Pesticide Biochemistry and Physiology 95 (2009) 33–37

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

Quantitative prediction of AHAS inhibition by pyrimidinylsalicylate based herbicides

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ARTICLE INFO

Article history:
Received 15 October 2008
Accepted 4 June 2009
Available online 9 June 2009

Keywords:
AHAS
Agrochemicals
Enzyme inhibitors
QSAR
Semiempirical calculations

ABSTRACT

Acetohydroxyacid synthase (AHAS) inhibition by pyrimidylsalicylate based herbicides is studied within the framework of quantitative structure-activity relationship (QSAR) methodology. A general model for this family of herbicides is developed to predict molar pI_{50}, i.e. the logarithm of the reciprocal molar concentration of herbicide required for 50% inhibition of the AHAS activity. The model, which involves only four descriptors: two geometric and two quantum chemical, accounting for the steric, electrostatic and hydrogen bonding interactions responsible for the binding of the herbicide to the enzyme; predicts the molar pI_{50} with a squared correlation coefficient of 0.89 and a standard deviation of 0.43. The training set includes 30 structures of substituted *O*-(4,6-dimethoxypyrimidin-2-yl) salicylic acids and thio analogs, covering a pI_{50} range from about 3 to 8 U. The model is validated with an external set of 13 structures not included in the training set.

1. Introduction

The necessity of increasing the productivity per cultivation area is a peremptory demand since, on hand, the available surface is limited, even worse it has diminished due to the degradation of the soil; and on the other hand, it is necessary to supply the food demand of a steadily increasing population.

To supply this demand of the current world population, about 6000 million, it is required to produce more and more. To do this is necessary to use massively chemicals, known generically as agrochemicals (insecticides, fungicides, acaricides, nematocides and herbicides). The use of these chemicals has allowed for significant reduction of the agriculture plagues and consequently increased the productivity. Among the pesticides, the herbicides deserve special attention since, due to the resistance developed by weeds, new products have to be steadily introduced to market [1,2].

Inhibition of essential amino acid biosynthesis in plants is one of the most prominent and attractive mode of action of herbicidal activity. Acetohydroxyacid synthase, AHAS, the first common enzyme in the biosynthetic route to the branched chain amino acids, valine, leucine and isoleucine, has been identified as the target of action of several structurally different types of chemicals (sulfonylureas, sulfonamides, imidazolinones and pyrimidylsalicylates) with high herbicidal activity. These four classes of herbicides, all obtained by traditional screening methods, have the attributes of low application rates, good crop selectivity, environmental safety and low mammalian toxicity. These herbicides act inhibiting the acetohydroxyacid synthase enzyme leading to the starvation of the plant for these amino acids, it is this starvation that is thought to be the primary mechanism by which these chemicals cause plant death. Since these inhibitors do not resemble the substrates or products of the AHAS catalyzed reaction it has been suggested that they do not bind to the active site but at the entrance of a tunnel leading to this site making multiple interactions with amino acid side chains in this tunnel [3–5].

Despite of the large number of papers on AHAS published in recent years, most of them by Duggleby and coworkers, on the interaction between herbicides and this enzyme there are some aspects of the inhibition that remain puzzling [6]. Thus, it is important to elucidate which structural features of the herbicides are responsible for the enzyme inhibition. This is essential for the design of novel herbicides since its properties may be predicted prior to synthesis and consequently the design may, in this way, be guided by the results of calculations.

Quantitative structure-activity relationship (QSAR) modeling has shown to be very effective for this purpose. The underlying assumption in a QSAR study is that the structural formula contains, in principle, coded within it all of the information which predetermines the chemical, biological and physical properties of the chemical. If we could elucidate in detail how these properties are determined by structure we were able to predict such properties simply from the molecular structure.

Unlike sulfonylureas and sulfonamides, AHAS inhibition by pyrimidylsalicilates has been scarcely studied by QSAR modeling

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doi:10.1016/j.pestbp.2009.06.001

[7,8]. In one of these papers several QSAR models, based on Verloop sterimol parameters, are reported for three families of pyrimidyl(thio)salicylate herbicides; however, a general model is not proposed. Surprisingly, considering the similarity of the compounds used to develop the models, they involve different number and types of descriptors, complicating the physical interpretation. On the other hand, the models are based on empirically derived descriptors which limit their application to new or developing chemicals.

In this study, we report a QSAR model to predict molar pI_{50}, the logarithm of the reciprocal molar concentration of herbicide required for 50% inhibition of the AHAS activity. The model involves only four descriptors, two geometric and two quantum chemical, accounting for the steric, electrostatic and hydrogen bonding interactions responsible for the enzyme inhibition.

2. Chemical data

The data set of the pI_{50} was taken from the data reported by Nezu et al. [8]. The set contains 46 structures of substituted *O*-(4,6-dimethoxypyrimidin-2-yl)salicylic acids and thio analogs inhibiting AHAS, including 6-substituted(thio)-, 5- and 6-substituted salicylic acids, Fig. 1; covering a pI_{50} range from about 3 to 8 U.

3. Methodology

Empirical evidences show that the acidic carboxyl group of these pyrimidylsalicylates is indispensable for AHAS inhibition; moreover, it has been suggested the carboxylate group is responsible for the binding of the inhibitor molecule to the enzyme [7]. Enzymes are proteins which are actives under relative mild reactions conditions: temperature below 100 °C, atmospheric pressure and nearly neutral pH. Therefore, at these conditions of pH these pyrimidylsalicylate based herbicides are in the anionic form, since their pK_a values go from about 3.5 to 5 [8].

Modeling was performed in order to set the anions in their lowest-energy 3D conformations. To achieve this goal, initial three-dimensional geometries of the chemical structures were generated using Hyperchem 7.0 molecular modeling package [9]. These 3D structures were refined later using Ampac 5.0, a semiempirical molecular modeling program [10], using AM1 parameterization. To determine the lowest-energy conformations for each molecule, geometry optimizations were carried out allowing one or more torsional angles to vary systematically. The keyword CHARGE= −1 was always used in all cases. The Ampac output files, containing the lowest-energy structures and the respective electron wave functions of individual compounds, were loaded into the Codessa program [11] to calculate the molecular descriptors. This pool of descriptors was reduced by removing descriptors that could not be calculated for every structure in the data set, and by eliminating one descriptor from those pairs highly correlated. Afterwards, from this reduced pool of descriptors the best multilinear correlation QSAR model was searched using the Sigmastat statistical package [12].

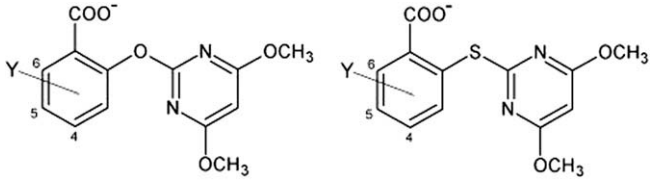

Fig. 1. General structures of *O*-(4,6-dimethoxypyrimidin-2-yl) salicylates and thio analogs.

4. Results

A total of 184 descriptors, 12 geometrical and 172 quantum chemical, were calculated for all compounds. The best regression equation found involves only four descriptors, two geometrical and two quantum chemical: S_M, the molecular surface area; S_{XY}, the normalized shadow area of the molecule projected on the XY plane; HOMO, the energy of the highest occupied molecular orbital; and FNSA, the fractional partial negatively charged surface area.

It is noteworthy that these descriptors individually correlate poorly with the property (R^2 = 0.22, 0.27, 0.11, 0.01 for S_M, S_{XY}, HOMO and FNSA, respectively), however, they collectively correlate pretty well with the property, R^2 = 0.89. This is an interesting result since, from the respective individual correlation coefficient, these descriptors are seemingly not relevant for predicting inhibition. Nevertheless, the relevance of these descriptors it is made evident only when the correlation with the collective is considered. This means that the interaction of information among the descriptors add an important additional predictive value which goes further from the simple sum of the information contained in the individual descriptors. This finding is in agreement with the widely accepted idea that inhibition is driven by several interactions (steric, electrostatic and hydrogen bonding) which occur simultaneously and synergically.

The best regression equation found is the following:

$$pI_{50} = 13.70 - 0.04\,S_M + 18.77\,S_{XY} + 0.79\,\mathrm{HOMO} - 4.99\,\mathrm{FNSA}$$

and its respective statistics is shown in Tables 1 and 2. In these tables the statistical parameters have the usual meaning. The *p*-values indicate that all descriptors are statistically significant at the 99% confidence level. On the other hand, the VIF values about 1 indicate there is no serious collinearity between the involved descriptors [13]. Therefore, it is concluded that all the independent variables included in the model are relevant.

The experimental and calculated values of pI_{50} along with the values of the descriptors involved in the model are shown in Table 3. The respective scatter plot is shown in Fig. 2.

To check the predictive capability of the model, it was tested with an external set of chemicals not included in the training set. The validation data set included 13 chemicals including 5- and 6-substituted salicylic acids as well as 6-substituted thio analogs. In Table 4, the values of the molecular descriptors along with the experimental and calculated values of pI_{50} for the validation set are shown. The statistics for the validation is as follows: R^2 = 0.84, s = 0.33, F = 59. These results confirm the prediction capability of the model.

5. Discussion

From the explanations suggested in literature, it seems to be logical to think the first requirement that the inhibitors must meet

Table 1
Statistical parameters for the best QSAR model.

	Coefficient	Std. error	*p*-value	VIF
Constant	13.70	2.76	<0.001	
S_M	−0.04	0.003	<0.001	1.31
S_{XY}	18.77	2.39	<0.001	1.81
HOMO	0.79	0.26	0.006	1.99
FNSA$^{(1)}$	−4.99	1.30	<0.001	1.11
R^2 = 0.89, R^2_{CV} = 0.85, R^2_{df} = 0.88, s = 0.43				