Cyanoacrylate Inhibitors as Probes for the Nature of the Photosystem II Herbicide Binding Site

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Three series of phenyl-substituted 2-cyanoacrylates were evaluated using simple quantitative structure activity relationships (QSAR) in an attempt to elucidate the nature of the regions of the binding site occupied by different parts of the molecules.

Inhibition of the Hill reaction by substituted 3-phenylamino-2-cyanoacrylates correlated well with the lipophilicity of the substituent. The hydrophobic effect was also dominant when the Hill activity of a series of 3-benzylamino-2-cyanoacrylates was analyzed, although potency was considerably higher in the latter series.

Lipophilicity and the electronic nature of the substituents were not major determinants in the Hill inhibitory activity of a series of substituted phenoxyethyl 2-cyanoacrylic esters. In this case, a significant correlation was found with the molar refractivity (MR) of *meta* substituents, a parameter reflecting substituent size.

The results indicate that the phenyl moiety of substituted 3-phenylamino- and 3-benzylamino-2-cyanoacrylates interacts with an essentially lipophilic binding domain, though it is likely that the two series are oriented differently with the 3-benzylamino series able to bind with greater affinity. In the phenoxyethyl ester series, the substituted phenyl group interacts with a different environment, wherein *ortho*- and *meta*-substitution is tolerated, dependent on the bulk of the substituent, but *para*-substitution is detrimental to affinity for this region of the site.

Introduction

A large number of chemically diverse commercial herbicides act by interfering with photosynthetic electron transport at the reducing side of photosystem II (PS II). These compounds, which include the urea, triazine and uracil herbicide families, all exert their effect by binding to the 32 kDa D 1 polypeptide of the PS II reaction centre, thereby inhibiting electron flow from a primary plastoquinone electron acceptor Q_A to a secondary acceptor Q_B [1]. It is generally accepted that all "amide-type" inhibitors compete directly with Q_B at its binding site on the D 1 polypeptide. However, studies with triazine-resistant mutants, in which particular plant biotypes lose their sensitivity to one group of PS II inhibitors (*e.g.* the triazines) while retaining full sensitivity to another (the phenylureas), led to the concept of overlapping binding domains for different families of inhibitors [2, 3].

Major advances have been made in recent years in understanding the structure of the PS II reaction centre, most notably the crystallization and X-ray structure determination of the reaction centre from photosynthetic bacteria [4, 5]. Similarities in the function and amino acid sequence of the bacterial centre and PS II led to new models for the PS II reaction centre [6, 7]. Although these models of the tertiary structure of the PS II reaction centre account for many observations as to the structure and properties of the herbicide binding site on the D 1 polypeptide, knowledge of the precise nature and orientation of herbicide binding is incomplete.

The uncertainty regarding the precise binding domains of different inhibitor types is compounded by obvious structural differences between the binding site in the bacterial reaction centre and the corresponding site in PS II [6]. X-ray crystallography has revealed the exact binding orientation of the triazine herbicide, terbutryn, in the reaction centre of *Rhodopseudomonas viridis* [8]. However, most PS II inhibitors either do not bind or bind very weakly to the bacterial system, making an assessment of the binding domains of the different chemical classes in PS II difficult. Nevertheless, information provided by studies with photoaffinity-labelled compounds and herbicide resistant mutants has allowed speculation as to the relative im-

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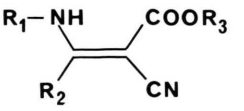
portance of different regions of D1 in herbicide binding. In fact, different inhibitor types have been classified according to their proposed mode of binding to the D1 polypeptide [6]. Amide-type herbicides were classified as the Ser_{264}-family, whereas phenols were classified as His_{215}-type. Recent work, however, has placed the binding site of ioxynil (a phenolic type) in a region remote from His_{215} [9]. Obviously, a more detailed understanding of the nature and location of the different herbicide binding regions is needed before further progress in the rational design of more effective inhibitors can be made.

From a chemical viewpoint, quantitative structure activity relationships (QSAR) can often provide some insight into the interaction of inhibitors with macromolecules. PS II inhibitors have been the subject of several such studies [10–14], with those using the urea family the most comprehensive. However, even within the same chemical family, for example, the phenylureas, discrepancies arise in the interpretation of QSAR data [10, 11]. While most studies highlighted the importance of hydrophobicity as the prime determinant of Hill inhibitory activity, the electronic effects of phenyl substituents also played a role in some circumstances. In a number of studies, steric effects imposed by the site were inferred [10, 13], but QSAR could not define these adequately.

2-Cyanoacrylates of general structure **1** are an established group of amide-type PS II inhibitors.



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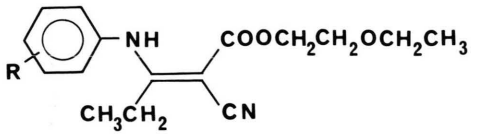
The more active members of the series are extraordinarily potent, but, as a class, their greatest value is that their activity in blocking photosynthetic electron transport is extremely sensitive to minor structural variation. They are, therefore, useful probes for investigating the nature and topography of the herbicide binding site, at least in respect of the binding domain of this generic structure.

A simple QSAR analysis of phenyl-substituted 2-cyanoacrylates has been carried out on three different basic structures, the *meta*- and *para*-substituted phenylamino series **2,** *meta*- and *para*-substituted benzylamino derivatives **3** and the *meta*-substituted phenoxyethyl ester derivatives **4.** Consideration of correlations between structure and Hill inhibitory potency allows some inferences to be drawn concerning the nature of the binding domain occupied by different parts of the molecule.

Materials and Methods

New compounds were synthesized by general methods described previously [15, 16]. All structures were confirmed by PMR spectra and key compounds gave satisfactory microanalyses. For



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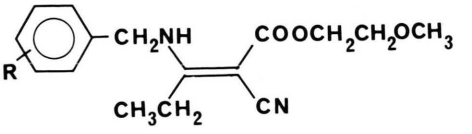

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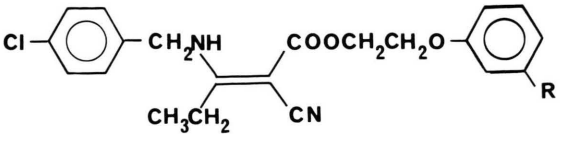

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clarity, substitution in the phenyl rings is referred to in the text using the *ortho (o), meta (m),* and *para (p)* notation for 2-, 3-, and 4-substitution.

Compounds were assayed for inhibition of the Hill reaction using chloroplast fragments isolated from the leaves of 21 day-old plants of *Pisum sativum* (*cv.* Victory Freezer), the electron acceptor being the indicator dye 2,3′,6-trichlorophenolindophenol. The experimental procedure was as described elsewhere [17]. The activity was expressed in terms of pI_{50}, *i.e.*, $-\log_{10} I_{50}$, where I_{50} was the molar concentration required to decrease the rate of dye reduction under illumination of saturating intensity to 50% that obtained in the absence of the compound. The pI_{50} values given are for reactions performed under coupled conditions and are the mean of at least three separate determinations, with a variation between experiments of less than ± 0.2 for each compound.

The physiochemical constants used in this study were those published by Hansch and Leo [18]. The π value for OC_6H_{13} (Fig. 1) was calculated using the value for OC_2H_5 [18] and adding four CH_2 groups at an incremental value of 0.5 per CH_2 unit.

Regression analyses of the data illustrated in Fig. 1–3 were recorded in Eqn. (1–3), where n is the number of compounds and r is the correlation coefficient. The numbers in brackets are the 95% confidence limits.

Results and Discussion

The observed pI_{50} data for *para*-substituted 3-phenylamino-2-cyanoacrylates **2** are plotted against the hydrophobicity constants for the various substituents in Fig. 1. A β-ethyl and an ethoxyethyl ester substituents were included in structure **2** to promote optimal interaction in the R_2 and R_3 regions [15, 19], so that the effect of substitution in the phenyl ring was maximized. Regression analysis of these data revealed a simple correlation of Hill inhibitory activity with π (Eqn. (1)).

$$pI_{50} = 1.17\ (\pm 0.18)\ \pi + 4.86\ (\pm 0.50) \qquad (1)$$
$$n = 13 \qquad r = 0.96$$

No significant correlation was detected between activity and the electronic effects of the substi-

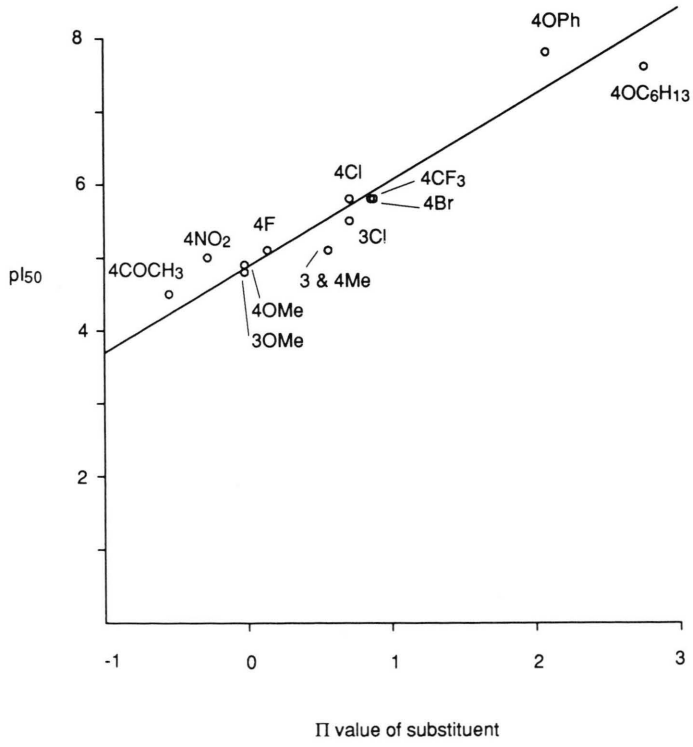

Fig. 1. Plot of measured Hill inhibitory activity (pI_{50}) for 3(*meta*)- and 4(*para*)-substituted phenylamino cyanoacrylates **2** *versus* lipophilicity of the substituent (π). Formulas are given in the text.

tuents (σ values) or group size (molar refractivity MR).

Substituted benzylamino-2-cyanoacrylates **3** are considerably more potent inhibitors of the Hill reaction than the corresponding phenylamino derivatives **2.** In fact, in the benzylamino series, the methoxyethyl ester substituent ($CH_2CH_2OCH_3$) was used to reduce activity by 10-fold compared with the corresponding ethoxyethyl esters, thus ensuring that the real activity (pI_{50}) of the most potent compounds remained in the 8.0–8.2 region, the maximum observable pI_{50} value of the assay system used [19]. Regression analysis of the pI_{50} data (Fig. 2) from these compounds showed a similar dependence on π (Eqn. (2)). Again, no correlation between σ or MR and the PI_{50} was observed.

$$pI_{50} = 1.08\ (\pm 0.22)\ \pi + 6.29\ (\pm 1.01) \quad (2)$$
$$n = 27 \qquad r = 0.86$$

The results tend to confirm previous studies of the QSAR of amide-type PS II inhibitors in that the hydrophobic effect of the substituents is the dominant feature of the regression. The coefficients of the π term in both Eqn. (1) and (2) suggest that compounds of general structure **2** and **3** partition into a lipophilic region of the binding site in much the same way as they would partition between water and octanol. However, closer examination of the data reveals some interesting anomalies. Of particular interest is the low activity of the unsubstituted benzylamino-2-cyanoacrylate. The pI_{50} value is considerably less than would be predicted by Eqn. (3). Furthermore, the nitro compounds (**3,** R = *m*-NO_2, *p*-NO_2) are more active than the unsubstituted compound (**3,** R = H), a result quite inconsistent with the expected effect of this substituent (an effect not observed with the corresponding phenylamino derivatives **2;** Fig. 1). The increase in activity in the presence of a substituent with favourable lipophilicity (*e.g.* *p*-Cl) is greater than would be expected; the pI_{50} of **3;** R = *p*-Cl is 7.1 compared with the unsubstituted compound **3,** R = H, where the pI_{50} is 4.8, an increase of 2.3 pI_{50} units. The expected increase in Hill inhibition by substitution with a *p*-chloro substituent would be 0.7 pI_{50} unit, if increased activity was due solely to increased hydrophobicity of the molecule. Thus, in comparison with the activity of the parent

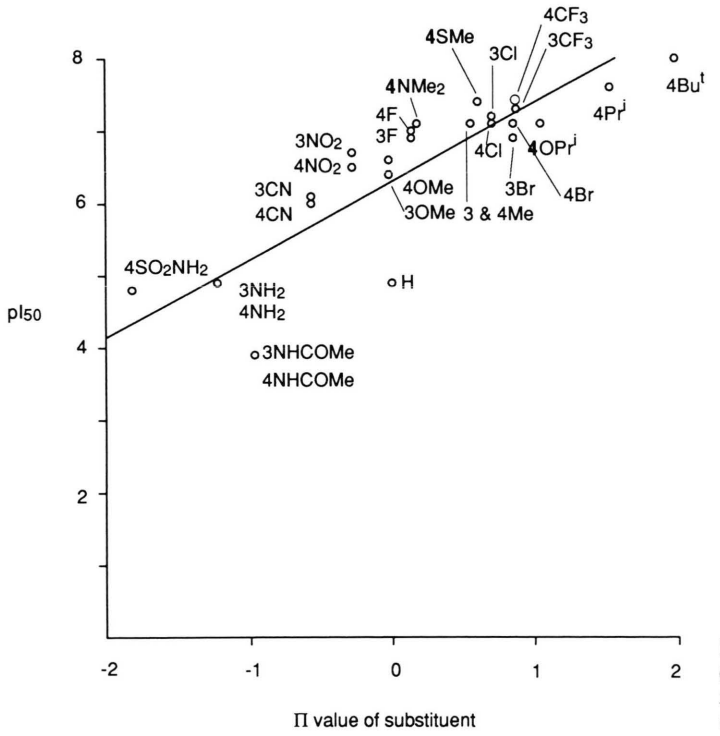

Fig. 2. Plot of measured Hill inhibitory activity (pI_{50}) for 3(*meta*)- and 4(*para*)-substituted benzylamino cyanoacrylates **3** *versus* lipophilicity of the substituent (π).

compound, the presence of almost any substituent produces an incremental increase in potency much higher than would have been predicted on the basis of lipophilicity alone.

The two series represented by structures **2** and **3** obviously have different binding characteristics although lipophilicity is the prime determinant for activity in both substituted phenylamino and benzylamino compounds. The methylene group between the amino function and the phenyl ring in series **3** allows greater flexibility in the orientation of the phenyl ring. The higher potency of compounds in series **3** compared with series **2** is evidence that this increased flexibility ensures a more favourable interaction with the site. Furthermore, the greater spatial freedom afforded the phenyl group appears to allow amplification of the beneficial lipophilic effect of a substituent in the *m*- or *p*-position.

Previous studies with phenylurea inhibitors identified the particularly adverse effect of *ortho*-substitution in the phenyl ring [14]. A similar situation pertains to both the phenylamino- and benzylamino-2-cyanoacrylate series, though the effect is quantitatively different. For example, in series **2,** replacement of a *p*-chloro- by a *o*-chloro-substituent reduces activity from pI_{50} 5.8 to 4.5. In the benzylamino series **3,** however, the same change reduces the pI_{50} value from 7.1 to 4.2, a reduction in activity of nearly 1000-fold. In the phenylurea case, computer graphics showed that *ortho*-substitution interfered strongly with the orientation of the planar urea group with respect to the phenyl ring [14]. Although a similar effect can be envisaged for *ortho*-substitution in the phenylamino series **2,** the linking methylene group renders such an explanation less likely in the case of the benzylamino series **3.** Nevertheless, the *ortho*-substituent must prevent the phenyl ring attaining the conformation necessary for optimum interaction with the receptor site.

QSAR analysis of the phenoxyethyl ester derivatives **4** presented quite a different picture from the simple dependence on lipophilicity described above. In this data set, only the *meta*-substituted series was used in the regression because of the very poor activity associated with *para*-substituted compounds. Unlike series **2** and **3** described above, no correlation between π and pI_{50} was observed. Furthermore, no significant relationship with σ was found. Only when the observed pI_{50} data were plotted against the MR of the substituent (Fig. 3) did a meaningful correlation emerge (Eqn. (3)). In general, a bulky substituent caused loss of inhibitory activity, though no *meta*-substituent significantly improved the potency of the unsubstituted compound **4** (R = H).

$$pI_{50} = -0.18\,(\pm 0.09)\,\mathrm{MR} + 8.27\,(\pm 1.51) \quad (3)$$
$$n = 11 \qquad r = 0.82$$

A comparison between the activity of isomers with a particular substituent at each of the three possible positions in the benzene ring again revealed a significant difference. *Ortho*-substituted compounds were equally potent with or more potent than the corresponding *meta*-substituted compounds, while *para*-substituted derivatives were weak inhibitors (Table I).

The data presented above indicate that the substituted phenyl moiety in **2** and **3** and the phenoxy

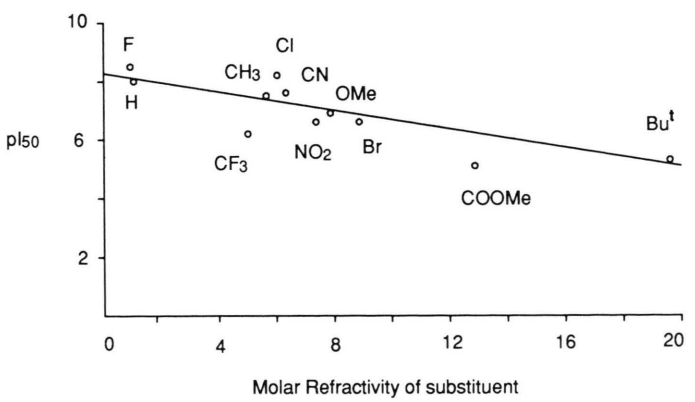

Fig. 3. Plot of measured Hill inhibitory activity (pI_{50}) for 3(*meta*)-substituted phenoxyethyl cyanoacrylic esters **4** *versus* molar refractivity (MR).

Table I. Hill inhibitory activity of *o*-, *m*- and *p*-substituted phenoxyethyl 2-cyanoacrylates **4***.

Substituent	pI_{50} Position of substituent		
	o	*m*	*p*
Cl	8.0	8.2	5.8
NO_2	7.9	6.9	5.5
CH_3	7.6	7.6	5.4

* See formulas in the text.

group in **4** interact with regions of the binding site having quite different characteristics. The phenyl- and benzylamino groups behave, in physicochemical terms, in the same way as does the phenyl group in, for example, the phenylureas. On the other hand, lipophilicity of the substituent is of little importance in determining activity in substituted phenoxyethyl ester derivatives.

It was recognized early in the study of cyanoacrylate structure-activity relationships that an ether oxygen atom in the ester side chain increased affinity for the binding site [15]. The increased activity associated with the ether oxygen atom can be explained by its ability to interact with hydrogen bond donor groups of water or peptide molecules. However, phenyl ethers form weaker hydrogen bonds than alkyl ethers and the high potency of *ortho*-substituted compounds would suggest, on steric grounds, that interaction involving the ether function in series **4** is minimal. In the absence of any effect on activity by the lipophilic or electronic properties of substituents, it appears likely that the phenyl ring in the ester side chain of series **4** is able to form a favourable π-bonding interaction which contributes significantly to binding. That this region of the site has stringent spatial limitations is evident from the weak activity of the *para*-substituted compounds when compared with the *ortho*- and *meta*-isomers (Table I).

The simple QSAR analyses reported above confirm earlier [19] structure-activity data which indicate the hydrophobic nature of the inhibitor binding site, particularly in the regions occupied by R_1 and R_2 (structure **1**). Previous studies also established that high affinity binding is critically dependent on the steric orientation of the groups attached to the cyanoacrylate molecules [19]. The QSAR of substituted phenylamino- and benzylamino-2-cyanoacrylates provides strong evidence that the phenyl rings of these two structural types interact differently with the lipophilic regions of the receptor, the increased flexibility of the latter series enabling the compounds to adopt a conformation permitting a more sensitive interaction with the site. Moreover, the phenyl ring in the ester side chain appears to bind differently. Lipophilic considerations are unimportant but the steric limitations of the binding domain play a significant role in determining activity. The fact that it is possible to identify different binding orientations within a chemically homogeneous group of inhibitors suggests that more detailed study of the cyanoacrylate class may be useful in establishing the relationship between the binding domains of other families of PS II inhibitors.

Acknowledgements

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