N-Methylanilinocyanoacrylate Photosystem II Inhibitors. Structure-Activity Relationships

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Cyanoacrylates, Photosystem II Inhibitors, Structure Activity

Many 3-N-methylanilino cyanoacrylate derivatives are potent inhibitors of photosynthetic electron transport at the PS II level in a thylakoid system isolated from *Pisum sativum*. These inhibitors are somewhat unusual in that they take more than 15 min to equilibrate with the binding site in contrast to most classical PS II inhibitors which tend to equilibrate rapidly *i.e.* within the mixing time of seconds. The effects of mono- and disubstituents in the aryl nucleus on inhibitory activity were studied. The nature and position of the substituent had no detectable influence on equilibration rate but a pronounced influence on inhibitory activity as measured by equilibrium pI_{50} values. Substituent position enhanced inhibitory activity in the order $3' > 2' >>> 4'$. As regards the nature of the substituent, size appeared to be the most important parameter – the smaller the substituent the greater the activity. Such influences were in contrast to those in analogous 3-anilino- and 3-benzylamino-2-cyano-acrylate series where the comparable order of substituent position effect was $3'$ and $4' >> 2'$ and where substituent hydrophobicity rather than size was the most important factor. Disubstituted fluoro derivatives have given interesting insights into the preferred orientation of these inhibitors at the binding site with the 2′- and 5′-positions interacting positively, the 4′-position, negatively, and the 3′-position neutrally.

Introduction

Structure-activity studies of inhibitors of photosynthetic electron transport at the PS II level are generally concerned with understanding the nature of the binding forces involved and/or the molecular architecture of the binding site. Such studies have included several cyanoacrylate series viz: 3-alkylamino-, 3-phenylamino(anilino)- and 3-benzylamino-2-cyanoacrylates [1, 2]. However, the structural requirements for activity within these different, though related, series vary greatly. This suggests that they interact in different ways with the D1 peptide. Of particular interest are the 3-methylphenylamino (N-methylanilino) derivatives where an unusual biphasic binding reaction has recently been observed [3]. Unlike most classical PS II inhibitors (ureas, triazines, etc.) and most other cyanoacrylate PS II inhibitors, all of which equilibrate rapidly with plant thylakoids, 3-N-methylanilino-2-cyanoacrylates bind in two stages – a rapid reaction within the time of mixing (seconds) followed by a much slower reaction taking 15–30 min to reach equilibrium. Earlier studies with these compounds [4] had not recognized the slow nature of the equilibrium process so that the "apparent" I_{50} values reported were determined using normal Hill reaction assay conditions. Such "apparent" I_{50} values tended to relate to the rapid binding phase of the interaction and are less relevant than "equilibrium" I_{50} values for interpreting binding behaviour and herbicidal potency.

This contribution is concerned with reporting equilibrium pI_{50} values for a series of secbutyl-3-(subst)N-methylanilino-2-cyanoacrylates using isolated pea thylakoids. The results are discussed in terms of the effects of the aryl substituents on the binding interaction.

Materials and Methods

The compounds reported in Table I and II were synthesized by first preparing a common starting intermediate, secbutyl-3-ethoxy-2-cyanoacrylate, by refluxing equimolar quantities of secbutyl cyanoacetate, triethylorthoformate and acetic anhydride at 140 °C for 2 h, removing volatile material *in vacuo* and then distilling under high vacuum the residual brown oil to give a clear yellow liquid (b.pt. 117–119 °C, 0.5 mm Hg). The intermediate was reacted with an equimolar quantity of an appropriately substituted aniline, either neat at

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140 °C for 1 h or in refluxing acetonitrile solution overnight, to give the corresponding secbutyl-3-(subst)-anilino-2-cyanoacrylate (>90% yield). This was then methylated under phase transfer conditions to give the secbutyl-3-N-methylanilino-2-cyanoacrylate (30–70% yield). Both intermediate and final products, if solid, were purified by recrystallization from ethanol otherwise by chromatography on a silica gel column. Compounds were characterized by PMR using a Jeol FX90Q spectrophotometer.

pI_{50} values were determined using a modification of a Hill reaction procedure described earlier [5]. The modification was necessary because of the slow equilibration of the N-methylanilino cyanoacrylates with the pea thylakoids. Instead of illuminating immediately after addition of the thylakoid suspension to the tris buffer containing the test compound and the indicator dye, the mixture was allowed to stand in the dark at room temperature for 30 min and then illuminated. Trials had indicated that the 30 min waiting period did not affect the control (no added compound) value but was sufficient to allow equilibrium between the compound and the thylakoids to be attained.

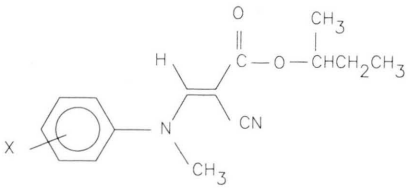
Results and Discussion

The effects of aromatic ring substituents on equilibrium pI_{50} values for a series of 3-N-methylanilino-2-cyanoacrylates are shown in Table I and II. The equilibrium pI_{50} of the parent compound, secbutyl-3-N-methylanilino-2-cyanoacrylate (6.9), when compared with the apparent pI_{50} value (6.0) reported for the same compound earlier [4] suggests that there is nearly a ten-fold difference between the apparent and equilibrium I_{50} values.

Substituents appeared to have no significant effect on the biphasic character of the binding interaction or on the rate of the slow binding step. However, both the nature and position of the substituent play an important role with the activities of the various substituted inhibitors ranging over a thousand-fold ($\Delta pI_{50} = > 3.0$ units). For a given mono substituent the 3′ position is the most active and the 4′ position least so – the general order of activity being $3' > 2' >>> 4'$.

Substituent size appears to be an important parameter – in general the smaller the size the greater the activity. This is most evident with 2′ derivatives. Not one of the 2′ substituted compounds studied is more active than its unsubstituted parent suggesting that the influences of other factors

Table I. The effect of 2′, 3′ and 4′ aryl substituents on the equilibrium pI_{50} values (pea thylakoids) of a series of secbutyl-3-N-methyl(subst)anilino-2-cyanoacrylates.



Substituent (X)	pI_{50} (equil)[a] 2′	3′	4′
H	6.9	6.9	6.9
F	6.7	7.4	3.6
Cl	6.7	7.4	<3.3
Br	6.5	7.1	–
Me	6.3	7.0	3.6
NH_2	–	7.0	–
I	–	6.8	–
NO_2	–	6.5	–
CF_3	5.9	6.4	–
OMe	–	6.3	–
CN	–	6.3	–
Et	–	6.0	–
OEt	–	4.6	–
COOMe	4.3	–	–
$COOPr_i$	–	3.8	–
Ph	4.1	–	–
Pr_i	4.0	–	–

[a] Determined after 30 min equilibration of compound and pea thylakoids in the dark at room temperature.

Table II. Equilibrium pI_{50} values for a series of aryl disubstituted secbutyl-3-N-methyl-anilino-2-cyanoacrylates.

Compound	pI_{50} (equil)[a]
2′5′-difluoro	7.5
3′5′-difluoro	7.3
2′6′-difluoro	7.0
2′3′-difluoro	6.7
2′4′-difluoro	5.0
3′4′-difluoro	<4.3
2′-fluoro-5′-methyl	7.0
2′-methyl-5′-fluoro	6.3
2′5′-dimethyl	4.8

[a] Determined after 30 min equilibration of compound and pea thylakoids in the dark at room temperature.

such as hydrophobicity and electronegativity are minimal. Further evidence of the size effect of 2′ substituents is shown by the relative activity of the 2′ halo (2′Cl > 2′Br > 2′I) and 2′ alkyl (2′Me > 2′Pr_i) derivatives. The effect of substituent size on the binding interaction normally reflects the size constraints of the binding niche with which the substituent interacts. However in the case of 2′ substituents, in particular, it may also influence the rotational freedom of the phenyl ring which in turn could affect the orientation of the aromatic nucleus with respect to the remainder of the inhibitor molecule. The larger the 2′ substituent, the greater the rotational restriction and the greater the interference with the ability of the molecule to adopt the optimum conformation for interacting with the receptor site.

Size also appears to be important with 3′ substituents. This is indicated by the relative activity of halo (3′F > 3′Cl > 3′Br), alkyl (3′Me > 3′Et) and alkoxy (3′OMe > 3′OEt) derivatives. However with 3′ substituents other factors may also play a role since the 3′-fluoro-, chloro-, bromo-, methyl- and amino-derivatives are all more active than the unsubstituted parent.

It is not possible to observe a relationship between the size and activity of 4′ substituents since all such derivatives are virtually inactive. It can however be concluded from the large activity difference ($\Delta pI_{50} = > 2.6$) between the unsubstituted parent (4′H) and the 4′-fluoro derivative that size is critical and that the 4′ position must interact with a binding niche capable of accommodating a hydrogen atom but not much else.

The influence of both the nature and position of aryl substituents on PS II inhibitory activity is different in the 3-N-methylanilino-2-cyanoacrylates to that in the related 3-anilino and 3-benzylamino series. Thus the relative effectiveness of substituent position in enhancing activity follows the order 3′ > 2′ >>> 4′ in the former but 3′ and 4′ >> 2′ in the latter. Likewise substituent size is the most important parameter determining activity with N-methylanilino derivatives – the smaller the substituent the more active the inhibitor, while substituent hydrophobicity is most important with 3-anilino and 3-benzylamino derivatives – the greater the hydrophobicity of the substituent the more active the inhibitor [2]. Such contrasts in structure-activity behaviour coupled with the differences in binding kinetics suggest that the interaction of N-methylanilino derivatives with the D1 peptide is different to that of other cyanoacrylates and this has been attributed to differences in H-bonding interactions [6].

Table II records equilibrium pI_{50} values for a series of disubstituted fluoro derivatives with the relative order of activity being:

$$2'5' > 3'5' > 2'6' > 2'3' >>> 2'4' \text{ and } 3'4'.$$

The inactivity of the 2′4′ and 3′4′ analogues is consistent with the generally weak activity of the *para* (4′) substituted derivatives noted in Table I and reinforces the conclusion that any substituent in the 4′ position interferes with the ability of these inhibitors to fit the binding site.

The observation that the *ortho*/*meta* (2′5′) difluoro derivative ($pI_{50} = 7.5$) is more active than either the *ortho* (2′) ($pI_{50} = 6.7$) or *meta* (3′) ($pI_{50} = 7.3$) mono fluoro compound suggests that fluoro groups in both the 2′ and 5′ positions can make a positive contribution to the binding interaction concurrently. On the other hand, the activity of the *ortho*/*meta* (2′3′) difluoro analogue ($pI_{50} = 6.7$) is similar to that of the *ortho* (2′) ($pI_{50} = 6.7$) but less than that of the *meta* (3′) derivative ($pI_{50} = 7.3$) indicating that in this situation only the 2′ substituent makes a positive contribution to the binding interaction, the 3′ substituent being neutral. It would seem therefore that only one of the two possible *meta* fluoro positions is able to react positively with the receptor site at any given time. This is in accord with the activity of the *meta*/*meta* (3′5′) difluoro compound ($pI_{50} = 7.3$) being comparable to that of the *meta* (3′) monofluoro derivative ($pI_{50} = 7.3$).

Table II also includes data for the 2′5′-dimethyl, 2′-methyl-5′-fluoro and the 2′-fluoro-5′-methyl derivatives. The weaker activity of the *ortho*/*meta* (2′5′) dimethyl derivative ($pI_{50} = 4.8$) as compared with both the *ortho* (2′) ($pI_{50} = 6.4$) and *meta* (3′) ($pI_{50} = 7.0$) mono methyl compounds indicates that the presence of a second methyl group reduces the binding affinity. This is in contrast to the stronger activity of the *ortho*/*meta* (2′5′) difluoro analogue as compared with the mono *ortho* and *meta* fluoro compounds. This difference in behaviour may be attributed to the larger size of the methyl as compared with the fluoro group and its greater interference with the rotational freedom of

the phenyl ring. This could be responsible for the lower inhibitory activity of the 2′-methyl derivatives viz: 2′-methyl (6.3), 2′-methyl-5′-fluoro (6.3) and 2′5′-dimethyl (4.8) as compared with the corresponding 2′-fluoro analogues viz: 2′-fluoro (6.7), 2′-fluoro-5′-methyl (7.0) and 2′5′-difluoro (7.5).

In general the patterns of activity of the disubstituted derivatives suggest that 2′ and 5′ substituents interact positively with the binding site, 3′ substituents interact with free space (*i.e.* neutrally) and 4′ substituents interact negatively with a small binding niche.

[1] J. N. Phillips and J. L. Huppatz, Z. Naturforsch. **39c,** 335–337 (1984).
[2] J. L. Huppatz, H. G. McFadden, and L. F. McCaffery, Z. Naturforsch. **45c,** 336–342 (1990).
[3] J. Phillips, Curr. Res. Photosynth., Proc. Int. Conf. Photosynth., 8th **1,** 587–590 (1989).
[4] J. L. Huppatz, J. N. Phillips, and B. M. Rattigan, Agric. Biol. Chem. **45** (12), 2769–2773 (1981).
[5] B. T. Brown, J. N. Phillips, and B. M. Rattigan, J. Agric. Food Chem. **29,** 719–722 (1981).
[6] J. N. Phillips and W. K. Banham, Z. Naturforsch. **48c,** 132–135 (1993).