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Novel Ligand-Based Approach to Screening of Large Databases for Paramphistomicide Lead Generation

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Y. Marrero-Ponce†‡ , A. Huesca Guillén∞, Y. Echevarría Diaz†, F. Torrens Zaragozá‡ and F. Ibarra Velarde∞

† Unit of Computer-Aided Molecular 'Biosilico' Discovery and Bioinformatic Research (CAMD-BIR Unit). Central University of Las Villas, Santa Clara, 54830, Villa Clara, Cuba.
‡ Institut Universitari de Ciència Molecular, Universitat de València, Edifici d'Instituts de Paterna, P. O. Box 22085, 46071, Valencia, Spain.
∞ Departament of Parasitology, Faculty of Veterinarian Medicinal and Zootecnic. UNAM, México, D.F. 04510, México.

ABSTRACT

In this report, non-stochastic and stochastic 2D atom-based linear indices were used to the discrimination of paramphistomicide compounds from inactive ones. Two linear classification-based QSAR models were obtained. These equations, performed considering both non-stochastic and stochastic TOMOCOMD-CARDD descriptors, classify correctly 88.57% of chemicals in database, for a good Mathew's correlation coefficient of 0.77. A few anthelmintics compounds and other drugs from the Merck Index, Negwer handbook, and Goodman & Gilman were selected/identified by the models as possible paramphistomicide, one of them was found in the recent literature as possessing this activity. The results demonstrate the usefulness of TOMOCOMD-CARDD method for drug discovery of new lead paramphistomicide compounds.

KEYWORDS: *linear discriminant analysis, TOMOCOMD-CARDD method, atom-based linear indices, QSAR, virtual screening, paramphistomicides.*

RESUMEN

En este informe se emplearon índices lineales estocásticos y no estocásticos en 2D, basados en átomos, para discriminar los compuestos de acción paramfistomicida de los inactivos. Se obtuvieron dos modelos lineales QSAR basados en la clasificación. Estas ecuaciones, llevadas a cabo teniendo en cuenta descriptores TOMOCOMD-CARDD tanto estocásticos como no estocásticos, clasifican correctamente el 88,57% de los elementos químicos de la base de datos, arrojando un buen coeficiente de correlación de Mathews del 0,77. Los modelos seleccionaron/identificaron algunos compuestos antihelmínticos y otros fármacos del índice Merck, del manual Negwer y de Goodman & Gilman como posibles paramfistomicidas, y la literatura reciente incluye a uno de ellos como poseedor de esta actividad. Los resultados demuestran la utilidad del método TOMOCOMD-CARDD para el descubrimiento de fármacos y de nuevos compuestos líderes de acción paramfistomicida.

PALABRAS CLAVE: *análisis discriminante lineal, método TOMOCOMD-CARDD, índices lineales basados en átomos, QSAR, cribado virtual, paramfistomicidas.*

INTRODUCTION

"Models are to be used, not believed".
Menger, F.M *J.Am.Chem.Soc*. 107 (1985) 3105

Paramphistomiasis is one of the important groups of parasitic diseases in several continents.[1-4] This illness caused by a number of species of paramphistomes is responsible for sporadic epizootics of acute parasitic enteritis accompanied with persistent fetid diarrhea in ruminants.4 Adult flukes in the rumen (first stomach or paunch) or reticulum (second stomach or honeycomb) for instance, are not known to cause clinical disease. However, heavy infections with immature flukes in the upper small intestine can cause serious ill-health and death. To control paramphistomiasis, regular use of anthelmintics is still the most practicable method.[5] Chemotherapeutic trials have been conducted under both natural and experimental conditions with variable results.

There is a danger of being swept up in the hype and excitement surrounding the area of bioinformatic and it is clear that in silico predictive modeling does not represent a panacea for the industry. However, nowadays is accepted that the implementation and integration of the opportunity presented by in silico modeling needs to be carried out in a "rational" and systematic manner.[6] The creation of new lead-paramphistomicide compounds by this experimental approach (probe and error method) is a long and complicated process which is based on several factors.[6] An alternative to the "real" world of synthesis and screening of compounds in the laboratory is an in silico "virtual" world of data, analysis, hypothesis and design that reside inside a computer. By this means, "the expensive commitment to actual synthesis and bioassay is made only after exploring the initial concepts with computational models and screens".[7]

Predictive in silico models could be used for structural subsystems identification (from large databases or libraries), accelerating the selection/identification of lead-paramphistomicide compounds.[6]

Therefore, predictive modeling has the potential to transform early-stage drug discovery. In connection, computer-aided drug design has emerged as a rational alternative in the search for novel drugs[7-8] and medicinal chemists are called to developing more efficient strategies for the search of novel candidates to be assayed as paramphistomicide drugs. In relation with it, ***TOMOCOMD-CARDD*** (acronym of the ***TO***pological ***MO***lecular ***COM***puter ***D***esign-***C***omputed-***A***ided ***R***ational ***D***rug.

Design) method has demonstrated to be a useful approach for discovery (identification/selection) of new lead or drug-like compounds with desirable activities.[9-20]

The main aims of this paper follow: 1) to develop quantitative models that discriminates paramphistomicide compounds from the inactive ones using ***TOMOCOMD-CARDD*** approach (2D atom-based linear indices) and Linear Discriminant Analysis (LDA), and 2) to perform a virtual (computational) screening for the search of new lead-paramphistomicide compounds.

In order to assure an adequate extrapolation power for the LDA models, a data set with a great molecular diversity was chosen. We have selected 35 organic chemicals for making up the data set, 20 with paramphistomicide activity, considering different modes of action, and the rest, 15, without this parasiticide activity (inactives).[21-70] Table SD1 gives the names of all the drugs studied (see supplementary data).

It is remarkable to stand out that, the most critical aspect of the construction of the training (learning) sets is to warranty a great molecular and action modes diversity in this data set. Figure 1 depicts a representative sample of such drugs. Both active and inactive compounds are representatives of most of the different structural patterns and modes of action of anthelmintic drugs, such as: a) agonist at nicotinic acetylcholine receptors (levamisole and metyridine); b) cholinesterase antagonists (profenofos and trichlorfan); c) glutamate-gated chloride receptor potentiators (moxidectin); d) increased calcium permeability (praziquantel); e) Inhibition of microtubule formation (albendazole, netobimin and fenbendazole); f) proton ionophores (bithionol, niclosamide, and rafoxanide); g) inhibition of phosphoglycerate kinase and mutase (clorsulon), and so on.[71-72]

Later, the molecular structure of each organic-chemical compounds in dataset was coded using non-stochastic and stochastic 2D atom-based linear indices.[10-12,14,20] These MDs were calculated using the 'in house' ***TOMOCOMD-CARDD*** software.[9] The total and local (atom, group, and atom-type) linear indices for small-to-medium sized organic compounds have been explained in some detail in the literature.[10-12,14,20] The atom-based ***TOMOCOMD-CARDD*** MDs computed in this study were the following: i) k^{th} (k = 15) total (global) non-stochastic atom-based linear indices not considering and considering H-atoms in the molecule [$f_k(\overline{x})$ and $f_k^H(\overline{x})$, respectively], ii) k^{th} (k = 15) local (group = heteroatoms: S, N, O) atom-based linear indices not considering and considering H atoms in the molecule, $f_{kL}(\overline{x}_E)$ and $f_{kL}^H(\overline{x}_E)$, correspondingly. These local descriptors are putative molecular charge, dipole moment, and H-bonding acceptors, iii) k^{th} (k = 15) local (atom-type = H atoms bonding to heteroatoms: S, N, O) atom-based linear indices considering H atoms in the molecule, $f_{kL}^H(\overline{x}_{E\text{-}H})$,. These local descriptors are putative H-bonding donors (hydrogen bonding capacity), lipophilicity, and so on, and iv) The k^{th} total [$^sf_k(\overline{x})$ and $^sf_k^H(\overline{x})$] and group [$^sf_k(\overline{x}_E)$, $^sf_k^H(\overline{x}_E)$ and $^sf_k^H(\overline{x}_{E\text{-}H})$] atom-based stochastic linear indices were also computed. Here, we used the symbols $f_k(\overline{x})$ and $^sf_k(\overline{x})$ for non-stochastic and stochastic atom-based linear indices, respectively.[10-12,14,20]

LDA, an *heuristic* algorithm capable of distinguishing among two or more categories of objects, is a useful technique to find discriminant functions with the ability to distinguish between two groups or populations.[73] To derive discriminant functions that permit the classification of lead-like compounds as positive (presence of paramphistomicide activity) or negative (absence of paramphistomicide activity), we used LDA in which non-stochastic and stochastic atom-based linear indices were used as independent variables. For obtaining LDA-based QSAR models, we used the statistic package STATISTICA.[74] Forward stepwise procedure was fixed as the strategy for variable selection and the principle of parsimony (Occam's razor) was taken into account as strategy for model selection.[75] The quality of the models was mainly determined by examining Wilk's lambda (λ) parameter (U-statistic) and the Mahalanobis distance (D^2). We also inspected the Fisher ratio (F), the p level (p), and the ratios between the cases and the variables in the equation and variables to be explored in order to avoid overfitting or chance correlation as well as the percentage of good classification in the training and test sets.[74,75] Validation of the models was also corroborated by means of a leave-one-out (LOO) cross-validation procedure. We also developed the linear discriminant canonical analysis by checking the following statistic: Canonical regression coefficient (R_{can}), Chi-squared and its p-level [$p(\chi^2)$].[76]

Discriminant ability was assessed in terms of the proportion of correct classifications in each set. The classification of cases was performed by means of the posterior classification probabilities. By using the models one compound can be then classified as active, if ΔP% > 0, being ΔP% = [P(Active) - P(Inactive)]x100 or as inactive otherwise. P(Active) and P(Inactive) are the probabilities that the equations classify a compound as active and inactive, respectively. The probability that a case belongs to a particular group is basically proportional to the Mahalanobis distance from that group centroid. In closing, the posterior probability is the probability, based on our knowledge of the values of others variables, that the respective case belongs to a particular group.

The classification obtained models are given below together with the LDA-statistical parameters:

$$\textbf{\textit{Class}} = 3.34 + 2.76\text{x}10^{-2} f_3(\overline{x}) - 7.57\text{x}10^{-9} f_{15}(\overline{x}) - 4.01 f_1(\overline{x}) - 7.16\text{x}10^{-5} f_{11L}(\overline{x}_{Hal}) + 1.99\text{x}10^{-6} f_{14L}(\overline{x}_{Hal}) \quad (\mathbf{1})$$

N = 35 λ = 0.445 F(5.29) = 7.1266 R_{can} = 0.74
χ^2 = 24.44

Mean (+) = -0.93 Mean (-) = 1.24 C = 0.77
Q = 88.57% p<0.0002

$$\textbf{\textit{Class}} = 3.79 + 1.39 f_0^H(\overline{x}) - 2.98 f_2^H(\overline{x}) + 10.28 f_{11}^H(\overline{x}) + 1.14 f_{14}^H(\overline{x}) - 9.87 f_{15}^H(\overline{x}) \quad (\mathbf{2})$$

N = 35 λ = 0.49 F(5.29) = 5.9642 R_{can} = 0.71
χ^2 = 21.57
Mean (+) = 0.85 Mean (-) = -1.13 C = 0.77
Q = 88.57% p<0.0007

where N is the number of compounds. The classification results (including the canonical scores) for the database (active and inactive ones) with the models **1** and **2** is given as Table 1. In addition, we provide a plot with the ΔP% for the actives and inactives using the non-stochastic and stochastic atom-based linear indices (see Figures 2 and 3).

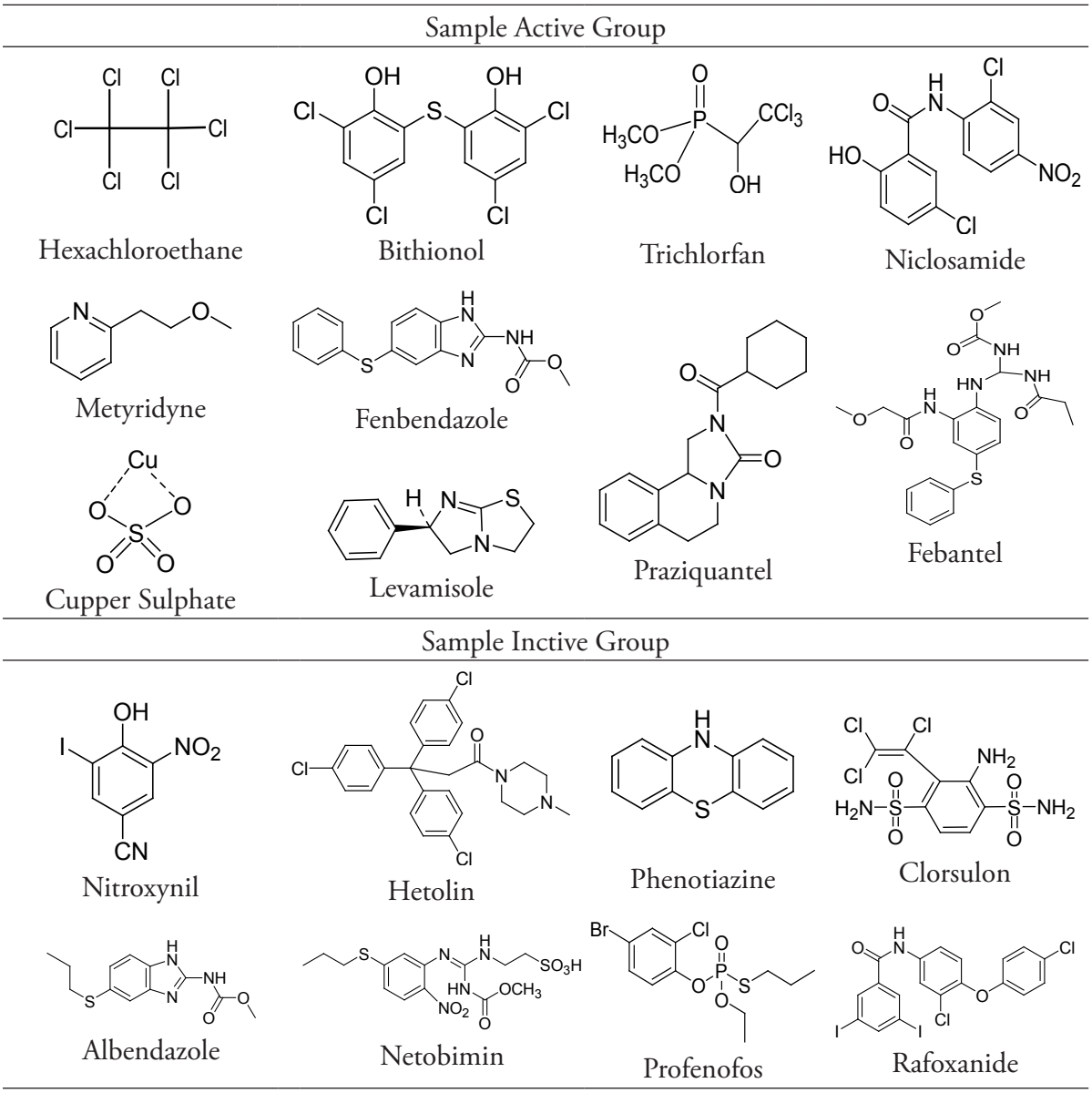

Figure 1. Random, but not exhaustive, sample of the molecular families of compounds studied here.

In Table 2 we illustrate the results obtained in the classification of compounds of the data set using both MDs. As it can be observed in Table 2 both fitted models exhibit good results. These two models correctly classified the 88.57% (accuracy) of the training set and showed high Matthews correlation coefficients (***C***) of 0.77. Table 2 also depicts the values of specificity, sensitivity and false positive rate (also known as 'false alarm rate'), statistical parameters very used in QSAR studies.[77]

To assess the predictability of the discriminant models, a LOO cross-validation was carried out. This methodology systematically removed one data point at a time from the data set. A discriminant model was then constructed on the basis of this reduced data set and subsequently used to predict the removed data point. This procedure was repeated until a complete set of predicted classification was obtained. The global classification of the LOO cross-validation procedure was the same that for data set in both equations (accuracy of 88.57%).

On the other hand, the massive cost of developing new drugs, coupled with candidate attrition rates during the discovery and development processes, highlights the need for a 'sea change' in the drug discovery paradigm.[6] In order to reduce costs, pharmaceutical companies have to find new technologies to replace the old 'hand-crafted' synthesis and testing new chemical entities (NCE) approaches.[78] In this sense, cheminformatics can be used to analyze data from high-throughput screening (HTS) and other forms of chemistry, thereby aiding in the identification of optimal lead structures.[79] In this sense, ligand-based *in silico* screening[80] has emerged as an interesting alternative to HTS.[81,82] By this means, computational techniques are used to select a reduced number of potentially active compounds from large available chemical or virtual combinatorial libraries. The main aim of this approach is to discriminate potent candidate molecules from inactive ones. This kind of *in silico* studies avoid the expensive commitment to actual synthesis and bioassays which are made only after exploring the initial concepts with computational model.[8]

Table 1. Name, Posterior Probabilities (ΔP%) and Canonical Scores of Chemicals in Data Set by Obtained Models.

Drug names[a]	ΔP%[b]	Canonical Scores[c]	ΔP%[b]	Canonical Scores[c]
	Non-Stocastic		**Stocastic**	
	Active Group			
Cupper Sulphate	97,98	-2,14	79,78	0,96
Tetrachloromethane	91,22	-1,45	83,31	1,07
Hexachloroethane	4,92	-0,08	85,08	1,13
Hexachlorophene	99,56	-2,84	95,26	1,73
Bethionol	75,16	-0,93	86,04	1,16
Trichlorfan	93,84	-1,62	93,00	1,52
**Niclosamide	86,12	-1,22	-1,73	-0,16
Oxyclozanide	90,27	-1,40	90,86	1,39
Niclofolan	69,94	-0,83	45,93	0,36
Brotianide	53,73	-0,58	96,50	1,88
*CGA (2-tertiary-butylbenzthiazole)	-66,54	0,71	86,30	1,17
Resorantel	50,03	-0,54	15,00	0,01
*Triclabendazole	-4,77	0,01	73,28	0,80
Levamisol	88,53	-1,32	9,61	-0,05
Thiobisdichloropheno	79,22	-1,02	86,71	1,19
Febantel	29,93	-0,31	96,91	1,95
Bethionol sulphoxide	98,17	-2,19	87,50	1,22
**Metyridine	37,86	-0,40	-33,35	-0,49
Fenbendazole	29,95	-0,32	4,19	-0,10
Dichlorophen	16,76	-0,19	44,53	0,34
	Inactive Group			
Hexachloroparaxilol	-80,73	1,00	-71,42	-1,04
*Phenotiazine	52,08	-0,56	-9,86	-0,24
Hetolin	-99,86	3,33	-99,59	-3,25
Closantel	-85,03	1,13	-60,89	-0,85
Moxidectin	-99,13	2,47	-98,31	-2,55
**Clorsulon	-99,89	3,43	63,55	0,61
Albendazole	-82,48	1,05	-81,04	-1,28
Profenofos	-97,38	1,96	-95,98	-2,10
Netobimin	-87,68	1,22	-99,20	-2,92
Mebendazole	-91,23	1,39	-3,12	-0,17
Rafoxanide	-77,95	0,93	-23,52	-0,38
Oxfendazole	-84,52	1,11	-2,03	-0,16
Mintic	-0,25	-0,03	-59,62	-0,83
***Nitroxynil	11,80	-0,14	71,30	0,76
Praziquantel	-41,91	0,38	-98,64	-2,65

[a]Incorrect classifications from Eqs. **1** or **2** are marked with (*) or (**), respectively. [b]**Δ**P% = [P(+) - P(-)]*100, where P(+) is the posterior probability with which the chemical is predicted as paramphistomicide and P(-) is the posterior probability with which the chemical is predicted as inactive. [c]Canonical scores obtained using canonical analysis.

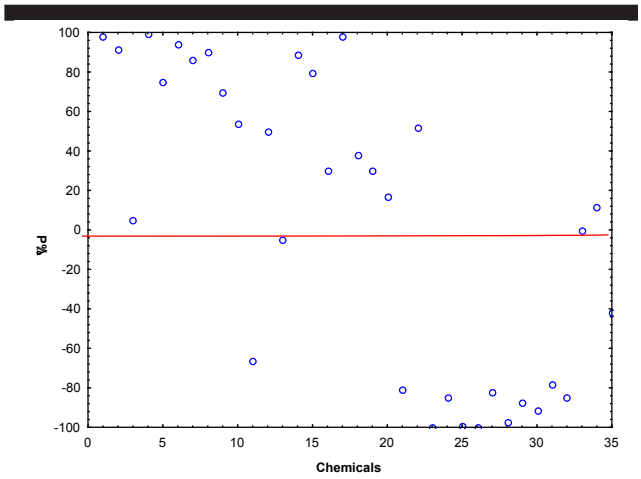

Figure 2. Plot of the ΔP% from Eq. 1 (using non-stochastic atom-based linear indices) for each compound in the database. Compounds 1-20 are active (paramphistomicides) and chemicals 21-35 are inactive (non-paramphistomicides).

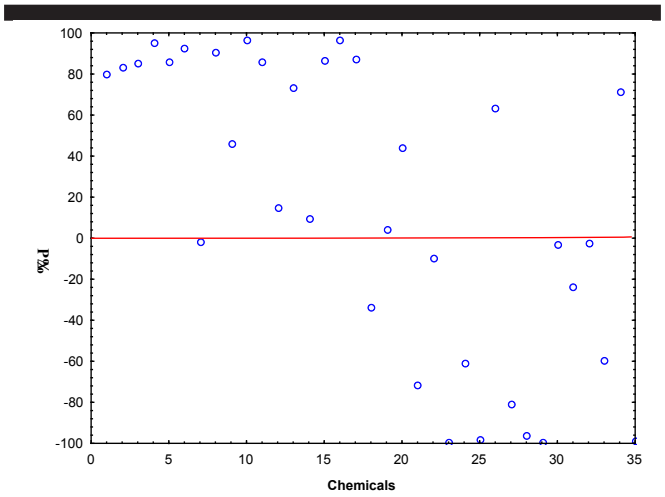

Figure 3. Plot of the ΔP% from Eq. 2 (using stochastic atom-based linear indices) for each compound in the database. Compounds 1-20 are active (paramphistomicides) and chemicals 21-35 are inactive (non-paramphistomicides).

Here we develop a virtual search of paramphistomicide compounds by using the discriminant functions obtained through the ***TOMOCOMD-CARDD*** method. Firstly, we select compounds to which had been reported anthelmintic activity,[83-85] but not have been assay as paramphistomicide. Table SD2 (see supplementary data) depicts the result of the ***TOMOCOMD-CARDD*** classification of anthelmintics in an external set. A few anthelmintics were selected by the discriminant function as possible paramphistomicides. We also looked for these compounds in the literature in order to determine if they have been reported as possessing the paramphistomicide activity. In connection, *diamphenethide* was identified as inactive by obtained models and this molecule has been reported in the literature as ineffective (at 10^{-1}M) *in vitro* test.[29] In the same *in vitro* experiment, the *sodium arsenite* show high paramphistomicide activity and this organic chemical was identified successful by developed ***TOMOCOMD-CARDD's*** models. This result is the most important validation for these QSAR equations, because it has been able to detect a series of anthelmintics as paramphistomicide from a database and some of these compounds have shown the predicted activity at *in vitro* assays.[29]

Finally, we had performed an exhaustive search in the Merck Index,[83] Negwer handbook,[84] and Goodman & Gilman[85] looking for organic-chemical compounds to be evaluated in the models. These chemicals are drugs or drugs-like compounds, which have well-established methods of synthesis and in many cases their toxicological, pharmacodynamical and pharmaceutical properties are well-known. A few compounds were identified by the classification function as possible paramphistomicides, among them we can find known drugs with other pharmacological properties (for more detail see Table SD3 at supplementary data). There is great variability in the functions of these chemicals and also there is great variability in their molecular structures. Nevertheless, most of these compounds identified as actives but not reported in the literature as anthelmintic or paramphistomicides are now in experimental test in order to demonstrate their pharmacological activity.

In conclusion, in this study two models was obtained and successfully applied to the search for drugs-like compounds, exhibiting significant paramphistomicide activity in addition to other pharmacological properties. We therefore conclude that simple, straightforward *in silico* tests such as these described here afford useful means for the initial selection of new chemicals for further more detailed evaluation as possible leads to the development of new and specific veterinary anti-paramphistomun drugs.

Table 2. Classification (confusion) matrices and prediction performances for LDA-based QSAR models.

	% Correct	(-)	(+)	% Correct	(-)	(+)
	Non-Stocastic (Eq. 1)			**Stocastic (Eq. 2)**		
Inactive Group (-)	86.66	13	2	86.66	13	2
Active Group (+)	90.00	2	18	90.00	2	18
Total Set	88.57	15	20	88.57	15	20
	Matthews Corr. Coefficient (*C*)	**Accuracy 'Q_{Total}' (%)**		**Specificity (%)**	**Sensitivity 'hit rate' (%)**	**False positive Rate (%)**
Non-Stocastic (Eq. 1)	0.77	88.57		86.67	86.67	10.00
Stocastic (Eq. 2)	0.77	88.57		86.67	86.67	10.00

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"A bibliography is provided not only as a guide to further reading, but also in acknowledgment of works I have consulted and used"
R. A. Close

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