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QSAR study of anticoccidial activity for diverse chemical compounds: Prediction and experimental assay of *trans*-2-(2-nitrovinyl)furan

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Abstract—In this work we report a QSAR model that discriminates between chemically heterogeneous classes of anticoccidial and non-anticoccidial compounds. For this purpose we used the Markovian Chemicals in silico Design (MARCH-INSIDE) approach [González-Díaz et al. *J. Mol. Mod.* **2002**, *8*, 237–245; *J. Mol. Mod.* **2003**, *9*, 395–407]. Linear discriminant analysis allowed us to fit the discriminant function. This function correctly classifies 86.67% of anticoccidial compounds and 96.23% of inactive compounds in the training series. Overall classification is 94.12%. We validated the model by means of an external predicting series, with 86.96% of global predictability. Remarkably, the present model is based on topological as well as configuration-dependent molecular descriptors. Therefore, the model performs timely calculations and allows discrimination between *Z*/*E* and chiral isomers. Finally, to exemplify the use of the model in practice we report the prediction and experimental assay of *trans*-2-(2-nitrovinyl)furan. It is notable that lesion control was 72.86% at mg/kg of body weight with respect to 60% at 125 mg/kg for amprolium (control drug). The back-projection map for this compound predicts a high level of importance for the double bond and for the nitro group in the *trans* position. We conclude that the MARCH-INSIDE approach enables the accurate fast track identification of anticoccidial hits. Moreover, *trans*-2-(2-nitrovinyl)furan seems to be a promising drug for the treatment of coccidiosis.

1. Introduction

The protist phylum Apicomplexa comprises obligate intracellular parasites of medical and veterinary significance (e.g., *Eimeria*, *Cryptosporidium*, *Plasmodium*, and *Toxoplasma*). The largest subgroup of the phylum contains organisms collectively referred to as the coccidia. Predominantly intestinal parasites, coccidia infect most phyla of invertebrates and all vertebrate classes. The disease they cause, coccidiosis, is recognized as the major health hazard in domestic animal husbandry, in zoo environments, and in wild animal populations.[1]

Since *Eimeria tenella* has a similar drug-susceptibility profile, anticoccidial drugs can also be viewed as a potential source of new antitoxoplasma therapies. Toxoplasmosis is a cosmopolitan zoonotic infection caused by the obligate intracellular protozoan *Toxoplasma gondii*.[2] Toxoplasmosis produces clinical symptoms in few immunocompetent individuals, although as many as 70% of adults in the United States are seropositive. The primary treatment for toxoplasmosis is the antifolate combination pyrimethamine–sulfadoxine given over long periods. Alternative therapy includes the combination of the antibiotics spiramycin, clindamycin, and trimetrexate. Atovaquone was recently introduced for the treatment of *Pneumocystis carinii* pneumonia, is active against both the tachyzoite and cyst forms of *T. gondii*, and may prove to be effective in preventing reactivation of previous latent infections in AIDS patients. Chemotherapy of Toxoplasmosis may be complicated by differences in drug susceptibility among different clinical isolates.[2]

Keywords: QSAR; Anticoccidials; Markov model; Stochastic matrix; Chiral topological indices; *Z*/*E* isomerism; Vinylfurans; *Eimeria tenella*.
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However, from veterinarian and economical points of view coccidiosis is a protozoan disease that costs the U.S. poultry industry about $ 400 million annually and the worldwide cost is estimated at about $ 800 million. Control of coccidiosis is primarily through the use of anticoccidial drugs. In recent years, pharmaceutical companies have not brought new anticoccidials to market. However, with the rise in drug resistance shown by the coccidia, new methods to combat this problem are becoming increasingly important.[3]

All of the reasons outlined above justify the search for novel antimicrobial agents, including anticoccidial and/or antitoxoplasma lead compounds. In this sense, quantitative structure–activity relationships (QSARs) have become an efficient tool for reducing the time and resources required for drug discovery. QSAR techniques are based on the use of so-called molecular descriptors, which are numerical series that codify useful chemical information that can be correlated statistically with biological properties or even physicochemical properties. QSAR techniques have proven successful in the discovery of antimicrobial agents for chemotherapy, including anti-bacterial, anti-parasitic, anti-helmintic, and other antimicrobial compounds.[4–10]

Various QSARs have been reported in connection with anticoccidial and/or antitoxoplasma lead compounds through computer-aided molecular design, e.g., see the work of Rhyu et al., and others. In this sense we can use this as a paradigm for our work. Unfortunately, with a few exceptions, almost all previous QSAR studies of anticoccidials are based on datasets of structurally similar compounds. For instance, the models reported by Gozalbes et al., are based on a heterogeneous series of compounds and focus only on antitoxoplasma activity and not on anticoccidial action. To the best of our knowledge, the one previous QSAR model concerning anticoccidial activity based on a heterogeneous series of compounds was sought using molecular negentropies. Given the above information it is apparent that there is a gap in medicinal chemistry methods for QSAR selection of antiprotozoal compounds. This gap is related to the lack of accurate models for the in silico discovery of anticoccidial compounds with diverse structural patterns.[2,11–14]

On the other hand, our research group recently introduced Markovian Chemicals 'in silico' Design (MARCH-INSIDE) as a novel approach for the virtual screening of drugs, including antimicrobial leads. The method provides a relatively quick method for the calculation of different molecular descriptors such as molecular electronegativity, hydrophobicity, polarizability, electronic entropy, and spectral moments. The QSAR MARCH-INSIDE approach has been used for the prediction of central nervous system, anti-cancer, anti-viral, and antibacterial drugs as well as for the study of drug side effects.[15–22]

In a previous study (see Ref. 14) we explored the utility of electronic entropies calculated by the MARCH-INSIDE approach to predict anticoccidial compounds. This previous study opened the door to the study of other stochastic molecular descriptors in this field. As a consequence, the present work deals with the use of stochastic spectral moments for the QSAR-based selection of novel anticoccidial compounds. First, we developed and validated a linear QSAR model to classify compounds as anticoccidial or not. Finally, we exemplified the use of the model by the prediction and biological assay of *trans*-2-(2-nitrovinyl)furan as a new potential anticoccidial.

2. Results and discussion

Among the QSAR techniques applied in this field, molecular descriptors based on spectral moments are important because of their broad range of applicability. In general, moment analysis has been widely used in many other different contexts of solid, theoretical, and bioorganic chemistry. Estrada and Molina carried out local structure–property studies and used a Markovic approach, with total indices, to highlight interesting applications for moments in chemistry. Gutman used this type of technique to study the structure of benzenoids. Molina et al., employed moments in the design of antibiotics. Morales et al., predicted the mutagenicity of dental monomers. Gónzalez has reported numerous interesting applications of spectral moments in bioorganic and medicinal chemistry. Other authors, such as Cabrera-Pérez et al., focused on pharmacokinetics and biopharmacy applications. Lastly, Vilar et al., reported interesting QSAR approaches to elucidate the mechanism of action of anti-HIV drugs using spectral moments.[23–32]

However, to the best of our knowledge the potential of spectral moments to seek a useful QSAR model for anticoccidial activity has not been investigated. We report here, the application of MARCH-INSIDE stochastic spectral moments, which encode not only topology but molecular chirality and *Z*–*E* isomerism, to discriminate anticoccidials from non-active drugs. This kind of configuration-encoding descriptor is very useful to discriminate between active and non-active stereoisomers, an issue that constitutes a major drawback for pure topologic indices.[33] The best discriminant function found in this work was:

$$\text{Anti-Cocci. Actv.} = -0.532 \times {}^{\text{SR}}\pi_0 + 1.799 \times {}^{\text{SR}}\pi_9 + 16.335 \qquad R_c = 0.90 \quad F = 304.5 \quad p < 0.01 \tag{1}$$

Where R_c is the canonical regression coefficient, F is the Fisher ratio, p is the level of error, and ${}^{\text{SR}}\pi_0(\omega)$ is the 0-step stochastic spectral moment, that is, the sum of the probabilities with which atoms retain electrons (movement of length 0, in terms of topologic distance $k = 0$). The term ${}^{\text{SR}}\pi_9$ is the sum of the probability with which electrons return to all the atoms in the molecule after nine steps (i.e., after going to nine different atoms and returning to the original atom). This parameter (${}^{\text{SR}}\pi_9$) is a measure of very long-range intramolecular electronic delocalization.[17,19,21] Prior to the statistical

analysis we transformed both variables into orthogonal descriptors using the so-called Randič procedure to avoid collinearity problems.[34–36] Consequently, we were able to determine the contribution of both molecular descriptors to the activity directly from the coefficients of the equation. It can be detected that, at least for our data, the probability with which a drug can act as anticoccidial decreases with the order-0 moment. This moment is proportional to the number of vertices in the molecular graph. In this case, we are dealing with small molecules so it means that the descriptors are proportional to the number of atoms in the molecule.[37] Indeed, many anticoccidial drugs do not have particularly large structures. It should also be noted that $^{SR}\pi_9$ has a positive contribution to the activity. As outlined before, this descriptor codifies long-range movements of electrons. Long-range movements of electrons are typical of aromatic systems with large π-electron clouds. Consequently, one would expect that small aromatic molecules could act, depending on their stereochemical structure, as efficient anticoccidials. For a detailed summary of all classification results, see Table 1.

Briefly, this model proved to be statistically efficient in the training and predicting series due to:

(a) This function gave rise to a very good classification of many anticoccidial compounds (86.67%) and almost all inactive compounds (96.23%) in the training series, with an overall classification of 94.12%.
(b) Validation of the model was carried out by means of an external predicting series. In this test, the model showed 89.96% of global predictability. The names of all compounds used in training (a) and validation (b) series, as well as their subsequent probabilities, are shown in Tables 2 and 3.
(c) Fisher tests showed a clear separation of both groups of chemicals at a 5% level of probability of error. Furthermore, there are only 3.3% of unclassified compounds, that is, six cases in 182 (combined training and predicting series). Unclassified compounds are those with $-5 < \Delta P\% < 5$, see Section 4. Both aspects clearly indicate good recognition of both groups by the model.

Finally, we describe an example of the use of the model in an actual search for anticoccidial leads. We calculated the $^{SR}\pi_k$ values and predicted the anticoccidial activity of numerous compounds contained in the CBQ data bank, which is not currently available to the public. As an example we selected one of these compounds for testing: *trans*-2-(2-nitrovinyl)furan. The model predicted this compound to have a high probability of activity. We also corroborated this result experimentally; see Table 4 for details. As summarized in this table there is an excellent match between the prediction and experiment results. It should be noted that *trans*-2-(2-nitrovinyl)furan was tested at a lower dose than amprolium (the control compound) and this represents a good result in terms of the biological activity.

It is important to point out that the compound assayed shows *Z*/*E* isomerism. Unfortunately, the same result is always obtained for both isomers when predicting the biological activity of this kind of compound or chiral molecule using classic topological indices. In this sense, some authors have recently reported the so-called chiral indices. One very important example of chiral topological indices was reported by Golbraikh et al., and this system has been extended to *Z*/*E* isomerism.[38,39] Other important examples in this sense are the chiral linear and quadratic indices reported by Marrero-Ponce et al.[40,41] One of the advantages of the spectral moments used in this text is that they can differentiate *Z*/*E* or chiral isomers.[33]

Finally, the use of atom stochastic moments enables back-projection of the model onto the biological activity.[21] This means that we can, for instance, draw a map projecting the contribution of each atom or group of atoms to the biological activity. This analysis is of major importance to guide future synthetic work.[42–45] Such a map for *trans*-2-(2-nitrovinyl)furan is depicted in Table 4. It appears that both the nitro group and the double bond play an important role, as does the oxygen of the furan ring. However, the current lack of knowledge about the mechanism of action of this compound makes it difficult to provide an explanation for this finding.

Table 1. Results of the statistical analysis

Observed	Predicted				Parameters
		Non-active	Anticoccidial	Total	
Classification matrix for training series					
Non-active	96.23	**102**	4	106	D^2: 6.34, Fisher ratio: 72.61
Anticoccidial	86.67	4	**26**	30	
Total	**94.12**	106	30	136	
Observed	Predicted				
		Non-active	Anticoccidial	Total	
Classification matrix for predicting series					
Non-active	89.47	**34**	4	38	*p*-level: 0.000, Wilks' Lambda: 0.48
Anticoccidial	75	2	**6**	8	
Total	**86.96**	36	10	46	

Compounds correctly classified by the model and percentages of good classification are depicted in boldface.

Table 2. Results for non-active compounds in training and predicting series

Compound	$\Delta P\%$[a]	Compound	$\Delta P\%$[a]	Compound	$\Delta P\%$[a]
		Training series			
CAM	−98.92	Calusterone	−93.40	Magestrol Acetate	−98.04
2,4 DEP	−92.50	Captodiamine	−83.64	MCN-2840	−40.87
Acetoxycycloheximidine	−17.51	Caramiphen	−40.15	Meclizine	−92.35
A-Denopterin	−77.15	CB-10252	−9.60	Mepensolate	−57.06
AL-1965	−59.71	Chlorasquin	−9.31	Mesyldegranol	−60.94
Ambunol	−76.77	Citostal[U]	0.26	Methasquin	−33.07
Amedin	−33.54	Cyanocyline A	−93.32	Mitopodozide	−98.36
Aminohexan	−16.83	Cycrimine	−66.29	Mitoxantrone	−95.53
Aminopterin	−28.55	Demecolcine	−49.42	Nicosin	−70.56
A-Ninopterin	−65.10	Denopterin	−68.22	Osayin	−60.27
Asaline	−98.47	Dibucaine	−70.90	Pentaquine	−29.06
Asamet	−98.86	Diethylstilbestrol[b]	11.24	Phansazin	−92.84
Asazol[U]	4.90	Dimetfolamide	−45.39	Phenaline	−73.66
AT-16	−35.18	Dimezol	−13.85	Pipazethate	−86.62
Azotomycin	−14.13	Diphenhidramine	−86.06	Thiphenamil	−25.73
Bimolane	−99.55	Disulfbumide	−94.31	Tiodazosin	−34.32
Biperiden	−88.13	Drostanolone	−99.50	Toromycin	−97.21
Bisantrene + A239	−75.35	Dyclonine	−32.06	TR 35	−7.47
Bremfol	−33.75	Estramustine	−99.54	Trestolone acetate	−91.63
Brusine	−96.54	Estramustine	−98.58	Triazinate	−75.60
Bupiracaine	−32.45	Euparotin Acetate	−39.24	Trifluoperazine	−77.70
Burseran	−82.89	Eupochlorin A	−22.10	Trimethobenzamide	−89.22
Butastezine	−91.62	Fenafan	−92.65	Trimetrexate[U]	−2.78
Butaverine	−29.50	Fenastezin	−49.76	Spergualin	−99.19
Bututricin	−76.03	Fluoroquine	−6.88	Spirazidin	−95.21
Calcio Mefolinas	−10.25	Fluphenazine	−93.43	Spirogermanium	−93.21
Fotetramine	−89.31	Pipenzolate	−85.83	Sulfinpyrazone	−29.81
Hexacaine	−32.62	Piperidolate	−61.27	Ketotrexate	−92.01
Hexestrol Diphosphate	−34.85	Pramoxine	−52.87	Lofenal	−28.52
Homocoralyne	−56.59	Propiomazine	−31.13	Lomenin-2[b]	15.82
Hydroxizine	−82.81	Quinaspar	−14.36	M-83	−48.32
Idarubucin	−98.60	Rabdophilin G	−91.47	Sibiromycin	−99.13
Imipramine	−79.86	Rotenone	−56.83	Isopropylcad	−98.98
Isopentaquine	−23.66	Rufocromycin	−81.19	V-100[U]	−2.86
Tylophorine	−93.15	Vinervine	−31.78	Votracon	−53.49
Zimet 54/79	−32.69				
		Predicting series			
Alifedrine	−25.95	Hisfen	−46.83	Prosfidium	−99.63
Amino Anfol[b]	24.67	Holacanthone	−74.93	QFI	−75.31
Aminotreofol[U]	0.94	Irisquinone A	−99.56	Rexamid	−13.21
Amygdalin	−93.84	LSD	−47.21	TABAC	−41.38
Bufumustine	−53.00	Macaine	−96.18	Valethamate Br	−77.47
Butacaine SO_4	−73.65	Medorubicin	−98.92	Diampromide	−63.61
Butodicin[U]	−4.47	Methopterine	−53.48	Doxapram	−97.50
Carbetapentane	−89.05	Methotrexate	−23.76	Fentirin[b]	18.53
Chlorbutifenicillin	−97.17	Nannosulfan	19.50	Fluorasquin[b]	20.09
Clofencilan	−42.79	Pentapiperide	−19.58	GEA-29	−84.92
Colchicine	−23.95	Phenadoxone	−89.17	Promicil	−48.30
Coralyne Chloride	−10.71	Phenamet	−73.66	Cyclomethycaine	−96.49
CRC-7001	−81.96	Prochlorperzine	−59.16		

[a] See Section 4.
[b] Misclassified compound.

3. Conclusions

The use of QSAR techniques to minimize the time and financial costs, as well as human and animal resources, has become a new alternative to massive screening in bioorganic and medicinal chemistry. The present results demonstrate the potential of MARCH-INSIDE in the specific virtual screening of anticoccidial drugs. The method is not only accurate but can be used as an idea generator by means of back-projection analysis. We have also provided an illustrative practical example of the use of this method for medicinal and bioorganic chemists.

4. Materials and methods

4.1. Stochastic spectral moments

The MARCH-INSIDE methodology uses MCH to codify information about molecular structure. This

Table 3. Results for active compounds in predicting and training series, as well as for the experimentally assayed compound

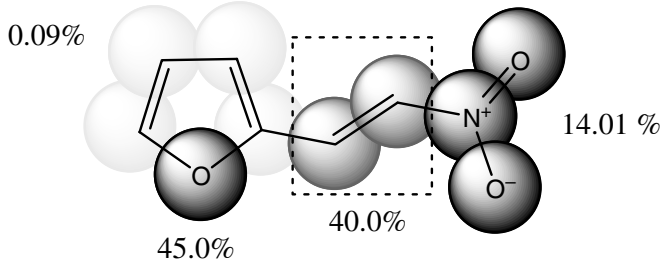
Compound	$\Delta P\%$[a]	Compound	$\Delta P\%$[a]	Compound	$\Delta P\%$[a]
		Training series			
NPA-acid	99.15	Bay g 7183	88.20	Sulfaclozine	99.89
Amquinate	37.00	Methiotriazamine	98.65	Tiazuril	95.08
Bitipazone[b]	−99.91	Nequinate[b]	−31.40	Toltrazuril	82.15
Ciproquinate[b]	−16.45	Nicarbazin	99.58	Tosulur	99.25
Clopidol	100.0	Nitrophenide	99.89	Etopabate	99.56
CP-25415	99.08	Nitromide	100.0	Febrifugine	74.16
Decoquinate[b]	−99.40	Proquinolate	75.76	Glycarbylamide	100.00
Diaveridine	98.63	Robenzidine	98.38	Sch 18545	71.78
Dimethalium cholride	99.61	Romet-30	96.65	Sulfabenz	99.74
Dinitolmide	99.99	Sufaquinoxaline	98.87	Dinsed	82.06
		Predicting series			
Aklomide	100.0	Lasadocid[b]	−100.0	Buquinolate[b]	−16.46
Amprol	95.89	Sulfametrole	99.88	Cocciden	95.95
Arprinocid	99.83	Beclotiamine	98.93		
trans-2-(2-Nitrovinyl)furan					

[a] Differential percentage of subsequent probability (see the text).
[b] Misclassified compound. See Table 4 for structural details of *trans*-2-(2-nitrovinyl)furan.

Table 4. Results of in vivo anticoccidial activity measured in chickens infected with *E. tenella*, chemical structure, and back-projection map analysis of the anticoccidial for *trans*-2-(2-nitrovinyl)furan

Group*	Dose (ppm)	Lesions index	Lesions control (%)	Weight increment (%)
1	2	$1.45 \pm 0.17^{(a)}$	58.57	$80.08 \pm 1.52^{(a)}$
2	4	$1.15 \pm 0.21^{(a)}$	67.14	$86.78 \pm 1.04^{(b)}$
3	8	$0.95 \pm 0.12^{(a)}$	72.86	$83.86 \pm 2.22^{(c)}$
4	125	$1.40 \pm 0.17^{(a)}$	60	$75.19 \pm 1.32^{(c)}$
5	—	$3.50 \pm 0.08^{(b)}$	0	$60.32 \pm 0.67^{(d)}$
6	—	0	100	$100^{(e)}$

trans-2-(2-Nitrovinyl)furan structure and back-projection map



[(a,b,c,d)]There are statistically significant difference between groups at p-level < 0.05.
*Groups 1, 2, and 3, *trans*-2-(2-nitrovinyl)furan; group 4, control drug (amprolium); group 5, infected but not treated animals; and group 6, neither treated nor infected animals.

procedure considers as states of the MCH the external electron layers of any atom core in the molecule (valence shell). The method uses the matrix $^1\Pi$ as the source of molecular descriptors and this matrix has the elements p_{ij}. The matrix is called the one-step electron-transition stochastic matrix. $^1\Pi$ is built as a squared table of order n, where n represents the number of atoms in the molecule. The elements ($^1p_{ij}$) of the one-step electron-transition stochastic matrix are the transition probabilities:[17]

$$^1p_{ij} = \frac{\chi_j \cdot e^{\omega_j}}{\sum_{k=1}^{\delta+1} \chi_k \cdot e^{\omega_k}} \quad (2)$$

Where χ_j is the electronegativity of the atom a_j, which is bonded with atom a_i. The elements of $^1\Pi$ ($^1p_{ij}$) are defined to codify information about the electron-withdrawing strength of atoms to withdraw electrons from their neighbors in the molecule. In the present context, ω is a symmetry codification factor used to specify the 3D environment of each atom in the molecule. Specifically, $\omega = 1$ for *R*-chiral atoms (following Cahn–Ingold–Prelog notation), non-chiral atoms in axial positions in rigid rings or atoms involved in *E*-double bonds. Conversely, $\omega = -1$ for *S*-chiral atoms, atoms in equatorial positions in rigid rings or atoms involved in *Z*-double bonds. Otherwise, $\omega = 0$ for atoms having non-specific 3D environments such as C atoms in $-CH_2-$ groups.[46]

The previously described 3D approach can be exploited in the generation of different topological indices. The

configuration-dependent stochastic spectral moments that will be used here are defined as:

$$^{SR}\pi_k = \sum_{i=1}^{g} {}^{k}p_{ii} = \mathrm{Tr}[(^{1}\pi)^{k}] \quad (3)$$

These molecular descriptors are the traces or moments of the kth-step-electron-transition stochastic matrices ($^{k}\Pi$). These matrices are the successive powers of $^{1}\Pi$. The trace (Tr) or moments can be calculated by summing up the main diagonal elements ($^{k}p_{ii}$) of $^{1}\Pi$. The calculation of $^{SR}\pi_k$ for any organic or inorganic molecule was implemented using the software MARCH-INSIDE.[47]

4.2. Statistical analysis

The approach outlined in the previous sections can be used to try to develop a simple-linear QSAR using MARCH-INSIDE with the general form:

$$\text{Anticoccidial} = b + b_0{}^{SR}\pi_0 + b_1{}^{SR}\pi_1 + b_2{}^{SR}\pi_2 + \cdots\cdots + b_k{}^{SR}\pi_k \quad (4)$$

Here, b_k are the discriminant function coefficients fitted by linear discriminant analysis. The model deals with the discrimination of anticoccidial chemicals from inactive ones. Examination of the Canonical regression coefficient (R_c), Fisher ratio (F), and the p-level (p) determines the quality of the model. We also considered the percentage of good classification. Finally, predictability in an external prediction set validates the model; these compounds were never used to develop the classification function.[48–50]

Each compound was scored in terms of activity by means of the differential probability percentage ($\Delta P\%$). This value was calculated as follows: $\Delta P\% = [P(+) - P(-)] \times 100$, where $P(+)$ is the probability that the drug is predicted as active and $P(-) = 1 - P(+)$ is the probability that the drug is predicted as an inactive compound by the model. This is a rigorous statistical index, which permits us to make a quote for the error. As the model p-level threshold limit is 0.05, we can perfectly classify as anticoccidial those compounds with $\Delta P\% > 5$. Conversely, those chemicals for which $\Delta P\% < -5$ must be classified as inactive. On the other hand, chemicals in the range $5 > \Delta P\% > -5$ must be considered as unclassified by the model at this p-level.[22]

4.3. Biological activity data

Here, we considered a general data set composed of 182 organic chemicals. This original set was split at random to design two different series of anticoccidial chemicals and two additional series of non-anticoccidial ones. A total of 30 anticoccidial drugs and 106 inactive chemicals formed the training series. The remaining chemicals were used in the cross validation. Both the anticoccidial activity and chemical structure of each compound were verified by different references—see a previous study and references cited therein.[14]

4.4. Biological assay

Sufficient quantities of G-0 (of analytical purity) for biological assays were purchased from the Chemicals Bioactive Center. Battery efficacy testing against *E. tenella* was based on the drug screen described previously. We used female animals of the white Leghorn line that were 1-day-old. Maintenance, labeling, and ethical requirements were strictly obeyed in the experiments. Neither anticoccidial additives nor growth promoters were used in the food. A careful selection of animals to ensure homogeneity of groups was carried out when animals were 12 days old. A total of six groups of animal were formed (see previous work and references cited therein[14]):

1. Infected and treated with *trans*-2-(2-nitrovinyl)furan diluted at 2 μg/ml.
2. Infected and treated with *trans*-2-(2-nitrovinyl)furan diluted at 4 μg/ml.
3. Infected and treated with *trans*-2-(2-nitrovinyl)furan diluted at 8 μg/ml.
4. Infected and treated with Amprolium diluted at 125 μg/ml.
5. Infected but not treated.
6. Neither infected nor treated.

Medicated water was given for 2 days beginning at the time of infection. Infection was by oral gavage of sporulated oocyst of *E. tenella*. Each animal was infected with 1×10^5 oocyst. Compounds were administered in water and doses expressed as micrograms of drug per milliliter of water. Efficacy was evaluated 6 days after infection by scoring cecal lesions on scale of 0 (normal) to 4.0 (most severe). Weight gains were also recorded for uninfected group, nonmedicated birds in control groups. Birds were individually weighed and pen-feed consumption was recorded. Activity was defined as a reduction in the lesion score of 1 U in comparison to the lesion score in infected nonmedicated groups (lesion control). The Johnson and Reid technique was used in the experiments and allows an estimate of the macroscopic lesion index. Results for each weight group were statistically analyzed by means of the ANOVA technique and the Wilcoxon technique was used for significance analysis of the lesion index. Differences between groups were determined on the basis of a 0.05 p-level. For further information on the techniques discussed in this section, please see previous work and references cited therein.[14]

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References and notes

1. Duszynski, D. W.; Upton, S. J. In *Parasitic Diseases of Wild Mammals*; Samuel, W. M. P. M. J., Kocan, A. A., Eds., 2nd ed.; Iowa State University Press, 2001; p 416.

2. Gozalbes, R.; Brun-Pascaud, M.; Garcia-Domenech, R.; Galvez, J.; Pierre-Marie, G.; Jean-Pierre, D.; Derouin, F. *Antimicrob. Agents Chemother.* **2000**, *44*, 2771.
3. Allen, P. C.; Fetterer, R. H. *Clin. Microbiol. Rev.* **2002**, *15*, 58.
4. Gopalakrishnan, B.; Khandelwal, A.; Rajjak, S. A.; Selvakumar, N.; Das, J.; Trehan, S.; Iqbal, J.; Kumar, M. S. *Bioorg. Med. Chem.* **2003**, *11*, 2569.
5. Karki, R. G.; Kulkarni, V. M. *Bioorg. Med. Chem.* **2001**, *9*, 3153.
6. Bandyopadhyaya, A. K.; Johnsamuel, J.; Al-Madhoun, A. S.; Eriksson, S.; Tjarks, W. *Bioorg. Med. Chem.* **2005**, *13*, 1681.
7. Marrero-Ponce, Y.; Marrero, R. M.; Torrens, F.; Martinez, Y.; Bernal, M. G.; Zaldivar, V. R.; Castro, E. A.; Abalo, R. G. *J. Mol. Model* **2005**, 1, Online.
8. Marrero-Ponce, Y.; Iyarreta-Veitia, M.; Montero-Torres, A.; Romero-Zaldivar, C.; Brandt, C. A.; Avila, P. E.; Kirchgatter, K.; Machado, Y. *J. Chem. Inf. Model* **2005**, *45*, 1082.
9. Meneses-Marcel, A.; Marrero-Ponce, Y.; Machado-Tugores, Y.; Montero-Torres, A.; Pereira, D. M.; Escario, J. A.; Nogal-Ruiz, J. J.; Ochoa, C.; Aran, V. J.; Martínez-Fernández, A. R.; García-Sánchez, R. N. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3838.
10. Marrero-Ponce, Y.; Castillo-Garit, J. A.; Olazabal, E.; Serrano, H. S.; Morales, A.; Castañedo, N.; Ibarra-Velarde, F.; Huesca-Guillen, A.; Sánchez, A. M.; Torrens, F.; Castro, E. A. *Bioorg. Med. Chem.* **2005**, *13*, 1005.
11. Gozalbes, R.; Gálvez, J.; García-Domenech, R.; Derouin, F. *SAR QSAR Environ. Res.* **1999**, *10*, 47.
12. Rhyu, K. B.; Patel, H. C.; Hopfinger, A. J. *J. Chem. Inf. Comput. Sci.* **1995**, *35*, 771.
13. Ling, Y.; Sahota, G.; Odeh, S.; Chan, J. M.; Araujo, F. G.; Moreno, S. N.; Oldfield, E. *J. Med. Chem.* **2005**, *48*, 3130.
14. González-Díaz, H.; Bastida, I.; Castañedo, N.; Nasco, O.; Olazabal, E.; Morales, A.; Serrano, H. S.; de Armas, R. R. *Bull. Math Biol.* **2004**, *66*, 1285.
15. Santana, L.; Uriarte, E.; González-Díaz, H.; Zagotto, G.; Soto-Otero, R.; Méndez-Alvarez, E. *J. Med. Chem.* **2006**, *49*, 1149.
16. González-Díaz, H.; Vina, D.; Santana, L.; de Clercq, E.; Uriarte, E. *Bioorg. Med. Chem.* **2005**.
17. González-Díaz, H.; Torres-Gomez, L. A.; Guevara, Y.; Almeida, M. S.; Molina, R.; Castanedo, N.; Santana, L.; Uriarte, E. *J. Mol. Model (Online)* **2005**, *11*, 116.
18. González-Díaz, H.; Tenorio, E.; Castanedo, N.; Santana, L.; Uriarte, E. *Bioorg. Med. Chem.* **2005**, *13*, 1523.
19. González-Díaz, H.; Cruz-Monteagudo, M.; Vina, D.; Santana, L.; Uriarte, E.; De Clercq, E. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1651.
20. González-Díaz, H.; Cruz-Monteagudo, M.; Molina, R.; Tenorio, E.; Uriarte, E. *Bioorg. Med. Chem.* **2005**, *13*, 1119.
21. Gia, O.; Marciani Magno, S.; González-Díaz, H.; Quezada, E.; Santana, L.; Uriarte, E.; Dalla Via, L. *Bioorg. Med. Chem.* **2005**, *13*, 809.
22. González-Díaz, H.; Gia, O.; Uriarte, E.; Hernadez, I.; Ramos, R.; Chaviano, M.; Seijo, S.; Castillo, J. A.; Morales, L.; Santana, L.; Akpaloo, D.; Molina, E.; Cruz, M.; Torres, L. A.; Cabrera, M. A. *J. Mol. Model (Online)* **2003**, *9*, 395.
23. Estrada, E.; Molina, E. *J. Mol. Graph Model* **2001**, *20*, 54.
24. Markovic, S.; Markovic, Z.; McCrindle, R. I. *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 112.
25. González, M. P.; Teran, C. M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3077.
26. Molina, E.; González-Díaz, H.; González, M. P.; Rodriguez, E.; Uriarte, E. *J. Chem. Inf. Comput. Sci.* **2004**, *44*, 515.
27. Helguera, A. M.; Cabrera Perez, M. A.; González, M. P.; Ruiz, R. M.; Gonzalez-Diaz, H. *Bioorg. Med. Chem.* **2005**, *13*, 2477.
28. Pérez, M. A.; Sanz, M. B.; Torres, L. R.; Avalos, R. G.; González, M. P.; González-Díaz, H. *Eur. J. Med. Chem.* **2004**, *39*, 905.
29. González, M. P.; González-Díaz, H.; Molina Ruiz, R.; Cabrera, M. A.; Ramos de Armas, R. *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 1192.
30. Pérez-González, M.; Morales Helguera, A. *J. Comput. Aided Mol. Des.* **2003**, *17*, 665.
31. Vilar, S.; Santana, L.; Uriarte, E. *J. Med. Chem.* **2006**, *49*, 1118.
32. Vilar, S.; Estrada, E.; Uriarte, E.; Santana, L.; Gutierrez, Y. *J. Chem. Inf. Model* **2005**, *45*, 502.
33. Gonzalez-Diaz, H.; Sánchez, I. H.; Uriarte, E.; Santana, L. *Comput. Biol. Chem.* **2003**, *27*, 217.
34. Randič, M. *New J. Chem.* **1991**, *15*, 517.
35. Randič, M. *J. Chem. Inf. Comput. Sci.* **1991**, *31*, 311.
36. Randič, M. *J. Comput. Chem.* **1993**, *14*, 363.
37. González-Díaz, H.; Uriarte, E. *Biopolymers* **2005**, *77*, 296.
38. Golbraikh, A.; Bonchev, D.; Tropsha, A. *J. Chem. Inf. Comput. Sci.* **2002**, *42*, 769.
39. Golbraikh, A.; Bonchev, D.; Tropsha, A. *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 147.
40. Marrero-Ponce, Y.; Castillo-Garit, J. A. *J. Comput. Aided Mol. Des.* **2005**, *19*, 369.
41. Marrero-Ponce, Y.; González-Díaz, H.; Zaldivar, V. R.; Torrens, F.; Castro, E. A. *Bioorg. Med. Chem.* **2004**, *12*, 5331.
42. Cruz-Monteagudo, M.; González-Díaz, H. *Eur. J. Med. Chem.* **2005**, *40*, 1030.
43. Stiefl, N.; Baumann, K. *J. Chem. Inf. Model* **2005**, *45*, 739.
44. Stiefl, N.; Baumann, K. *J. Med. Chem.* **2003**, *46*, 1390.
45. Stiefl, N.; Bringmann, G.; Rummey, C.; Baumann, K. *J. Comput. Aided Mol. Des.* **2003**, *17*, 347.
46. González-Díaz, H.; Marrero, Y.; Hernandez, I.; Bastida, I.; Tenorio, E.; Nasco, O.; Uriarte, E.; Castanedo, N.; Cabrera, M. A.; Aguila, E.; Marrero, O.; Morales, A.; Perez, M. *Chem. Res. Toxicol.* **2003**, *16*, 1318.
47. González-Díaz, H.; Molina, R.; Hernandez, I.; Chemicals Bio-actives Center, Central University of Las Villas, Cuba., March, 2006.
48. Marrero-Ponce, Y.; Castillo-Garit, J. A.; Olazabal, E.; Serrano, H. S.; Morales, A.; Castanedo, N.; Ibarra-Velarde, F.; Huesca-Guillen, A.; Jorge, E.; del Valle, A.; Torrens, F.; Castro, E. A. *J. Comput. Aided Mol. Des.* **2004**, *18*, 615.
49. García-García, A.; Gálvez, J.; de Julian-Ortiz, J. V.; García-Domenech, R.; Munoz, C.; Guna, R.; Borras, R. *J. Biomol. Screen* **2005**, *10*, 206.
50. García-García, A.; Gálvez, J.; de Julian-Ortiz, J. V.; García-Domenech, R.; Munoz, C.; Guna, R.; Borras, R. *J. Antimicrob. Chemother* **2004**, *53*, 65.