_3	Br	CH_3	OCH_3	CH_3	4.71
10	CO-O-CH_3	H	CH_3	OCH_3	CH_3	4.71
11	CO-O-Et	H	CH_3	OCH_3	CH_3	4.70
12	CO-O-Pr	H	CH_3	OCH_3	CH_3	5.16
13	CO-O-Bu	H	CH_3	OCH_3	CH_3	5.35
14	CO-O-*i*-Bu	H	CH_3	OCH_3	CH_3	5.19
15	CO-O-CH_2-Ph	H	CH_3	OCH_3	CH_3	5.48
16	CO-O-CH_2-P	H	H	OCH_3	H	5.49
17	CO-O-CH_2-P	CH_3	H	OCH_3	H	5.29
18	CO-O-CH_2-P	CH_3	H	CH_3	H	5.36
19	CO-O-CH_2-P	CH_3	CH_3	Br	H	5.53

Table II. Structures and antioxidant activities (pIC_{50}) of flavonoid derivatives



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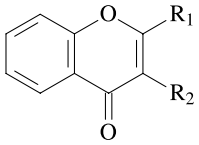
Compounds	R_1	R_2	OH position	pIC_{50}
20	C_6H_3-3,4′-OH	OH	5,7	4.43
21	C_6H_4-4′-OH	H	5,7	4.16
22	C_6H_4-3′-OH	H	5,7	4.28
23	C_6H_4-4′-OCH_3	H	6	3.86



Compounds	R_1	R_2	OH position	pIC_{50}
24	C_6H_3-3′,4′-OH	OH	3,5,7	4.93
25	C_6H_3-2′,4′-OH	OH	3,5,7	4.24
26	C_6H_5	OH	3,5,7	3.92
27	C_6H_5	H	6	3.72
28	C_6H_5	H	7,8	4.22
29	C_6H_5	H	5,7	4.17
30	C_6H_5	H	5,6,7	3.80
31	H	C_6H_4-4′-OH	7	4.10

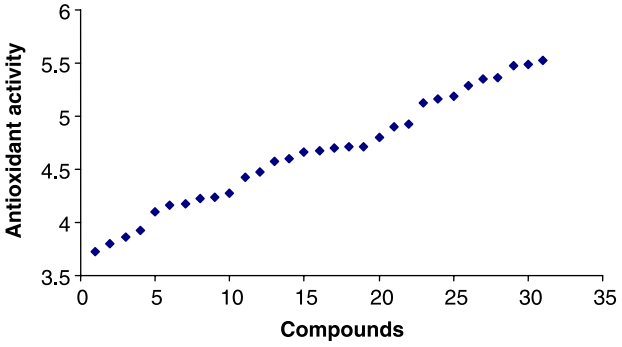
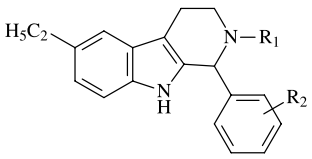


Figure 1. Distribution of pIC_{50} of the compounds.

peroxidation *in vitro*, whereas the ability of pinoline to protect rat brain homogenates against H_2O_2-induced lipid peroxidation was higher than that of melatonin [22,23,24]. Pinoline was also found to be more active than melatonin in reducing nitric oxide-induced lipid peroxidation in rat retinal homogenates [25], and to protect DNA against chromium(III) and H_2O_2 oxidative damages [26]. On the other hand, both pinoline and melatonin participate in the stabilization of hepatic microsomal membranes owing to their capacity to inhibit membrane lipid peroxidation [27]. Therefore it appears that pinoline is endowed with antioxidant activity although this depends on the nature of the radical to scavenge and the tissue to protect. In previous works [14,15,16], some 1-aryl-1,2,3,4-tetrahydro-β-carbolines have been synthesized and their ability to prevent low density lipoprotein (LDL) copper-induced peroxidation in comparison with melatonin and pinoline using quantitative structure-activity relationships has been investigated [15]. The best compound of the series, identified as [6-ethyl-1-(3-methoxyphenyl)-2-propyl-1,2,3,4-tetrahydro-β-carboline] chlorhydrate (GWC22), displays an ethyl group in the 6 position of the β-carboline, that increased the antioxidant activity [16]. This work aimed to draw two dimensional quantitative structure-activity relationships on the effects of different substitutents on the nitrogen atom in position 2 and the aromatic ring, onto the antioxidant activity. In order to build a predictive model of the antioxidant activity of these compounds, we used hexahydropyridoindoles [28] and flavonoids [29] reported from the literature. The chemical structure of the 19 hexahydropyridoindoles studied by Rackova et al. is very close to that of our β-carbolines and the introduction of flavonoids



R_1,R_2 = alkyl groups.

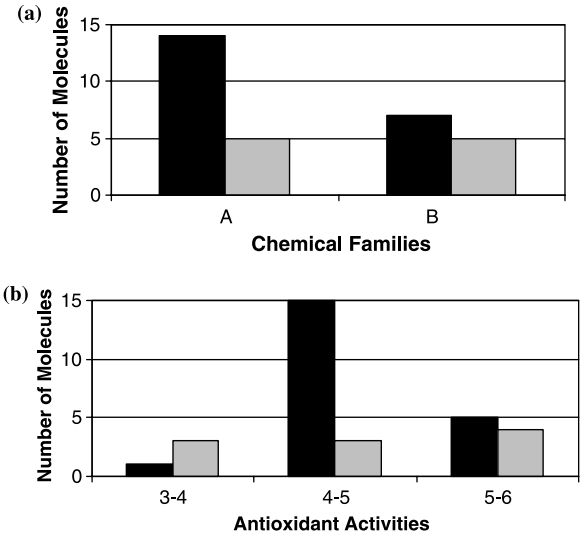


Figure 2. Distributions of chemical families (a) and biological activities (pIC_{50}) (b) *versus* number of compounds for the training (black) and test (grey) sets of model 1.

in this model can allow us to obtain a wider spectrum of prediction.

Materials and methods

Selection of compounds

Rackova et al. synthesized 31 compounds. Their antioxidant efficacy against lipid peroxidation of liposomal membranes was studied in a biphasic system comprising suspension of dioleoylphosphatidylcholine (DOPC) liposomes and peroxyl radicals continually generated from 2,2-azobis(2-amidinopropane) hydrochloride (AAPH). The antioxidant activity was expressed in terms of pIC_{50} or $\log(1/IC_{50})$ (Tables I and II). The 31 molecules selected belong to two different chemical families: hexahydropyridoindoles [28] (Family A: 19 compounds) and flavonoids [29] (Family B: 12 compounds). A homogenous repartition of the antioxidant activity between the two chemical families is a prerequisite if meaningful results are to be obtained from a quantitative structure-activity relationships study (Figure 1 and 2). A multi-model approach was used to assess the predictive power of the final model. Approximately two thirds of the 31 compounds were divided into 5 individual training sets of 21 compounds each and the remaining third was used as test sets with 10 compounds each. Compounds were randomly split between training and test sets by following Oprea et *al.* suggestions [30].

Molecular modeling

Molecular modeling studies were performed using SYBYL 6.9.1 [31] running on Silicon Graphics workstations. Three-dimensional models of compounds

Table III. Statistical results for the five 2D-QSAR models.

	Model 1	Model 2	Model 3	Model 4	Model 5
q^2 [a]	0.794	0.876	0.890	0.898	0.880
N [b]	2	5	2	3	2
s_{cv} [c]	0.213	0.209	0.203	0.185	0.209
r^2 [d]	0.888	0.929	0.931	0.934	0.929
s [e]	0.157	0.158	0.161	0.148	0.161
F [f]	71.708	39.245	121.992	80.705	117.327
r^2_{pred} [g]	0.952	0.918	0.907	0.901	0.939

[a]Crossvalidation correlation coefficient; [b]Number of components; [c]Standard error of prediction; [d]Correlation coefficient; [e]Standard error of estimate; [f]F-ratio; [g]Predicted correlation coefficient.

were built from a standard fragments library and their geometry was subsequently optimized with the semi-empirical MOPAC 6.0 package using the hamiltonian AM1 (keywords: PRECISE, NOMM, PARASOK, XYZ) and Coulson atomic charges were calculated using the same method [32].

Selection of the descriptors

The sofware DRAGON 2.1 [33] was employed to select the best descriptors among 18 families of descriptors. 35 descriptors were retained due to their usefulness in a correct correlation with the pIC_{50} of the compounds. They belong to constitutional, topological, charge, empirical descriptors, aromaticity indices, functional groups and properties. Among them, only 5 descriptors were chosen based on their high correlation with the biological activity (Table V).

χ_2: the 2nd order connectivity index, usually known as Kier-Hall connectivity indice (topological descriptors). It is a variant of the Randic connectivity index and can be interpreted as the contribution of one molecule to the biomolecular interaction arising from the encounters of bonds of two identical molecules.

IC_5: the 5th order information content index or neighbourhood information content is based on neighbour degrees and edge multiplicity, and is an indice of neighbourhood symmetry (topological descriptors).

ICR: the radial centric information index quantifies the degree of compactness of molecules by distinguishing between molecular structures organized differently with respect to their centres, i.e. long linear chains or short ramified substitution pattern (molecular descriptor).

nCT: total number of tertiary carbons (functional group descriptors).

nCO: total number of aliphatic ketones (functional group descriptors).

These five descriptors were chosen because they took into account the structural differences of the studied compounds and gave the highest crossvalidated q^2 with the smallest number of components N. Although electronic, energetic and lipophilic parameters such as Hammett sigma (σ^+), hydratation energy (E_{HYDR}), enthalpy of formation (ΔH), energy of the lowest occupied molecular orbital ϵ(LUMO), energy of the highest occupied molecular orbital ϵ(HOMO) or the partition coefficient Clog$P$ are often used in the calculation of the antioxidant activity in QSAR studies, they did not show an increase of q^2 value in this case, probably because they did not accurately handle the differences into each family of compounds.

2D-QSAR studies

The 2D-QSAR equation was derived by using a linear regression analysis. The partial least square (PLS) method implemented in the QSAR module of SYBYL was used to build and validate the models. The optimal number of components N retained for the final PLS analysis was defined as the one that yielded the highest crossvalidated q^2 value and which normally had the smallest standard error of prediction s_{cv}. The robustness of the model was internaly evaluated by calculating r^2, s and F values from the training sets and was externally validated by calculating r^2_{pred} from the test set.

Presentation of the results

The results obtained for the best model are expressed as a representation of the predicted values of pIC_{50} *versus* experimental values of pIC_{50} for the training and test sets.

Results and discussion

In order to obtain the best predictive 2D-QSAR model we used a multi-model approach.

Training and test sets

A great attention was paid to the distribution of biological activities and structural classification of compounds in both training and test sets. All models show homogeneity in the distribution of antioxidant activity and structural caracteristics of their compounds, which were split between training and test sets randomly as presented for model 1 in Figure 2.

D-QSAR models

Five different training sets were used to derive five separate 2D-QSAR models. The results obtained are represented in Table III. Although the predictivity of a model can only be accessed validating it using a test set, the results indicate the robustness of the models, which yield crossvalidated correlation coefficients q^2

Table IV. Predicted values *versus* actual values for the test set of model 1.

Compounds	Experimental pIC_{50}	Predicted pIC_{50}	Difference
5	4.66	4.68	0.02
13	5.35	5.26	0.09
14	5.19	5.27	0.08
18	5.36	5.36	0.00
19	5.53	5.36	0.17
22	4.28	4.10	0.18
23	3.86	4.00	0.14
27	3.72	3.91	0.19
28	4.22	4.00	0.22
30	3.80	4.00	0.20

(from 0.794 to 0.898) with reasonable respective standard errors of prediction s_{cv} (from 0.185 to 0.203). A q^2 value of 0.3 corresponds to a confidence limit greater than 95%, which minimizes the risk of finding correlation just by mere chance [34]. Our five models yielded high conventional r^2 (from 0.888 to 0.934) with relatively low standard errors of estimate s (from 0.148 to 0.161) by using the optimal number of components (2).

Predictivity of the models

To validate our models, we attempted to predict the activities for the 10 compounds of the test sets. The calculated correlation coefficient r^2_{pred} are given in Table IV. Among the five models, model 1 yielding a good r^2_{pred} (0.952) appears to be the best predictive one. Low differences between the experimental pIC_{50} and the predicted pIC_{50} are obtained for the test set of the model 1 (Table IV) and all compounds were predicted with an error lower than 0.3. More over the predicted pIC_{50} values *versus* the experimental pIC_{50} values for both training and test sets of the model 1 are linear and without outliers (Figure 3).

The 2D-QSAR equation obtained for model 1 is the following:

$$\begin{aligned} pIC_{50} &= 2.698 + 0.093\,\chi_2 - 0.183\,ICR \\ &\quad + 0.400\,IC_5 + 0.405\,nCT - 0.675\,nCO \\ n &= 21,\ r^2 = 0.888,\ s = 0.157,\ F_{2,18} = 71,708 \end{aligned} \quad (1)$$

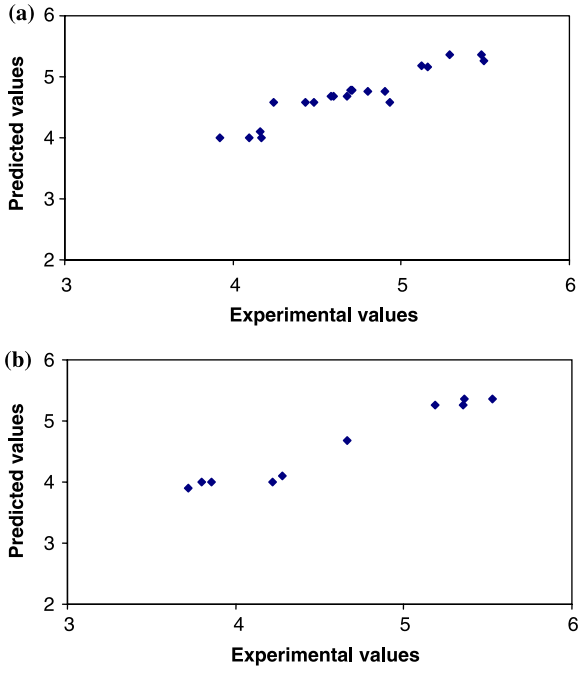


Figure 3. Predicted values *versus* experimental values for the training (a) and test (b) sets of model 1. General structure of 1,2,3,4-tetrahydro-β-carbolines R_1, R_2 = alkyl groups.

Table V. Values of the 5 descriptors chosen.

Compounds	χ_2	ICR	IC_5	nCT	nCO
1	6.00	1.00	4.00	0.00	0.00
2	8.00	2.00	4.00	0.00	0.00
3	7.00	1.00	4.00	0.00	0.00
4	7.00	1.00	4.00	0.00	0.00
5	8.00	2.00	4.00	0.00	0.00
6	6.00	1.00	4.00	0.00	0.00
7	7.00	2.00	4.00	0.00	0.00
8	9.00	2.00	4.00	1.00	0.00
9	9.00	2.00	4.00	0.00	0.00
10	9.00	2.00	4.00	0.00	0.00
11	9.00	2.00	4.00	0.00	0.00
12	9.00	2.00	5.00	0.00	0.00
13	10.00	2.00	5.00	0.00	0.00
14	10.00	2.00	4.00	1.00	0.00
15	11.00	2.00	5.00	0.00	0.00
16	10.00	2.00	5.00	0.00	0.00
17	11.00	2.00	5.00	0.00	0.00
18	11.00	2.00	5.00	0.00	0.00
19	11.00	2.00	5.00	0.00	0.00
20	10.00	2.00	5.00	0.00	1.00
21	10.00	2.00	5.00	0.00	1.00
22	10.00	2.00	5.00	0.00	1.00
23	8.00	2.00	4.00	0.00	1.00
24	7.00	2.00	4.00	0.00	1.00
25	8.00	2.00	4.00	0.00	1.00
26	8.00	2.00	4.00	0.00	1.00
27	8.00	2.00	4.00	0.00	1.00
28	9.00	2.00	4.00	0.00	1.00
29	9.00	2.00	4.00	0.00	1.00
30	8.00	2.00	4.00	0.00	1.00
31	8.00	2.00	4.00	0.00	1.00

Coefficients obtained for this equation show that the 2^{nd} order connectivity index, the 5^{th} order neighbourhood information content and the total number of tertiary carbons increase pIC_{50}. That means that the more ramified the substitution pattern and the higher the number of tertiary carbons are, the higher the antioxidant activity will be. On the contrary, the radial centric information index and the total number of aliphatic ketones decrease pIC_{50}, what means that compact molecules with aliphatic ketones must be avoided to keep a good antioxidant activity. This last point agrees with the experimental values of pIC_{50} for the 12 flavonoids. Most of them display a pIC_{50} inferior to the worst of hexahydropyridoindoles (see Tables I and II).

Conclusion

In conclusion, a series of 19 hexahydropyridoindoles and 12 flavonoids, reported from the literature, were used in the present study to generate and validate 2D-QSAR models. Topological descriptors (χ_2, IC_5), molecular descriptors (ICR) and functional group parameters (nCT, nCO) showed the highest predictive power and a highly significant predictive correlation ($r^2_{pred} = 0.952$) was obtained, whereas electronic, energetic or lipophilic parameters were expected to give the best results but failed. This demonstrates that although it is important to take account of electronic and energetic parameters and lipophily for an antioxidant activity, topological, molecular and functional groups parameters are also very important and can sometimes give a better view of the structural differences of various families of compounds. It highlights that the antioxidant activity can be governed by topological, molecular and functional groups parameters. This model could be further applied for the prediction of the antioxidant activity of 22 1,2,3,4-tetrahydro-β-carbolines designed in our laboratory but it will be finally validated only when the designed compounds are synthesized and evaluated.

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