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QSAR STUDY ON THE ANAESTHETIC ACTIVITY OF SOME BARBITURATES AND THIOBARBITURATES

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Multiple regression analysis was used to perform new statistical models for modeling anaesthetic activity of some barbiturates and thiobarbiturates in terms of electronic and geometrical structural parameters. There was found that for a limited number of compounds, three-parametric models display relevant regression features and quality of correlation either for trial and tested set of molecules, proving therefore their predictive reliability, while the hydrophilic influence was not found with relevant influence at all in anesthesia processes.

INTRODUCTION

The importance of barbiturates and thiobarbiturates is well established in pharmaceutical chemistry and drug design. This class of drugs exhibit hypnotic, anticonvulsant and anaesthetic activity.[1,2] However, despite their clinical importance, the molecular features responsible for anaesthetic activity are still inadequately understood. Following earlier QSAR study of barbiturates reported in literature that deals with the correlation molecular properties-anticonvulsant activity,[3] we have studied the molecular features responsible for anaesthetic activity of barbiturates and thiobarbiturates.

METHODS

The goal of QSAR methodology is to develop several models to predict activity using correlation analysis employing statistical techniques. [4,5] In this regard, mathematical multilinear models, which have the advantage of formalizing the quantum superposition principle, are formed to correlate molecular structure with recorded activity,[6-10] while being useful for predicting activity for new synthesized compounds from their computed structural information as well.

Our statistical models correlate anaesthetic activity of studied compounds with descriptors of physical-chemical, electronic and geometrical nature. Among the physical-chemical descriptors we used the octanol/water partition coefficient, LogP, as a descriptor developed by the classic Hansch QSAR approach[12] since it describes the molecular ability of a given compound (drug) to penetrate biological membranes. Information in ligand-receptor binding is supplied by descriptors from electronic class due to encoding electron distribution properties and, because the recognition of a molecule, by its biological receptor, is principally an electrostatic effect. Among the used electronic descriptors were polarizability, dipole moment or the energy of valence molecular orbitals (HOMO and LUMO). Finally, the drug-substrate interaction is driven by geometrical descriptors throughout the steric influences they carry. As such, the volumes of substituents R_1, R_2 and the angle from C_5 of pyrimidinic ring in structures of barbiturates allow for refining the correlation between the structures of compounds studied and their anaesthetic activity.

The biological activity, the median anesthetic doses AD_{50} measured on rats by intraperitoneal injection,[13-19] were employed for trial correlation.

RESULTS AND DISCUSSION

Table 1 lists the structures, the median anaesthetic doses (AD_{50}) in absolute units, and the actual working activities for the 15 trial molecules used in this study to elaborate statistical models.

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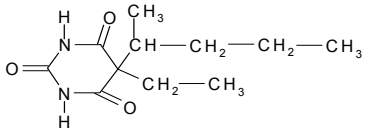
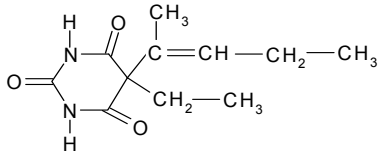
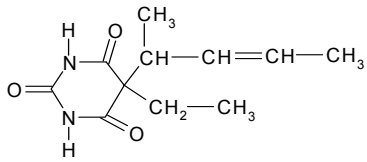
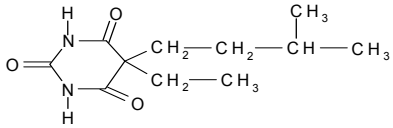
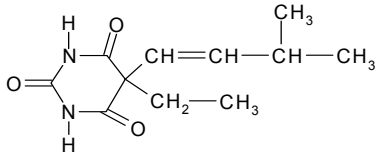
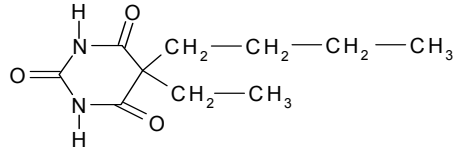
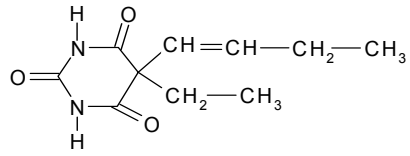
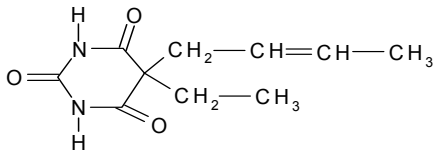
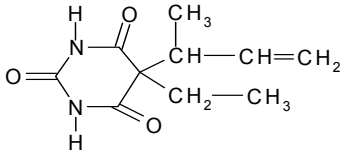
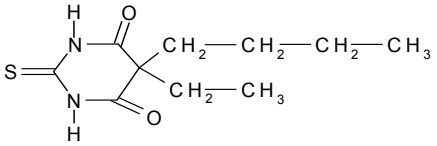
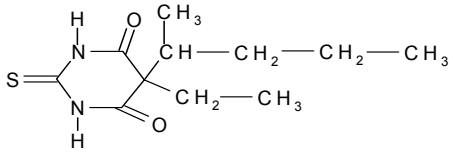
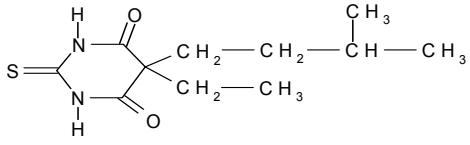
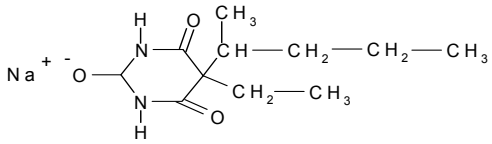
Table 2 shows the structures, the median anesthetic doses (AD_{50}), in absolute units, and the activities for the testing compounds necessary to verify the proposed models.

Table 1

Molecular trial structures measured, median anaesthetic doses and working activities of the studied compounds used to elaborate actual statistical models

No.	Compound	AD_{50}	$A_i = \log\frac{1}{AD_{50}}$	$A_i^* = \frac{A_i + 10}{2}$
1	 5-Ethyl-5-(1'-methyl-butyl) barbituric acid (pentobarbitone)	128	-2.108	3.946
2	 *trans*-5-Ethyl-5-(1'-methylbut-1'-enyl) barbituric acid	180	-2.259	3.870
3	 *trans*-5-Ethyl-5-(1'-methyl-but-2'-enyl) barbituric acid	167	-2.229	3.885
4	 5-Ethyl-5-(3'-methyl-butyl) barbituric acid	235	-2.376	3.811
5	 *trans*-5-Ethyl-5-(3'-methyl-but-1'-enyl) barbituric acid	269	-2.431	3.784
6	 5-Ethyl-5-butyl barbituric acid	264	-2.431	3.784

7	 *trans*-5-Ethyl-5-(but-1'-enyl)-barbituric acid	280	-2.455	3.772
8	 5-Ethyl-5-(but-2'-enyl) barbituric acid	280	-2.455	3.772
9	 5-Ethyl-5-(1'-methyl-prop-2'-enyl) barbituric acid	268	-2.431	3.784
10	 5-Ethyl-5-butyl-2-thiobarbituric acid	300	-2.481	3.759
11	 5-Ethyl-5-(1'-methyl-butyl)-2-thiobarbituric acid (thiopentone)	119.65	-2.301	3.849
12	 5-Ethyl-5-(3'-methyl-butyl)-2-thiobarbituric acid	400	-2.602	3.699
13	 Sodium-5-ethyl-5-(1'-methyl-butyl) barbiturate (sodium pentobarbital)	120.83	-2.086	3.957

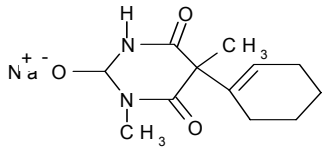
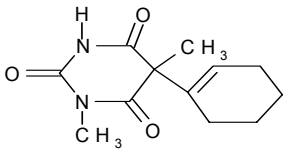
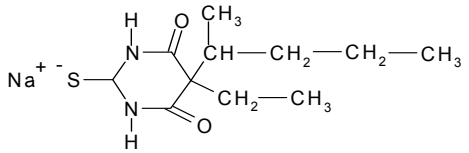
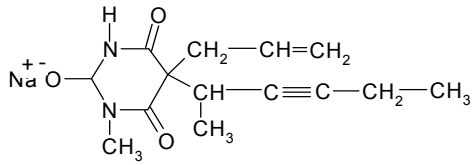
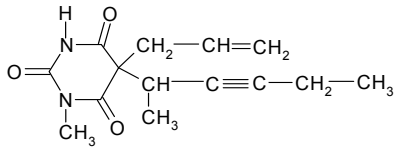
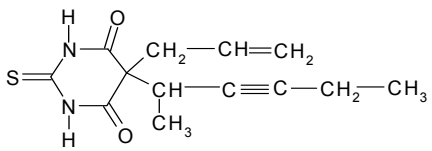
14	 Sodium-3-methyl-5-methyl-5-cyclohexenyl barbiturate (hexobarbital sodium)	387.19	-2.588	3.705
15	 3-Methyl-5-methyl-5-cyclohexenyl barbituric acid	423.24	-2.627	3.686

Table 2

Molecular test structures, measured median anaesthetic doses and working activities of the studied compounds used to verify the predictive power for proposed models

No.	Compound	AD_{50}	$A_i = \log \frac{1}{AD_{50}}$	$A_i^* = \frac{A_i + 10}{2}$
I	 Sodium 5-ethyl-5-(1'-methyl-butyl) thiobarbiturate (thiopental sodium)	149.54	-2.23	3.88
II	 Sodium 5-(2'-propenyl)-5-(1'-methyl-pentin-2'-inyl) barbiturate(methohexital sodium)	46.78	-1.67	4.17
III	 3-Methyl-5-(2'-propenyl)-5-(1'-methyl-pentin-2'-inyl) barbituric acid(methohexital)	57.56	-1.76	4.12
IV	 5-(2'-propenyl)-5-(1'-methyl-pentin-2'-inyl) thiobarbituric acid (thiohexital)	88.36	-1.90	4.05

Note that in Tables 1 and 2 the displaced activities A_i^* are considered for assuring positive activity measure in view of a better correlation and interpretation respecting the analysis based on the genuine activities A_i. However, the median anaesthetic doses fluctuation is within large limits indicating the presence of a complex anaesthetic mechanism towards probable unknown effects.

The computed values of the used descriptors are summarized in Table 3 for the 15 selected trial molecules of Table 1, and in Table 4 for the trial molecules of Table 2.

Table 3

Calculated values of descriptors for the selected anaesthetics of Table 1 used to elaborate statistical models

No.	Log P	Polarizability (Å^3)	Dipole moment (D)	HOMO (eV)	LUMO (eV)	Angle from C_5	Volume R_1 (Å^3)	Volume R_2 (Å^3)
1	1.86	23.14	1.626	-11.2097	-0.1642	113.522	238.11	166.47
2	1.43	22.95	1.989	-9.9700	-0.2007	111.857	235.64	166.47
3	1.60	22.95	1.824	-10.0203	-0.1524	112.241	237.88	166.47
4	1.86	23.14	1.573	-11.2492	-0.1826	111.088	236.78	166.47
5	1.60	22.95	1.620	-10.3765	-0.1334	109.909	239.12	166.47
6	1.53	21.31	1.525	-11.2590	-0.1869	111.154	216.39	166.47
7	1.27	21.12	1.654	-10.2913	-0.1717	109.189	217.65	166.47
8	1.27	21.12	1.689	-9.9635	-0.1638	111.25	214.86	166.47
9	1.25	21.12	1.656	-10.5197	-0.1797	112.317	215.47	166.47
10	2.18	24.47	2.309	-9.3793	-1.1829	111.202	215.76	166.47
11	2.51	26.30	2.534	-9.3564	-1.1697	111.874	238.11	166.47
12	2.51	26.30	2.356	-9.3755	-1.1791	111.101	236.78	166.47
13	3.01	23.08	8.077	-9.6683	0.7962	113.327	238.11	166.47
14	2.32	23.95	13.58	-8.9745	0.1289	109.471	232.25	137.56
15	1.17	24.01	1.724	-9.8757	-0.0533	113.478	232.25	137.56

Table 4

Calculated values of descriptors for the selected anaesthetics of Table 2 used to verify statistical models

No.	Log P	Polarizability (Å^3)	Dipole moment (D)	HOMO (eV)	LUMO (eV)	Angle from C_5	Volume R_1 (Å^3)	Volume R_2 (Å^3)
I	3.35	25.44	6.601	-8.85771	-0.3041	112.064	238.11	166.47
II	3.34	27.29	9.679	-9.3557	0.0662	113.485	266.37	193.42
III	2.20	27.35	2.173	-10.2864	-0.1002	111.457	266.37	193.42
IV	2.60	28.68	2.945	-9.3192	-1.1270	111.604	266.37	193.42

It is worth remarking that the values of LogP coefficient are higher for the salts of barbituric acids and for the thiobarbituric compounds than for the substituted barbituric acids, while the correlation between lipophilicity and barbiturate activity was previously observed.[20]

Going to electronic indices one may notice that: the polarizability of barbituric compounds depends on the unsaturation of substituents from C_5 and has smaller values than the polarizability of thiobarbituric compounds. Barbituric compounds have lower dipole moment than thiobarbituric compounds. Among the studied compounds, the salts of barbituric acids have the highest dipole moment. Thiobarbituric anesthetics have the values for HOMO higher and the values for LUMO smaller than barbituric anesthetics. The salts of barbituric acids have the highest values for LUMO.

For geometric parameters, the angle from the C_5 of the pyrimidinic ring is about 109^0 if only one of the two substituents attaches on the C_5 through a secondary or a tertiary carbon with a sp^2 hybridization.If the substituent is bonding to the C_5 through a secondary carbon with sp^3 hybridization, the angle becomes larger till 113.5^0. The biggest substituent at the atom C_5 has been noted being with R_1 while with R_2 the smaller substituent is recorded.

Multivariate regression analysis has been performed on trial molecules of Tables 1 computing mono-, bi- and three- parameter models with parameters of Table 3, according with Topliss-Costello rule.[21] The best attempts with mono and bi-parametric regressions are presented in Table 5, whereas those with three-parametric models give better results and are explicitly presented in what follows.

Table 5

Regression analysis and quality of correlation for selected mono and bi-parametric models for trial molecules of Table 1 with parameters of Table 3

Descriptors	R-Pearson Correlation	F-Fisher Test	S-Standard Error
Log P	0.26	0.95	0.0830
Angle_C_5	0.50	4.46	0.0742
VR_2	0.52	5.06	0.0730
VR_2, VR_1	0.68	5.36	0.0650
VR_2, Angle_C_5	0.72	6.67	0.0616
LUMO, VR_2	0.72	6.48	0.0621

The first (I) best three-parameter model found contains two geometric descriptors (the volume of substituent R_2 and angle from C_5) and an electronic descriptor (dipole moment) unfolds as:

$$A_I = -\ 1.\ 39472\ (\pm 1.239364) + 0.\ 00657\ (\pm 0.001645)\ VR_2 + 0.\ 03669\ (\pm 0.010517)\ Angle\_C_5 + 0.\ 01272\ (\pm 0.005117)\ Dip \quad (1)$$

n = 15, R = 0.834; F = 8.427; S = 0.051

showing that the anaesthetic activity of barbiturate and thiobarbiturate is mainly regulated by the C_5 angle mechanisms followed by the dipole moment, with a remnant degree by the magnitude of volume of substituent R_2, with an observed-prediction distribution depicted in Figure 1.

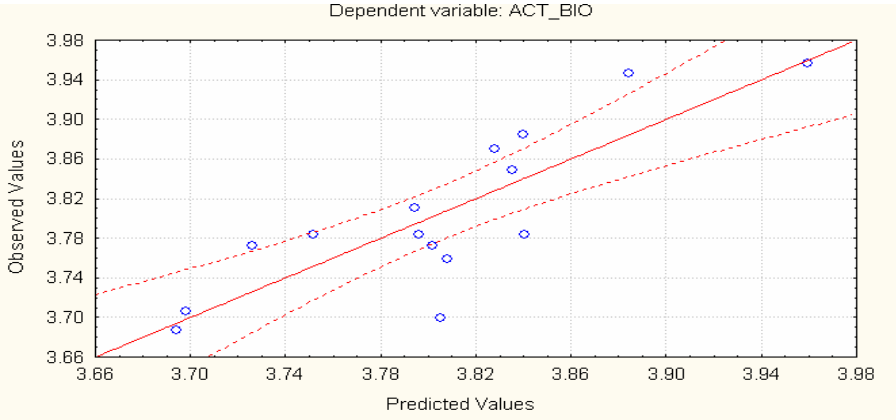


Fig. 1 – Correlation between observed and predicted activity for anaesthetics of Table 1 used to elaborate statistical model of Eq. (1) with parameter data of Table 3.

The next significant three-parameter statistical model (II) is found as based mainly on LUMO electronic descriptor influence, followed by the C_5 angle mechanism and to a lesser extent by the volume of substituent R_2 control:

$$A_{II} = 0.104058\ (\pm 1.181732) + 0.005102\ (\pm 0.001413)\ VR_2 + 0.025905\ (\pm 0.010513)\ Angle\_C_5 + 0.066700\ (\pm 0.027649)\ LUMO \quad (2)$$

n = 15, R = 0.830; F = 8.1759 ; S = 0.052

The regression given by Eq. (2) gives parameters and quality similar to those of Eq. (1), with the graphical observed-predicted illustration in Figure 2.

The third three-parameter statistical model (III) shown in Eq. (3) gives also good statistical results:

$$A_{III} = 2.086003\ (\pm 0.442058) + 0.027262\ (\pm 0.011709)\ POL + 0.137615\ (\pm 0.037370)\ LUMO + 0.006915\ (\pm 0.001593)\ VR_2 \quad (3)$$

n = 15; R = 0.823; F = 7.7241; S = 0.05309

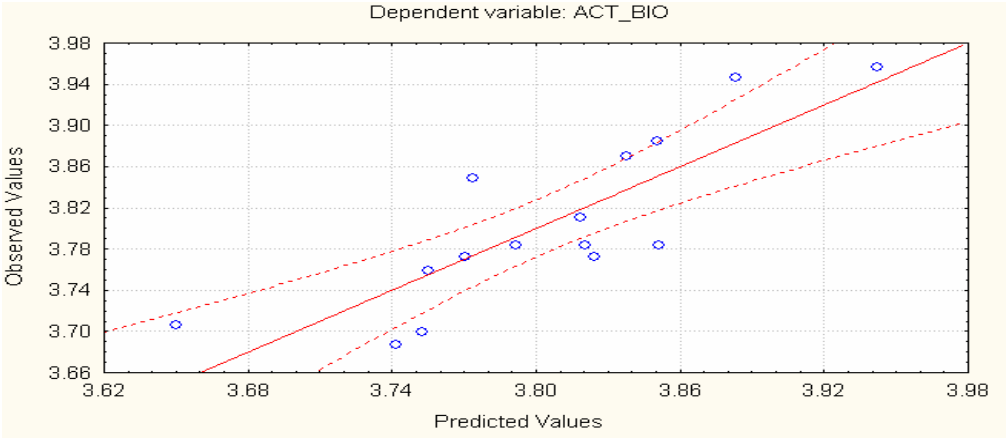


Fig. 2- Correlation between observed and predicted activity for anaesthetics of Table 1 used to elaborate statistical model of Eq. (2) with parameter data of Table 3.

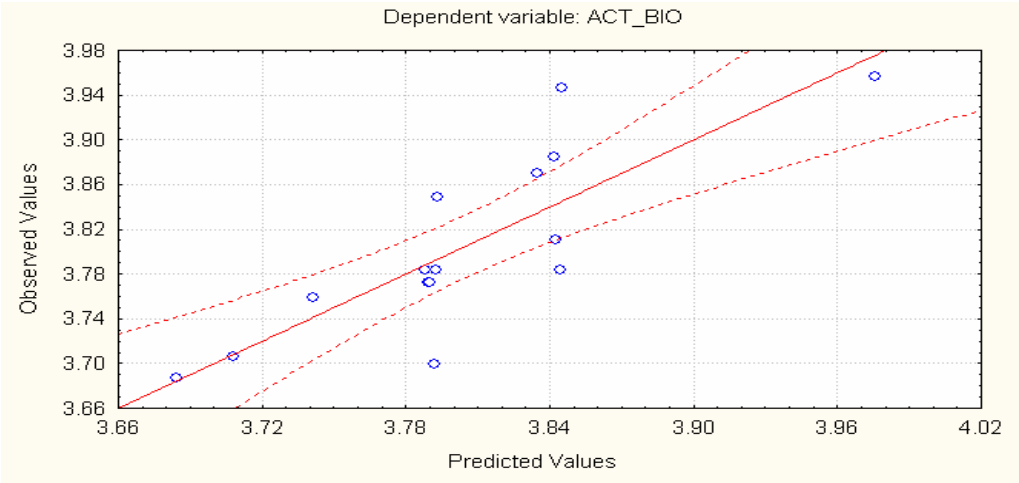


Fig. 3 – Correlation between observed and predicted activity for anaesthetics of Table 1 used to elaborate statistical model of Eq. (3) with parameter data of Table 3.

This time, the anesthetic activity is less dependent on the size of the smaller substituent, R_2, being more strongly associated with electronic effects (polarizability and energy of molecular orbitals, LUMO). The model (III) allows an acceptable correlation between observed and predicted activity as shown in Fig. 3.

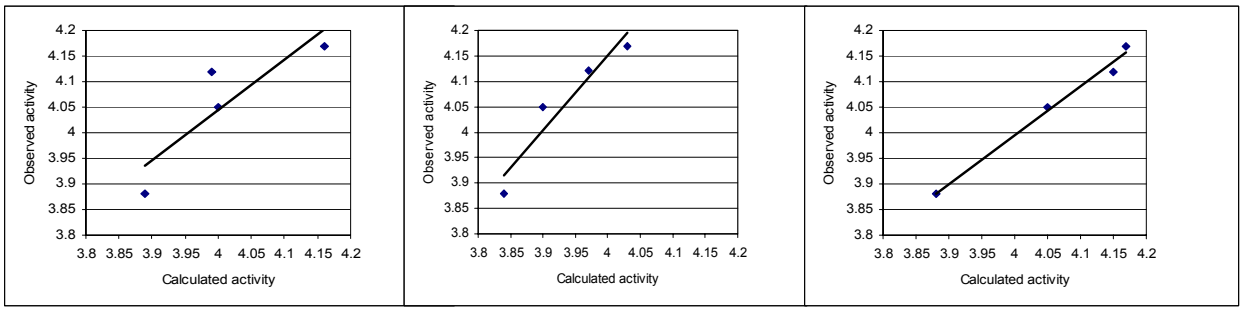
Next, in order to confirm the reliability of our results we have calculated the anaesthetic activity for the four compounds of Table 2, using models expressed by Eqs. (1)-(3). The results are displayed in Table 6 showing that the observed and the calculated activities are very close to each other.

Graphical representation of data presented in Table 6 is illustrated in Fig. 4.

Table 6

Calculated and observed activities for the test compounds of Table 2 with parameters of Table 4

Compound	Calculated activities (axis OX)			Observed activities (axis OY)
	Eq. (1)	*Eq. (2)*	*Eq. (3)*	
I	3.89	3.84	3.88	3.88
II	4.16	4.03	4.17	4.17
III	3.99	3.97	4.15	4.12
IV	4	3.9	4.05	4.05



a) b) c)

Fig. 4 – Correlation between observed and calculated activity for the test compounds of Table 2, according with the results of Table 6 for: a) model (I) of Eq. (1); b)) model (II) of Eq. (2); c) model (III) of Eq. (3).

Since the test analysis implies the monovariate correlations, one employs the relation between the slopes of the interpolation lines in Figure 4 and the associated correlation factors of the equation (see Appendix)

$$y^{Observed} = ay^{Computed} + b \tag{4}$$

providing the results resumed in Table 7.

Table 7

Statistical parameters associated with the equation (4); variances of observed σ_{Y-calc} and calculated σ_{Y-obs} activities (y's), correlation factor $r_{calc-obs}$ and computed slopes, respectively, according with the formulas presented in Appendix, (A3), (A4), (A5), and (A2), respectively, however being implemented within the standard deviation rule (with $n \to n-1$ for the number of observations)

Model	σ_{Y-calc} aka σ_x	σ_{Y-obs} aka σ_y	$r_{calc-obs}$ aka r_{xy}	Computed Slope a
Eq. (1)	0.128	0.146	0.4907	0.556484
Eq. (2)	0.095	-//-	0.537396	0.823171
Eq. (3)	0.153	-//-	0.559309	0.534468

The results confirm the idea that, when the standard deviations approach to each other, the correlation factor is close to the slope of correlation, the case of models upon Eqs. (1) and (3), and particularly for the case of Eq. (3). Another interesting feature is that, for the *test set*, the hierarchy of the correlation factors

$$r_{calc-obs}^{Test-Model-Eq.(1)} < r_{calc-obs}^{Test-Model-Eq.(2)} < r_{calc-obs}^{Test-Model-Eq.(3)} \tag{5}$$

is reverse order than that recorded for the *trial set* which is:

$$RI = 0.834 > RII = 0.830 > RIII = 0.823 \tag{6}$$

According to the present analysis, one may conclude that although the model of Eq. (3) has the lowest correlation factor on trial set it has the highest correlation factor on the test set, i.e., it provides better predictions. Therefore, one may assume it as the best model to be considered for further estimation or for molecular mechanism explanation; in this respect it affirms that the structural electronic parameters as POL and LUMO have more intrinsic influence on anesthetic activity than the geometrical factors entering in models of Eqs. (1) and (2).

CONCLUSIONS

The barbiturates have been used for more than 80 years in modern pharmacology, though a full understanding of the relationship structure-anaesthetic activity of barbiturates and

thiobarbiturates is still in debate. Aiming to provide a molecular mechanistic picture for the biological-chemical interaction of barbiturates with organisms, both trial and test series of compounds have been employed towards multi-linear correlation with hydrophobic, electronic and steric structural influences. The present results shows that the hierarchy of structural parameters in ligand-receptor mechanism may eventually be electronic (through valence orbitals) ≥ geometric angles >> geometric volumes, with an order degree differences between the last two geometrical factors, while LogP appears with the lowest importance in anesthetics metabolic processes. Further studies are thus necessary to better assess the hydrophobicity role in barbiturates' biological activities.

APPENDIX: Monovariate Analysis

Given the data:

y_{obs}	x_{given}
⋮	⋮

there can be proved[22] that the correlation equation

$$y = ax + b \tag{A1}$$

has its slope with the form

$$a = \frac{n\sum_i x_i y_i - \left(\sum_i x_i\right)\left(\sum_i y_i\right)}{n\sum_i x_i^2 - \left(\sum_i x_i\right)^2} = \frac{\sigma_y}{\sigma_x} r_{xy}, \tag{A2}$$

or, written in terms of *standard deviations*:

$$\sigma_x = \sqrt{\frac{1}{n}\sum_i \left(x_i - \bar{x}\right)^2} = \sqrt{\frac{1}{n}\sum_i x_i^2 - \frac{1}{n^2}\left(\sum_i x_i\right)^2} \tag{A3}$$

$$\sigma_y = \sqrt{\frac{1}{n}\sum_i y_i^2 - \frac{1}{n^2}\left(\sum_i y_i\right)^2} \tag{A4}$$

or also, of *the correlation factor*:

$$r_{xy} = \frac{\frac{1}{n}\sum_i x_i y_i - \frac{1}{n^2}\left(\sum_i x_i\right)\left(\sum_i y_i\right)}{\sigma_x \sigma_y}. \tag{A5}$$

With these, it is clear that the monovariate linear correlation has the correlation factor as the direct information included in its slope; indeed, if the *x*- and *y*-standard deviations are considered approximately the same,

$$\sigma_x = \sigma_y \tag{A6}$$

that happens in the ideal case when both the *x*- and *y*- data sets are described by the same normal distribution, it results in the identity:

$$a = r_{xy}. \tag{A7}$$

However, since, in general, we have the case

$$\frac{\sigma_y}{\sigma_x} \neq 1 \tag{A8}$$

it is clear that this ratio "modulates" the correlation slope a to provide the correct, sub-unitary, correlation factor:

$$r_{xy} = \frac{\sigma_x}{\sigma_y} a \leq 1 \tag{A9}$$

this explaining why, even in the cases of a slope higher than unity ($a > 1$), the correlation factor still behaves as sub-unitary.

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