Understanding the Topography of the Photosystem II Herbicide Binding Niche: Does QSAR Help?

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QSAR, Photosystem II Inhibitors, Cyanoacrylates

For thirty years the study of Quantitative Structure Activity Relationships (QSAR) has been an active area of research aimed at developing an understanding of the interactions between inhibitors of photosynthetic electron transport and the herbicide binding site in the Photosystem II (PS II) reaction centre. Many QSAR studies of PS II inhibitors with diverse chemical structures have emphasized the hydrophobic nature of the binding domain, with lipophilicity being the dominant determinant of Hill inhibition activity. The cyanoacrylate classes of PS II inhibitors also show a diversity of active structures and considerable variation in inhibition potency with minor alterations to structure. QSAR analysis and examination of chirality in cyanoacrylate inhibitors has also shown the importance of steric factors in determining activity. Different modes of binding for different classes of cyanoacrylates have been identified; a classical urea-type relationship between activity and hydrophobicity and another type of interaction in which the lipophilicity or electronic nature of phenyl substituents plays little part and the size of the substituents is of primary importance. Because size and shape are parameters of great importance in determining the topography of a binding site, QSAR studies of flexible PS II inhibitors such as cyanoacrylates will continue to be important in elucidating the intricacies of inhibitor/PS II interactions.

Introduction

A critical analysis of the use of QSAR (Quantitative Structure Activity Relationships) and molecular graphics in herbicide design was presented by Draber in the special issue of Zeitschrift für Naturforschung reporting the proceedings of the international workshop "Herbicides affecting chloroplast functions" held at Lake Placid, N.Y., in August, 1986 [1]. At that time, there was an optimistic expectation that a combination of classical QSAR analyses and the rapidly evolving techniques of computer generated molecular graphics would lead to new highly potent and commercially useful herbicides.

The application of QSAR methods attempting to link chemical structure and biological activity began with the pioneering work of Hansch and Fujita in the early 1960s [2]. The basis of the approach was to develop a set of numerical descriptors of physical parameters that can be related to the biological properties of a molecule. This allows determination of a quantitative relationship between compound structure and biological activity. Obviously, the problem of understanding the interaction of small molecules with specific macromolecular receptors is a formidable one. Nevertheless, attempts to quantify these interactions have resulted in an extensive QSAR literature. The resulting mathematical models can provide an insight into the nature and topography of the active site region of a biological receptor. Apart from leading to a greater understanding of binding interactions, these models may also allow the possibility of predicting the activity of new molecules.

A significant proportion of the QSAR studies with agrochemicals is concerned with photosynthetic herbicides. QSAR analysis of these compounds was facilitated by the availability of a quick, convenient method of measuring the intrinsic activity of inhibitors of photosynthetic electron transport; the Hill reaction in isolated chloroplasts. Although the use of the Hill reaction in such studies does have disadvantages in that there is not always a good correlation between activity in the Hill reaction and useful herbicidal activity, an extensive literature on QSAR of this type has accumulated.

This paper will examine some of these QSAR studies and attempt to assess their contribution to the current knowledge of the nature and topography of the photosystem II (PS II) herbicide bind-

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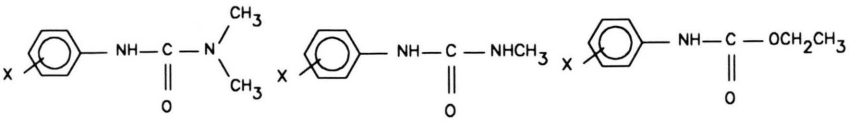
ing site. Whether these methods have contributed to the development of new herbicides, or even to the discovery of new inhibitors, is more difficult to assess since product development is a complex process shrouded in commercial confidentiality. Therefore attention will be focussed on the similarities and differences between classes of PS II inhibitors of the amide/triazine family and the value of QSAR studies in understanding the interactions of these inhibitors with the D 1 protein of PS II.

Discussion

The first significant use of QSAR to correlate the biological activity of PS II inhibitors with chemical structure appeared in 1966 [3]. Hansch and Deutsch analyzed inhibition of the Hill reaction by a series of ureas (**1**) and derived the following relationship:

$$pI_{50} = 1.29\pi + 0.54\sigma + 4.18 \qquad (1)$$

$$n = 12,\ r = 0.94,\ s = 0.37.$$



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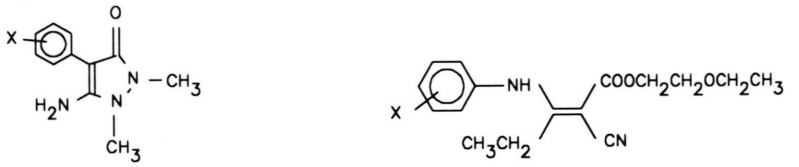

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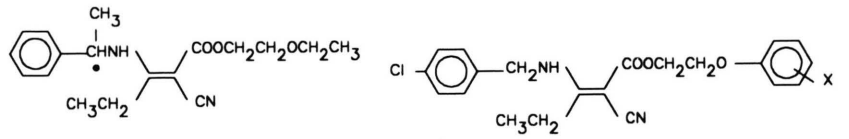

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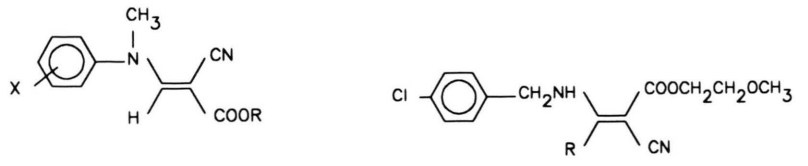


10 **11**

In equation (1), $pI_{50} = -\log_{10} I_{50}$, where I_{50} is the molar concentration of inhibitor required to reduce the photosynthetic electron flow in isolated thylakoid membranes to 50% of that obtained in the absence of inhibitor, π is the Hansch hydrophobicity parameter, σ is the Hammett constant reflecting the electronic nature of the substituent, n is the number of compounds in the set, r is the correlation coefficient and s is the standard deviation.*

This analysis established that a lipophilic aromatic or alicyclic ring was required for activity. In addition, a group having the potential for hydrogen bonding was needed for efficient binding. The most important variable was lipophilicity, suggesting that specific hydrophobic binding of the aromatic ring and its substituents was critical for interaction with the site of action.

Some further examples [5] of the correlation of biological activity with chemical structure of inhibitors of photosynthetic electron transport are given below.

N-Methylureas (**2**)

$$pI_{50} = 1.03\,(\pm\,0.19)\log P + 4.27\,(\pm\,0.28) \quad (2)$$
$$n = 15,\ r = 0.96,\ s = 0.19.$$

N-Methoxy-N-methylureas (**3**)

$$pI_{50} = 0.73\,(\pm\,0.12)\log P - 0.12\,(\pm\,0.06)\,\mathrm{BR} + 3.61\,(\pm\,0.42) \quad (3)$$
$$n = 38,\ r = 0.90,\ s = 0.59.$$

Carbamates (**4**)

$$pI_{50} = 0.89\,(\pm\,0.25)\log P + 1.13\,(\pm\,0.70) \quad (4)$$
$$n = 7,\ r = 0.97,\ s = 0.21.$$

Equations (2)–(4) again emphasize the relationship between lipophilicity (in this case the measured value of log P rather than the substituent coefficient, π) and Hill inhibition activity [5]. More sophisticated physical descriptors such as a steric parameter (BR) (Eq. (3)) can be used to improve the correlation. In this series, compounds with branched hydrocarbon groups in the 4-position *(para)* show lower activity than was anticipated on the basis of lipophilicity. A negative coefficient for BR (a parameter based on the molar refractivity of the substituent group) showed an inverse relationship between activity and size, providing evidence that bulky groups hindered binding.

Equations (5)–(7) below are more recent examples of QSAR derived for PS II inhibitors of more complex structure.

Phenylbiurets (**5**) [6]

$$pI_{50} = 1.67\,(\pm\,1.03)\log P - 0.22\,(\pm\,0.16)\,(\log P)^2 - 1.08\,(\pm\,0.54)\,\sigma + 4.16\,(\pm\,1.56) \quad (5)$$
$$n = 16,\ r = 0.91,\ s = 0.21.$$

Aminopyrazolones (**6**) [7]

$$pI_{50} = 0.71\,(\pm\,0.19)\,\pi + 0.81\,(\pm\,0.32)\,\sigma + 4.47\,(\pm\,0.12) \quad (6)$$
$$n = 11,\ r = 0.97,\ s = 0.13.$$

Phenylaminocyanoacrylates (**7**) [8]

$$pI_{50} = 1.17\pi\,(\pm\,0.18) + 4.86\,(\pm\,0.50) \quad (7)$$
$$n = 13,\ r = 0.96.$$

In the biuret series (Eq. (5)), Hill inhibition activity is, as usual, determined primarily by hydrophobicity. However, this series is unusual in that a 2-(*ortho*)F (or Cl) substituent enhances activity, though 2-phenyl substitution is normally a detrimental structural feature in amide inhibitors. The 2-F substituent in structure (**5**) confers an order of magnitude more potency on these molecules over the unsubstituted compounds. Electronic effects (measured using the paramter σ) are also significant in Eq. (5) accounting for more than 10% of the variance [6]. The activity of the aminopyrazolones (**6**) also conforms to the dependence on hydrophobicity (Eq. (6)) though, in this series, the amide function characteristic of this general class of inhibitor forms part of a cyclic structure.

The cyanoacrylates (**7**) are potent Hill inhibitors that do not contain a formal amide moiety but an arrangement in which the carbonyl and NH functions are separated by a double bond (a vinylogous amide). However, the similarity of the QSAR (Eq. (7)) of the cyanoacrylates to those of other examples of amide-type inhibitors (Eq. (1)–(6)) suggests that the vinylogous amide function appears to function in binding in the same way as the amide function of the classical urea inhibitors. However, the steric arrangements of individual atoms of the vinylogous amide group are profoundly different from that of a normal amide.

* For a more detailed discussion on the physico-chemical basis of QSAR, see the review by Bowyer *et al.* [4].

Other classes of PS II herbicides, including the heterocyclic *s*-triazine [9], 1,2,4-triazinone [10] and uracil [11] series have also been subjected to QSAR analysis. Again, there is a primary dependence on log *P*, with increases in lipophilicity giving rise to increases in potency of Hill inhibition.

The QSAR analyses of a chemically diverse range of PS II inhibitors enables some general conclusions to be made about the nature of the receptor site. Without exception, lipophilicity is the major determinant of activity. A bioactive functional group which has the potential for hydrogen bonding is also a common feature of the urea/triazine type of inhibitor. However, a striking feature of the majority of these analyses is a lack of any well defined steric effects. Hydrophobic space in the region of the binding site could be assumed to be large and largely unconstrained.

Steric effects

Steric effects, which should reflect the architecture of the binding site, are poorly defined in the QSAR of classical amide-type PS II inhibitors. This can be accounted for to some extent by the molecules themselves which tend to have relatively rigid, flat core structures. Iwamura and co-workers [12] assessed anilide, phenylurea, carbamate and triazine PS II inhibitors for steric interactions using a variation of the parameters developed by Verloop [13] as the STERIMOL program. Some steric constraints were identified in each series and the study pointed to differences in the interaction of each class with the receptor site.

The cyanoacrylate PS II inhibitors are subject to a number of steric constraints which should be significant in determining topographical features of the binding site. For example, chiral discrimination has been observed [14] in the optical pair (**8**), with the *S*-isomer being 200 times more active than the *R*-isomer. This phenomenon has also been observed in the urea [15] and triazine [16] series, though the level of discrimination between isomers was not nearly as great. The activity of cyanoacrylates as PS II inhibitors has been shown in a number of instances to be very sensitive to the arrangement of substituent groups. Apart from the activity difference associated with the stereochemistry of optical isomers, a number of other structural features indicate strong steric interaction between these molecules and the binding site.

Anilino-cyanoacrylates (**7**) follow the urea QSAR with pI_{50} showing a close dependence on the lipophilicity of the phenyl substituent (Eq. (7)). However, when a CH_2 group is inserted between the NH and phenyl groups, some important differences are revealed in the QSAR (Fig. 1). Although the relationship between pI_{50} and activity conforms well to the established pattern, the unsubstituted compound is an outlier. Almost any substituent, even some with negative values of π such as NO_2 and CN, confers an increase in activity. This phenomenon can be interpreted in terms of the increased flexibility introduced by the CH_2 "hinge" enabling the phenyl ring with its attached 3- or 4-substituent to achieve a positive interaction with a specific binding region.

The placement of groups near the ester carbonyl of the cyanoacrylate molecule can have a profound effect on activity (Fig. 2), though this type of steric interaction has not been defined quantitatively. The introduction of a methyl substituent on the carbon atom closest to the carbonyl function causes a dramatic loss in activity. The adverse steric effect is again evident when a 3-tetrahydrofurfuryl ring is attached directly to the ester function. Activity is reduced more than 100-fold compared with the compound with a 2-tetrahydrofurfuryl ring attached to the ester through a methylene group (Fig. 2).

Another type of binding interaction which can be defined by QSAR and which provides evidence that the PS II herbicide receptor site has the potential for several different interactions that may contribute to binding is described in Eq. (8).

$$pI_{50} = -0.18(\pm 0.09)\,MR + 8.27(\pm 1.51) \quad (8)$$
$$n = 11, r = 0.82.$$

In this instance, analysis of the substituent effects in a series of phenoxyethyl ester derivatives (**9**) of the cyanoacrylate molecule showed an inverse relationship between size (as quantified by the parameter *MR*; Molar Refractivity) and activity. However, lipophilicity or the electronic nature of the substituent had no quantifiable influence on activity. In this respect, Eq. (8) presents a completely different pattern to the classical urea QSAR. Further evidence for adverse steric interactions is provided by the effect of different phenyl substitutions in these molecules. 4-Substituents had a particularly detrimental effect on activity, being typically greater

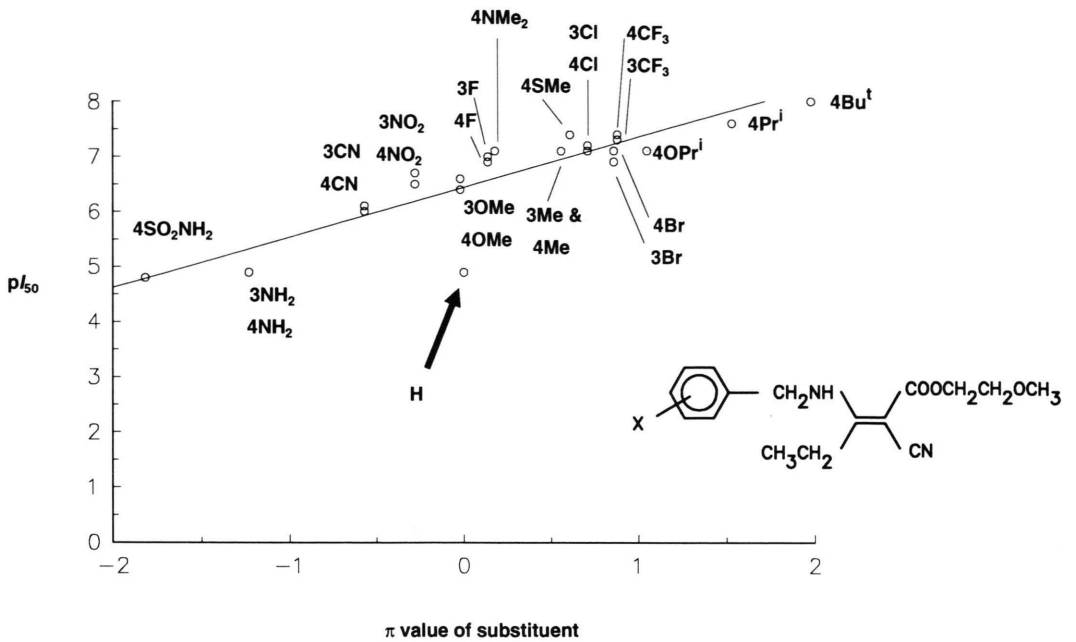
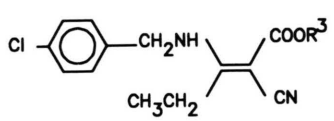


Fig. 1. Hill Inhibition Activity (pI_{50}) *vs* lipophilicity (π) of substituent X.

than 100 times less active than the corresponding 2- or 3-substituted derivatives [8].

Similar effects were observed in a series of aralkyl esters (Fig. 3). Here inhibitor potency varied



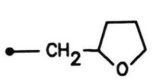
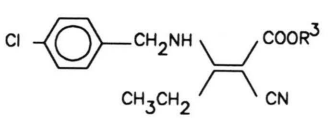
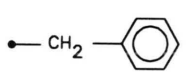
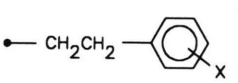
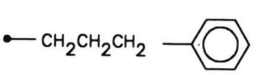
R^3	pI_{50}
$-CH_2CH_2OCH_2CH_3$	8·2
$-CH(CH_3)CH_2OCH_3$	5·6
	7·2
	5·1

Fig. 2. The adverse effect of substitution on the carbon adjacent to the ester function in cyanoacrylate esters.



R^3	pI_{50}
	5·5
	7·2 (X = H)
	5·7

X	pI_{50}	X	pI_{50}
$2-NO_2$	7·6	2–Cl	7·3
$3-NO_2$	7·6	3–Cl	7·5
$4-NO_2$	< 4	4–Cl	4·7

Fig. 3. The effect of alkyl chain length and aryl substitution in a series of aralkyl cyanoacrylate esters.

with an increasing length of the alkyl chain between the ester function and the phenyl ring, maximum activity being obtained with the phenylethyl esters. Substitution of the phenyl ring in this series again emphasizes the detrimental effect of 4-substitution (Fig. 3), indicating steric constraints imposed by a substituent in this position. Furthermore, from the limited data available, it appears that neither lipophilicity nor the electronic nature of phenyl substituents has any significant effect on activity.

N-Methylanilino cyanoacrylates (**10**) are also potent Hill inhibitors but again have quite different structural requirements for activity and also exhibit non-classical QSAR. Compared with cyanoacrylates of structures (**11**) the N-methylanilino derivatives (**10**) have different stereochemistry around the double bond. For biological activity, the nitrogen must be substituted by a methyl group and there must be no substituent at the β-position. Ester substitution is restricted to a small alkyl substituent (straight or branched chain of up to four carbon atoms) whereas compounds of type (**11**) have greater flexibility in ester substituents while still retaining high activity. In the *cis* series (structure **11**) the only significant determinant in the QSAR analysis is the lipophilicity of the substituent. Moreover, as with the urea series, 2-substituents lower potency considerably. In contrast, in the N-methylanilino series, 4-substitution virtually destroys activity but 2- and 3-substituted derivatives retain potency. As with the phenoxyethyl derivatives (**9**) and the phenylethylesters (Fig. 3) discussed above, activity cannot be correlated with any of the classical parameters. Lipophilicity and the electronic nature of the substituent have no effect and correlation with any size parameter is weak. Furthermore, members of the N-methylanilino series are slow binding inhibitors. The kinetics of their interaction with the site is different and the orientation of these molecules within the binding domain must differ from the classical amide inhibitors and even from other closely related cyanoacrylate structures.

Conclusion

The classical Hansch-Fujita approach and other physicochemical methods that have succeeded it provided a valuable insight into the nature of the PS II herbicide binding site. The dominance of hydrophobic interactions has been a recurring theme in QSAR studies of PS II inhibitors. The QSAR method has been less successful in identifying and quantifying steric interactions. This is in part due to the fact that the classical "amide-type" molecules were relatively rigid conformationally. More flexible systems like the cyanoacrylates allowed a more extensive probing of the receptor site based on steric interactions [17]. Although such studies have not resulted in the direct development of novel compounds of commercial significance, it is likely that the integration of QSAR and computer modelling based on inhibitors with a degree of flexibility and variable stereochemical features will continue to further our understanding of PS II/inhibitor interactions.

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