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Comparative Molecular Field Analysis (CoMFA) on [6] + [6] Fused Pyrazines with Nematocide Properties

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Abstract

Quantitative structure-activity relationship (QSAR) studies, using the Comparative Molecular Field Analysis (CoMFA), on a series of 6,7-diarylpteridine derivatives showing nematocide properties have been carried out. The CoMFA model generated in the study has been used to estimate the namatocide activity (MIC_{50}) of seven 6,7-diarylpteridines related to those previously studied. The model is highly predictive for all pteridine derivatives in the test set. Moreover, this model also predicts satisfactorily the nematocide activity of other [6] + [6] fused pyrazines (quinoxalines and pyridopyrazines) which were not represented in the training set. The analysis revealed the importance of steric factors (64.8%) and then the electrostatic ones (35.8%). Most pteridines under study were previously synthesized and tested as nematocide agents. Now, the synthesis and biological evaluation of 6,7-di-(2′-thienyl)-4(3H)-thioxo-pteridine **36** are reported.

Key words: Molecular Field Analysis (CoMFA), nematocide activity, *Caenorhabditis elegans*, pteridines, quinoxalines, pyridopyrazines.

1 Introduction

Resistance to drugs in use is a great problem in chemotherapy of helmintiasis [1] and therefore efforts to find new compounds showing antihelmintic activity must be carried out. Lately, we have focused our interest in developing new fused pyrazine derivatives showing nematocide activity. The synthesis and *in vitro* assays against *Trichinella spiralis* and/or *Caenorhabditis elegans* and *in vivo* tests against *T. spiralis* of pyrazinothiadizaine dioxide [2], pteridine [3], quinoxaline [4] and pyridopyrazine derivatives [5] were reported.

To obtain further insight into the relationship between the structure and the biological activity of pteridines as new nematocides, we have carried out quantitative structure-activity relationship (QSAR) studies using the Comparative Molecular Field Analysis (CoMFA) method introduced by Cramer et al. [6] in 1988. The CoMFA methodology of 3D-QSAR is based on the assumption that the interaction between an inhibitor and the enzyme is primarily noncovalent in nature and shape-dependent, and identifies the quantitative influence on potency of specific chemical features at particular regions in space [7]. The nematocide activity for most of the pteridines under study were already measured experimentally [3, 5] but structural data on the drug-receptor complexes are absent. Stimulated by the lack of structure-activity relationships, the present work was undertaken to investigate the utility of CoMFA as a quantitative tool for describing inhibitory activities. It was, therefore, hoped that CoMFA can help delineate the structural prerequisites for enhanced biological activity and thus guide the rational design for this new class of pteridines as nematocides.

2 Materials and Methods

2.1 Data set for Analysis

Published [3, 5] and unpublished *in vitro* nematocide properties against *C. elegans*, on a serie of pteridines, were used for this study. The structures of the 36 pteridines constituting the training set in the QSAR analysis are found in Figure 1. The compounds included in the model development differ from one to another in the nature of substituents in both heterocyclic moieties.

The test set of compounds (Figure 2) included several pteridines drawn from compounds that were synthesized together with the above training set as well as some quinoxaline, hexahydroquinoxaline and pyridiopyrazine derivatives.

Abbreviations

CoMFA: Comparative molecular field analysis, DMSO-d_6: dimethylsulfoxide hexadeuterated, DMF: dimethylformamide, Hz: hertz, MHz: Megaherzt, NMR: nuclear magnetic resonance, MIC: minimal inhibition concentration, h: hours, ppm: part per million, s: singlet, dd: double doublet, rms: root mean square, PLS: partial least square.

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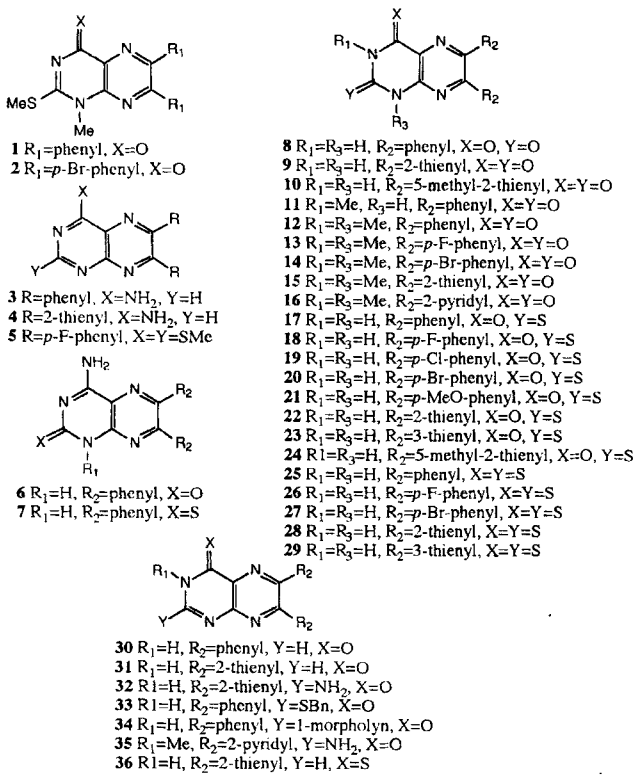

Figure 1. Pteridine derivatives for training set.

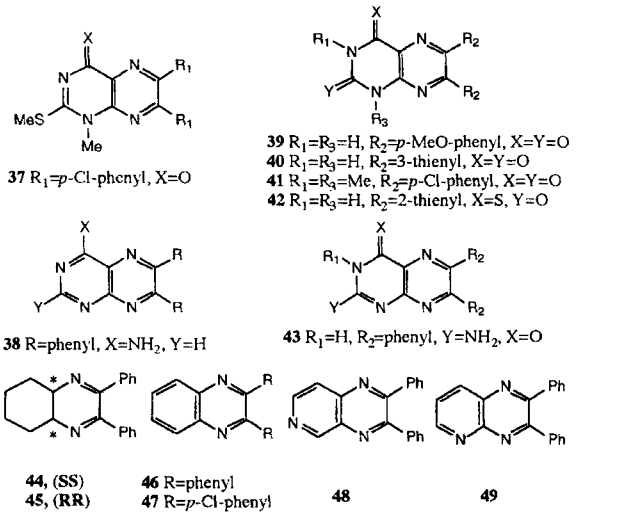

Figure 2. Pteridines (**37–43**) and other [6] + [6] fused pyrazines (**44–49**) for the test set.

2.2 Compounds

The synthesis and chemical characterization of some compounds under study (Figures 1 and 2) have been previously described, **1,3,6–12**, **15–17**, **22**, **24**, **28–31**, **33–35**, **38**, **4–43** [3]; **25** [8], **39** [9], **44**, **45** [4], **46**, **47** [10], **48** [5], **49** [11]. The synthesis of pteridines **2**, **4**, **5**, **13**, **14**, **18–21**, **23**, **26**, **27**, **32**, **37** and **40** will be described elsewhere. 6,7-Di-(2′-thienyl)-4(3H)-thioxopteridine (**36**) was obtained from the 4-oxo-pteridine **31** by thionation reaction using Lawesson reagent (2,4-bis-(*p*-methoxyphenyl)-1,3-dithia-2,4-diphos-phetane-2,4-disulfide) in dry dioxane under reflux for 6 h. Compound **36** was obtained in 41% yield, mp 268–70°C (DMF/H_2O), ^{1}H NMR (DMSO-d6) δ (ppm): 8.32 (s, 1H, H-2), 7.89 (dd, 1H, $^3J_{3',4'} = 5.0$, $^4J_{2',4'} = 1.1$ Hz, H-4′), 7.84 (dd, 1H, H-4″), 7.38 (dd, 1H, $^3J_{2',3'} = 3.7$, $^4J_{2',4'} = 1.1$ Hz, H-2′), 7.28 (dd, 1H, H-2″), 7.15 (dd, 1H, $^3J_{3',4'} = 5.0$, $^3J_{2',3'} = 3.7$ Hz, H-3′), 7.11 (dd, 1H, H-3″). Melting point was determined in a Reicher-Jung apparatus and is uncorrected. Microanalysis was performed with a Heraeus CHN-O-rapid elemental analyzer for C, H, N, S, and the result is within ±0.4% of the theoretical values. The ^{1}H-NMR spectrum was recorded in a Varian Germini-200 instrument at 200 MHz.

2.3 Nematocide Activity Screening

The *in vitro* tests were carried out following the technique of Simpking and Coles [12] with slight modifications [13]. Tests were carried out in tissue-culture 24 wells plates (Nunclon). Compounds were dissolved in dimethylsulfoxide (DMSO) and added to the well to make a 0.5%, or less, final concentration of the solution. All compounds were tested at concentration of 100 μg/ml; subsequently, compounds showing activity higher than 50% were tested at lower concentrations (50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39 and 0.20 μg/ml) to determine the minimal inhibition concentration required to obtain up to 50% (MIC_{50}) in the reduction of *C. elegans* growth. Eight wells were observed for each drug concentration; the same number of control wells without product and others containing 10 ml DMSO were included in all experiments. The effect of compounds on the development and reproductive capacity of *C. elegans* was determined by comparing the population levels attained in the control and test wells after an incubation period of seven days at 20 ± 1°C. Minimal inhibition concentration (MIC_{50}) was calculated from the reduction percentage of nematode population growth at different doses of test compounds in relation to controls. Mebendazole was used as standard nematocide drug (log $MIC_{50} = -2.57$ mM). Statistical analysis was made using the Student's t-test, p values less than 0.05 were considered significant.

2.4 Molecular conformation and Alignment

All molecular modeling techniques and CoMFA studies described herein were performed on Silicon Graphics workstations using the SYBIL 6.3 molecular modeling software [14] from Tripos, Inc., St. Louis, MO.

The compounds were built from fragments in the SYBYL database. Each structure was initially full optimized using the standard Tripos molecular mechanics force field. Partial atomic charges required for calculation of the electrostatic interaction energies were calculated using the Gasteiger-Marsili method [15]. These energy-minimized structures were then subjected to full geometry optimization following the AM1 semiempirical method [16] implemented in MOPAC 5.0 package [17], using the keyword "precise" and not taking into account the amide barrier correction.

CoMFA was then initiated using the minimum-energy conformations obtained as described above. Pteridine **2** was used as the template molecule on which to align the others. All the compounds

were aligned via root mean square (rms) fitting of the four nitrogens atoms of the pteridine ring. In this alignment, all the molecules show the same direction of the dipolar moment.

2.5 CoMFA Interaction Energy Calculation

For the alignment set, the steric and Coulombic potential energy fields were separately calculated at each lattice intersection on a regularly spaced grid of 2.0 Å units in all x, y and z directions. The steric term represents the van der Waals (6–12) interactions, while the Coulombic term represents the electrostatic interactions for which a distance-dependent dielectric expression $\epsilon = \epsilon_0 R_{ij}$ with $\epsilon_0 = 1.0$ was adopted. The grid pattern, generated automatically by the SYBYL/CoMFA routine, extended 5.0 Å units in all directions beyond the dimensions of each molecule.

A sp^3 carbon atom with a van der Waals radius of 1.52 Å and a +1.0 charge was selected as the probe to calculate the steric and electrostatic fields. Values of the steric and electrostatic energy were truncated at 30 kcal/mol.

2.6 Partial Least Squares (PLS) Analysis

To obtain a 3D-QSAR, partial least squares [18] (PLS) fitting was used. The PLS method has been applied successfully in numerous QSAR studies aiming to rationalize those structural features affecting biological activity. PLS regression seeks a relationship between **Y** and **X**, where **Y** is the response or dependent variable and **X** represents the descriptor data.

The PLS algorithm was initially used with the cross-validation option to obtain the optimal number of components needed for the subsequent analysis of the data. In the leave-one-out cross-validation, each compound is systematically excluded from the set and its activity predicted by a model that comes from the rest of compounds. The optimal number of components was then chosen as that which yielded either the smallest rms error or the largest cross-validated r^2 value. This generated a fitted correlation of the entire training set with conventional r^2 values. The 3D-QSAR model so derived was then employed to predict the inhibitory values of the 7 pteridine derivatives in the test set besides 6 related structures of diaryl [6] + [6] fused pyrazines (Figure 2).

Table 1. Summary of CoMFA-PLS results for Training Set of 36 pteridines

r^2 cross-validated	0.776
r^2 conventional	0.910
std error	0.232
no. of components	5
F value	107.896
Contributions: steric	0.642
electrostatic	0.358

Table 2. Observed versus Calculated MIC_{50} (mM) values for pteridines in the training set

Comp	log MIC_{50} (obs)	log MIC_{50} (calcd)	residual	Comp	log MIC_{50} (obs)	log MIC_{50} (calcd)	residual
1	−2.26	−2.13	−0.13	**19**	−2.12	−2.36	0.24
2	−2.71	−2.76	0.05	**20**	−2.04	−2.39	0.35
3	−1.20	−0.81	−0.39	**21**	−1.19	−1.33	0.14
4	−0.49	−0.66	0.17	**22**	−0.54	−0.71	0.17
5	−0.92	−0.91	−0.01	**23**	−0.66	−0.87	0.21
6	−0.80	−0.94	0.14	**24**	−1.40	−1.74	0.34
7	−0.64	−0.93	0.29	**25**	−1.84	−2.02	0.18
8	−0.50	−0.61	0.11	**26**	−2.28	−2.13	−0.15
9	−0.77	−0.63	−0.14	**27**	−3.00	−2.49	−0.51
10	−2.01	−1.72	−0.29	**28**	−1.26	−0.78	−0.48
11	−1.04	−0.58	−0.46	**29**	−1.01	−0.93	−0.08
12	−0.54	−0.61	0.07	**30**	−0.48	−0.59	0.11
13	−2.10	−2.16	0.06	**31**	−1.02	−0.83	−0.19
14	−2.52	−2.48	−0.04	**32**	−0.51	−0.76	0.25
15	−0.55	−0.69	0.14	**33**	−1.15	−0.84	−0.31
16	−0.43	−0.75	0.32	**34**	−0.59	−0.71	0.12
17	−0.76	−0.64	−0.12	**35**	−0.58	−0.72	0.14
18	−0.72	−0.77	0.05	**36**	−1.08	−0.89	−0.19

3 Results and Discussion

3.1 CoMFA of Pteridines with Nematocide Activity

The results of the CoMFA study are summarized in Table 1. The correlation between calculated and experimental MIC_{50} values (Table 2) is shown in Figure 3. This PLS regression analysis used five components for optimal fitting to explain the variance in the biological data. The relative contributions to the CoMFA model are 64.2% steric and 35.8% electrostatic, indicating that the variation in activity among the inhibitors is dominated by differences in the steric (van der Waals) interactions with the receptor.

3.2 Prediction for Compounds of the Test Set

The CoMFA model generated was used to estimate the MIC_{50} values of the test pteridines depicted in Figure 2. The observed and corresponding CoMFA-predicted MIC_{50} values (mM) for the test set compounds are listed in Table 3 and plotted in Figure 3. A comparison of these MIC_{50} values shows that CoMFA can generally predict the activity of this new family of nematocide pteridines to within a log unit of their measured value, regardless of whether they are more or less active taking as reference Mebendazole. As expected, the model was highly predictive for all pteridine derivatives in the test set, thus reflecting the structural composition of the training set. In addition, the model also satisfactorily predicted the activity of other test-set, [6] + [6] fused pyrazines (Figure 2), not represented in the training set. Consistent with the biological data, it predicted that (RR)-2,3-diphenyl-hexahydroquinoxaline (**45**) is more active than its (SS)-enantiometer (**44**). The model also successfully predicted the activities for diaryl quinoxalines (**46** and **47**) and diphenylpyridopyrazines (**48** and **49**) (Table 4).

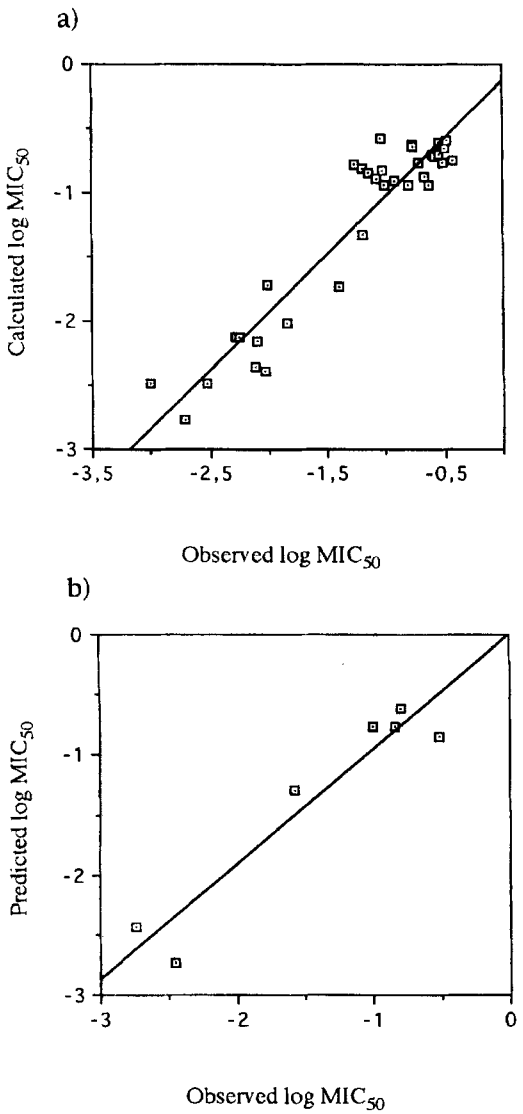

Figure 3. Plots of observed and predicted *versus* calculated log MIC_{50} values for training (a) and the test (b) set compounds.

Table 3. Observed versus predicted MIC_{50} values for pteridines in the test set

Comp.	log MIC_{50} (obs)	log MIC_{50} (calcd)	residual
37	−2.46	−2.74	0.28
38	−1.00	−0.76	−0.24
39	−2.74	−2.43	−0.31
40	−0.84	−0.76	−0.08
41	−0.52	−0.84	0.32
42	−1.57	−1.30	−0.27
43	−0.80	−0.62	−0.18

Table 4. Observed versus predicted MIC_{50} values for diaryl [6] + [6] fused pyrazines

Comp.	log MIC_{50} (obs)	log MIC_{50} (calcd)	residual
44	−0.73	−0.44	−0.29
45	−1.58	−0.95	−0.63
46	−2.52	−2.00	−0.52
47	−1.80	−2.23	0.43
48	−0.44	−0.78	0.35
49	−1.59	−1.93	0.34

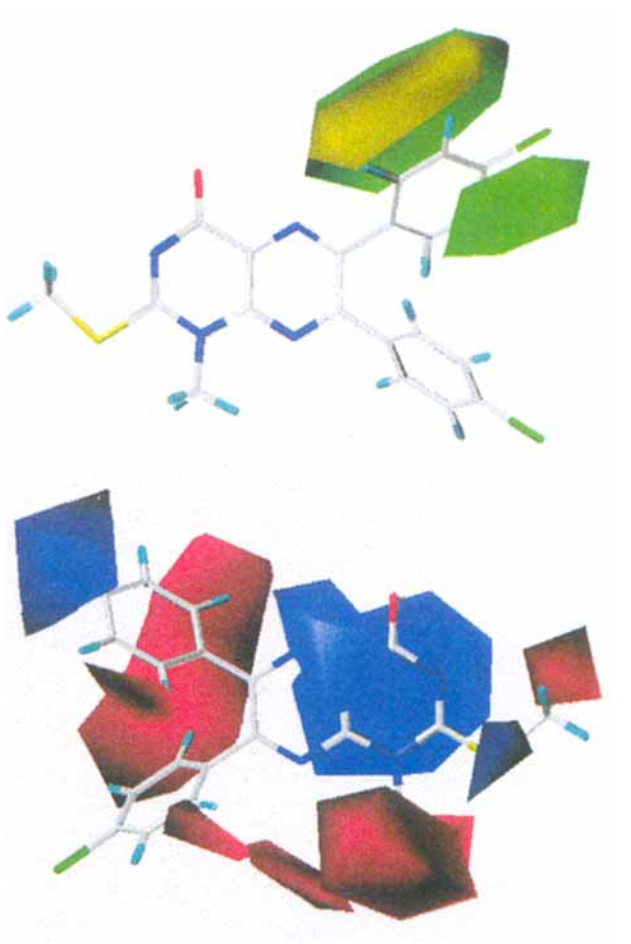

Figure 4. Views of the CoMFA steric and electrostatic contour plots: Regions where increased steric bulk is associated with enhanced activity are indicated in green, while regions where increased bulk is associated with diminished activity are indicated in yellow. Regions where increased positive charge is favourable for activity are indicated in blue, while regions where increased negative charge is favourable for activity are indicated in red.

3.3 CoMFA Fields

In order to visualize the information content of the derived 3D-QSAR model, CoMFA contour maps were generated by interpolating the products between the 3D-QSAR coefficients and their associated standard deviations. Figure 4 shows a view of the CoMFA steric field contribution contour map from the analysis

done using compound **2** as reference structure. The green and yellow polyhedra describe regions in space around the molecule where additional steric bulk in the molecules enhance or diminish anithelmintic properties. CoMFA field contributions highlight regions where steric interaction energies are enhanced by groups of increasing steric bulk in the neighbourhood. An examination of the CoMFA steric field revealed that position 6 of pteridine moiety is the most sensible to the steric properties. In this study, the steric field map also indicated that a different orientation of the aryl fragment in this position has a measurable effect on activity. When torsion angle between the 6-aryl moiety and the pteridine nucleus has a value around $+60°$ compounds are more active than those where this torsion angle is around $-60°$. Adding bulky groups at 6-position is predicted to enhance the nematocide activity, the torsion angle has a positive value always (see figure 4). Hence, methylthienyl derivatives **10** and **24** show more activity than the corresponding thienyl ones **9** and **22** respectively, or by changing phenyl (**3** and **43**) by thienyl moiety (**4** and **32**) a decreasing activity is shown. Consistent with this assessment, green contours in the CoMFA steric map surround the immediate portion of these substituents. Taking into account the commented steric factor, derivatives bearing bulky substituents at 6-aryl moiety should be more potent derivatives than unsubstituted ones.

Figure 4 shows a view of the same structure embedded in the CoMFA electrostatic contour maps. The red and blue polyhedra describe regions where a high electron density (i.e., negative charge or polarity) within the substrate structure enhances or diminishes activity, respectively. The electrostatic field map shows a red contour over 6-aryl substituent as well as a blue contour surrounding the *para* substituent of 6-aryl moiety. This fact indicates that a low electron density in *p*-substituent together with a high electron density in 6-aryl moiety will lead to enhanced potency. Additionally, the presence of the blue contour near of pteridine nucleus indicates that low electronic density at this nucleous enhance the activity. Therefore, an aromatic derivative must be more potent compound than its non-aromatic homologue. In fact, that is the case of quinoaxaline **46** and its hydrogenated **44** and **45**. On the other hand, in the case of pteridines we cannot predict *a priori* if an aromatic derivative, such as **3** or **4**, will be more or less potent than another non-aromatic derivative, **8** or **9**, since the different electronegativity of substituents in pteridine nucleus influences the charge on it.

4 Conclusion

The CoMFA method has been applied successfully to rationalize the nematocide activity of diarylpteridines in terms of their steric and electrostatic properties. The 3D-QSAR yielded a regression equation with a high degree of statistical significance and performed exceptionally well in predicting the activity (MIC_{50}) of more compounds in the test set, even those (e.g. quinoxalines and pyridopyrazine) not represented in the training set. However, it must emphasized that the alignment considered in this study was selected in the absence of experimental structural data on the nematode receptor-inhibitor complex for these compounds. All the information obtained from this study will be considered for the design of new nematocides.

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