Structure–Activity Studies on Amides Inhibiting Photosystem II

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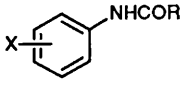
A series of *N*-phenylanilides and *N*-phenylureas have been synthesised and their *'in vitro'* photosystem II activity measured in a cell-free system. An attempt was made to correlate *'in vitro'* data with a novel hydrophobicity parameter π_{amide} (related to the substituent on the phenyl ring) and with the torsion angle θ between the planes of the phenyl and amide moieties.

Relating chemical structure to biological activity is an important aspect in the rational design of bioactive molecules. In this respect it is also desirable to seek information about the conformational factors that may affect the levels of biological activity. A study of the X-ray crystal structures of structurally related molecules can provide this information.

In only a few cases in the design of agrochemicals have the results of X-ray crystallography been correlated with biological activity. Anderson investigated the activity of a series of triazolyl ketone herbicides[1] whereas Taira studied the fungicidal activities of N-phenylsuccinamides.[2] In both studies a torsion angle between the planes of the phenyl moiety and that of one of its substituents was correlated with biological activity, although in none of these studies was any physical justification offered. As quantitative structure–activity relationships (QSAR) are linear free-energy relationships, the variables used implicitly have units of energy. Thus, if a torsion angle is used as a physicochemical variable it has to be assumed that this angle is some sort of measure of the relative energy differences between the molecules under consideration. This assumption has been used in the investigation we now report on the possible effects of conformation on a number of PSII inhibitors having the general structure **1**.



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Over the past 30 years a large number of compounds of structure **1** have been synthesised and tested for photosystem II (PSII) inhibition, by measuring the efficiency with which they disrupt the electron-transport chain in the process known as the Hill reaction.[3] Compounds have included carboxylic amides,[4] ureas[5–8] and carbamates.[4]

X is usually a *meta*- and/or *para*-substituent on the benzene ring and is an important factor in determining inhibition potency. As X is made more hydrophobic, *'in vitro'* activity increases until an optimum value is reached for large hydrophobic substituents.[6] *ortho*-Substitution considerably decreases actvity[7] and this may be because these substituents strongly interfere with the orientation of the planar amide group with respect to the phenyl ring.

Changing the nature of R in structure **1** also changes activity, although few QSAR studies[4,9,10] have been carried out where Hill inhibition has been monitored with a systematic change in R. These studies suggest that the size of R has an effect on the binding of these molecules at their site of action, presumably at the Q_B site of PSII.

Quantification of the steric effect of R on activity has been difficult and has relied upon the introduction of indicator variables and different coefficients for different steric parameters.[4,10] In view of these uncertainties in relating chemical structure to *'in vitro'* activity, we have attempted to tackle this problem directly by analysing the X-ray crystal structure of a number of *N*-phenylamides and phenylureas that show PSII activity. This was done in an attempt to elucidate trends in PSII inhibition in terms of the conformation of these molecules.

Experimental

Materials for PSII Inhibition and X-Ray Analysis.—Anilides and ureas were prepared according to the general procedures detailed below. Two methods were used to synthesise *N*-phenylamides (see Table 1). In method A the appropriate acid chloride (0.01 mol) was dissolved in acetone (25 cm^3) and added slowly to solution of the amine (0.01 mol) and triethylamine (0.01 mol) in acetone (50 cm^3). The solution was refluxed for 1 h and the white solid filtered off. The clear solution was poured into 1.5 mol dm^{-3} HCl (60 cm^3) and the precipitated solid collected and recrystallised from an appropriate solvent.

In method B the appropriate amine (0.01 mol) and triethylamine (0.01 mol) were dissolved in dichloromethane (50 cm^3) and the acid chloride (0.01 mol) added dropwise. After being refluxed for 1 h, the mixture was washed with HCl (3 mol dm^{-3} 2 × 30 cm^3), $NaHCO_3$ (30 cm^3) and water (30 cm^3), dried over $MgSO_4$, the solvent removed under reduced pressure and the crude product purified by recrystallisation.

Growth of Crystals for X-Ray Crystallography.—Crystals, suitable for X-ray crystallography, were grown by the vapour-diffusion method described by Jones [11] and using a binary or tertiary solvent system as required (Table 1).

X-Ray Crystallography.—Crystal data, data collection and refinement details are summarised in Table 2.* Data for all compounds were measured on a Nicolet R3m diffractometer, using Cu-Kα radiation (λ = 1.541 78 Å, graphite monochromator) using ω-scans. Data were corrected for Lorentz and polarisation factors and, where indicated, for absorption. With the exception of compound **7**, which was solved by the heavy-atom method, all structures were solved by direct methods.

* Lists of atomic co-ordinates (hydrogen and non-hydrogen, thermal parameters bond lengths and bond and torsion angles have been deposited at the Cambridge Crystallographic Data Centre. For details of the CCDC deposition scheme, see 'Instructions for Authors (1991),' *J. Chem. Soc., Perkin Trans. 2*, 1991, issue 1.

Table 1 Method of preparation and conditions for crystallisation

Compound	Method	Solvent	Precipitant
2	A	Ethyl acetate	Light petroleum (b.p. 30–40 °C)
3	A	Chloroform–ethanol (5:1)	Hexane
4	B	Chloroform–ethanol (5:1)	Light petroleum (b.p. 30–40 °C)
5	A	Toluene	Hexane
6	A	Ethyl acetate	Light petroleum (b.p. 30–40 °C)
7	A	Toluene	Hexane
8	B	Toluene	Hexane
9	B	Toluene	Hexane
10	A	Chloroform	Light petroleum (b.p. 30–40 °C)
11	B	Ethyl acetate	Light petroleum (b.p. 30–40 °C)
12	Urea	Chloroform	Light petroleum (b.p. 30–40 °C)
13	Urea	Chloroform	Light petroleum (b.p. 30–40 °C)
14	Urea	Chloroform	Light petroleum (b.p. 30–40 °C)
15	Urea	Ethanol	Light petroleum (b.p. 30–40 °C)

All non-hydrogen atoms were refined anisotropically. The positions of all the NH protons were located from ΔF maps, and refined isotropically subject to an NH distance constraint. The positions of all the remaining hydrogen atoms were idealised, C–H = 0.96 Å, assigned isotropic thermal parameters, $U(H) = 1.2U_{eq}(C)$, and allowed to ride on their parent carbon atoms. All methyl groups were refined as rigid bodies. The chirality of the structure of **7** was determined by the refinement of a free variable η which multiplies all f''. All computations were carried out on an Eclipse S140 computer using the SHELXTL program system (G. M. Sheldrick, SHELXTL, An Integrated System for Solving, Refining and Displaying Crystal Structures from Diffraction Data, Revision 5.2, 1985, University of Göttingen, FRG).

Octanol–Water Partition Coefficient (P) Measurements.—An accurately measured mass of the test compound was dissolved in octan-1-ol (presaturated with water) (20 cm^3). Aliquots (5 cm^3) were added to centrifuge tubes containing water (presaturated with octanol) (10 cm^3) and extra presaturated octanol (0.0, 1.0, 2.5 and 5.0 cm^3). The centrifuge tubes were shaken for 1 h at 20 °C, the layers separated and the octanol layers diluted as appropriate (usually by a factor of 10–50). Suitable standards of the compound in acetonitrile were prepared. Each layer from each tube (diluted where appropriate) was analysed together with the standards by HPLC (Hewlett Packard 1080B, 10 μm injection volume, 20 cm × 4.9 mm internal diameter column, packing Spherisorb 8–5 μm) monitoring at an appropriate wavelength. The standards were used to plot a standard curve of concentration against HPLC peak area and this was used to read off the unknown concentrations in the samples. The original concentrations in the centrifuge tubes were calculated, and hence the partition coefficients and log *P* values.

Photosystem II Inhibition: The Hill Reaction.—Leaves (20 g) from 10 day old pea plants (*Pisum sativum*) were homogenised at full speed for 20 s in a Waring blender in an ice-cold sucrose medium (20 cm^3, composition 0.35 mol dm^{-3} sucrose, 0.1 mol dm^{-3} potassium phosphate, 0.001 mol dm^{-3} magnesium chloride, pH 6.8). The homogenate was filtered through four layers of muslin and centrifuged for 7 min at 2000*g*. The pellet was resuspended in the sucrose medium (20 cm^3) and recentrifuged for 7 min at 2000*g*. The pellet was resuspended in the sucrose medium (20 cm^3) and recentrifuged under the same conditions. After resuspension in a dilute salt solution (20 cm^3; composition 0.1% sodium chloride, 0.1% magnesium chloride w/v), the ruptured chloroplasts were centrifuged at 12 500*g* for 15 min and the pellet resuspended in the salt solution (10 cm^3).

The chlorophyll content of the suspension was determined in duplicate according the following process. The suspension (100 mm^3) was shaken with 80% acetone (10 cm^3) and centrifuged at 2000*g* for 7 min. The absorbance of the supernatent at λ = 652 nm was determined and the concentration of chlorophyll estimated from the expression $[ch] = A_{652} \times 2.90$ mg ml^{-1}.

The assay medium for the Hill inhibitors consisted of tricine buffer (0.50 cm^3; composition 0.1 mol dm^{-3} tricine, 0.5 mol dm^{-3} methylamine, 0.002 mol dm^{-3} ammonium chloride, 0.002 mol dm^{-3} sodium azide pH 8.0), 2-acetamido-3-(isopropylamino)-1,4-naphthaquinone (an autoxidizable quinone), in Me_2SO (0.01 mol dm^{-3}; 1 mm^3), a suitable volume of chloroplast suspension to give a final chlorophyll concentration of 50 μg ml^{-1}, a sufficient volume of water to give 1 cm^3 of assay medium and between 0 and 10 mm^3 of a solution of the test compound in Me_2SO.

The rate of electron transport was monitored by measuring the oxygen uptake associated with the reoxidation of the quinone, using a Hansatech oxygen electrode at a constant temperature of 20 °C. The reaction vessel was illuminated with red light of saturating intensity (150 W quartz–iodine projector bulb with red filter) and the rate of electron transport in the presence of various concentrations of the test compound was compared with control rates to give values for % activity. Between four and six different concentrations were used and the PI_{50} was estimated graphically from a plot of % activity against log (concentration).

Results and Discussion

As hydrophobicity is an important variable in determining the activity of PSII inhibitors,[4–10] we first set out to find a measure of this physico-chemical parameter which would allow us to treat both the N-phenylanilides and the phenylureas in the same QSAR analysis. Parameters derived from the Hansch[12] parameter π for the substituents on the aromatic ring were found not to be suitable as these do not allow for the electronic interactions between the amide group and the aromatic substituent. We therefore derived a new parameter which we call π_{amide}, defined by eqn. (1).

$$\pi_{amide} = \log P_{substituted\ compound} - \log P_{unsubstituted\ compound\ (X=H)} \quad (1)$$

This parameter allows both for the contribution to hydrophobicity by the substituent X on the aromatic ring and for interactions between the amide moiety and the substituent. In fact, a different π_{amide} parameter is derived for each substituent in each position on the phenyl ring (*e.g.* *o*-Cl, 0.10; *m*-Cl, 1.11; *p*-Cl, 0.94) and for combinations of substituents (*m*, *p*-Cl_2, 1.83). The value for *m*,*p*-Cl_2 is lower than the sum of the *meta*- and *para*-chlorine values because the substituents alone

Table 2 Crystal data and data-collection parameters

	Compound											
	(3)	**(4)**	**(5)**	**(7)**	**(8)**	**(10)**	**(11)**	**(12)**	**(13)**	**(14)**	**(15)**	**(40)**
Formula	$C_{14}H_{13}NO$	$C_{14}H_{12}N_2O_3$	$C_{14}H_{12}FNO$	$C_{11}H_{12}BrNO$	$C_{11}H_{13}Cl_2NO$	$C_{14}H_{11}Cl_2NO_2$	$C_{15}H_{13}Cl_2NO$	$C_9H_{12}N_2O$	$C_{12}H_{18}N_2O$	$C_{12}H_{16}FN_2O$	$C_{10}H_{13}ClN_2O$	$C_{13}H_{20}N_2O$
M_w	211.3	256.3	229.3	254.1	246.1	296.2	294.2	164.2	206.3	223.3	212.7	220.3
Crystal system	Monoclinic	Monoclinic	Orthorhombic	Orthorhombic	Monoclinic	Triclinic	Monoclinic	Orthorhombic	Orthorhombic	Orthorhombic	Monoclinic	Monoclinic
Crystal dimensions/mm	0.07 × 0.10 × 0.27	0.27 × 0.27 × 0.47	0.17 × 0.27 × 0.67	0.13 × 0.23 × 0.33	0.08 × 0.20 × 0.23	0.12 × 0.14 × 0.27	0.17 × 0.17 × 0.80	0.27 × 0.36 × 0.38	0.18 × 0.25 × 0.27	0.09 × 0.17 × 0.19	0.04 × 0.27 × 0.47	0.08 × 0.13 × 0.15
a/Å	5.687(1)	13.890(3)	4.820(1)	8.258(1)	9.736(1)	5.610(2)	20.821(5)	10.503(2)	10.182(2)	10.325(2)	10.679(3)	9.870(2)
b/Å	25.318(7)	11.707(3)	19.774(2)	9.326(1)	11.630(8)	7.882(3)	4.942(1)	10.843(2)	11.008(3)	11.005(2)	9.080(2)	9.533(2)
c/Å	8.100(5)	16.241(3)	24.867(4)	14.302(1)	11.806(9)	15.064(6)	28.421(6)	15.324(2)	21.013(5)	21.339(4)	11.418(2)	14.934(2)
α/°	90	90	90	90	90	102.32(3)	90	90	90	90	90	90
β/°	91.97(1)	101.12(1)	90	90	111.41(5)	99.11(3)	99.99(2)	90	90	90	102.26(2)	108.18(1)
γ/°	90	90	90	90	90	92.03(3)	90	90	90	90	90	90
U/Å^3	1166	2591	2370	1101	1245	641	2880	1745	2355	2425	1082	1335
Z	4	8	8	4	4	2	8	8	8	8	4	4
Space group	$P2_1/n$	$P2_1/c$	$Pbc2_1$[a]	$P2_12_12_1$	$P2_1/a$	$P\bar{1}$	$C2/c$	$Pbca$	$Pbca$	$Pbca$	$P2_1/c$	$P2_1/a$
D_c/g cm^{-3}	1.20	1.31	1.28	1.53	1.31	1.53	1.36	1.25	1.16	1.22	1.31	1.10
F(000)	448	1072	960	512	512	304	1216	704	896	952	448	480
μ(Cu-Kα)/cm^{-1}	6	7	7	49	46	46	41	6	6	7	29	5
θ range/°	0–58	0–55	0–58	0–58	0–50	0–58	0–55	0–58	0–58	0–58	0–58	0–58
No. unique reflections	1557	3260	1639	778	1270	1728	1808	1179	1578	1634	1452	1803
No. obs. reflections	1321	2909	1589	767	1116	1591	1626	1105	1367	1414	1285	1515
No. variables	150	352	316	150	144	180	174	122	153	171	139	165
Absorption	None	None	None	Gaussian	Gaussian	Gaussian	Gaussian	None	None	None	None	None
Transmission factors	—	—	—	0.530, 0.327	0.682, 0.408	0.629, 0.431	0.612, 0.370	—	—	—	—	—
R	0.058	0.043	0.039	0.026	0.060	0.049	0.071	0.041	0.046	0.053	0.053	0.056
R_w	0.067	0.051	0.047	0.028	0.076	0.057	0.089	0.054	0.053	0.063	0.063	0.067
g	0.002 23	0.000 48	0.000 58	0.000 97	0.000 49	0.001 42	0.000 96	0.000 90	0.000 80	0.003 21	0.000 79	0.001 28
Extinction parameter, x	0.016(4)	0.013(1)	0.002(1)	0.012(1)	0.012(3)	0.028(5)	0.002(1)	0.009(2)	0.002(1)	0.007(1)	0.007(2)	0.002 9(4)

[a] Non-standard aspect of space group *Pca* 2, No. 29.

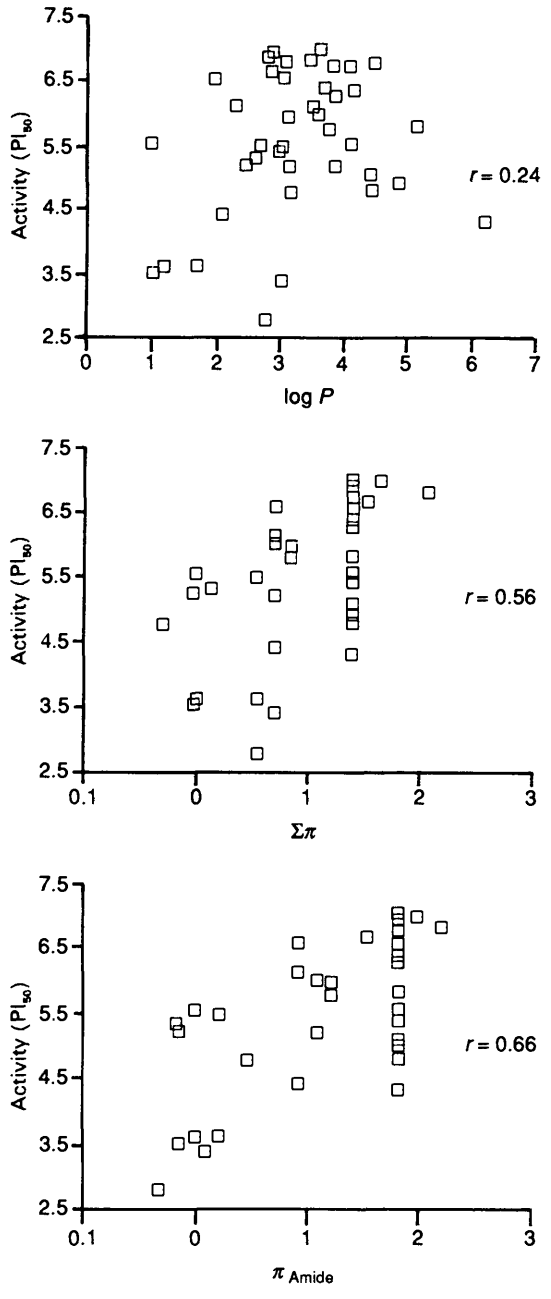

Fig. 1 *'In vitro'* activity of PSII inhibitors plotted against various measures of hydrophobicity: (*a*) $r = 0.24$; (*b*) $r = 0.56$; (*c*) $r = 0.66$

present a certain surface area to the solvent, and this contributes to the hydrophobicity; but when there are two adjacent substituents, the facing surfaces do not contact solvent and therefore do not contribute to the hydrophobicity.[13] This probably also accounts in part for the low value for *ortho*-chlorine, but this may also be influenced by steric interactions and possibly by hydrogen bonding between the chlorine and the amide group.

Fig. 1 shows plots of Hill inhibition activity (PI_{50}) against various measures of hydrophobicity, namely log *P*, $\Sigma\pi$ and π_{amide}, using data listed in Table 3. The best correlation coefficient, *r*, is obtained when PI_{50} values are plotted against π_{amide}. However, this parameter only explains 44% of the variance in the activity data, leaving the remaining 56% variability unexplained. As previous studies[4,10] appeared to suggest that the size and shape of the amide moiety might influence the PSII activity of these molecules, the X-ray structures of compounds **2**–**24** were analysed (Table 2).

Crystal structures for acetanilide **16**,[14] 4′-chloroacetanilide **17**,[15] 4′-methylacetanilide **18**,[16] 4′-methoxyacetanilide **19**,[17] 3′,4′-dichloro-2-methylpropananilide **31**,[18] *N*′-(4-chlorophenyl)-*N*,*N*-dimethylurea **37**,[19] *N*′-(4-chlorophenyl)-*N*-methoxy-*N*-methylurea **38**,[20] *N*′-(3,4-dichlorophenyl)-*N*,*N*-dimethylurea **39**,[21] 3′,4′-dichloroacetanilide **2**,[22] 3′,4′-dichlorocyclopropanecarboxanilide **6**,[22] and 3′,4′-dichlorophenyl-3-methylbutananilide **9**[23] are described in the literature. The compounds phenylacetanilide **3**, 4′-nitro(phenyl)acetanilide **4**, 2′-fluoro(phenyl)acetanilide **5**, 3′-bromo-1-methylcyclopropanecarboxanilide **7**, 3′,4′-dichloropentananilide **8**, 3′,4′-dichloro-4-methoxybenzanilide **10**, 3′,4′-dichloro-3-phenylpropananilide **11**, *N*,*N*-dimethyl-*N*′-phenylurea **12**, *N*′-(4-isopropylphenyl)-*N*,*N*-dimethylurea **13**, *N*′-(3-fluoro-4-isopropylphenyl)-*N*,*N*-dimethylurea **14** and *N*′-(3-chlorophenyl)-*N*-propylurea **15** were crystallised and crystal structures obtained.

A comparison of particular bond lengths and bond angles between different molecules did not yield any significant correlation with PSII activity. This was not surprising as these relatively simple molecules contain only one common hydrogen-bond donor/acceptor group. Further examination of the X-ray structures showed that the torsion angle (θ) between the phenyl and the amide planes varied considerably from one compound to another. Measured θ values are defined as angles between the plane of the six carbon atoms in the phenyl ring and the atoms in the C–(C=O)–N moiety. θ values for literature compounds have been recalculated on this basis for comparison. For example, in the three amides shown in Fig. 2, θ varies from 6.0° to 47.7°. Other θ values are listed in Table 3. For this reason, θ was investigated as a possible variable in describing biological activity. Table 3 shows that four of the compounds are given two values of θ. This is because these compounds have two molecules in the unit cell (in the case of **2**, **4** and **5**), or because there are two crystal forms known (in the case of **18**). Since in a structure–activity correlation, only one value of θ can be used in relation to one value of activity, the interpretation of these results causes a problem.

It has been noted by Du Plessis[24] that in the case of acetanilides (ArNHCOAr), crystal packing forces tend to cause both aryl rings to rotate out of coplanarity with the amide group. It seems reasonable to suggest that packing might have the same effect in the case of other molecules. Hence, in the four cases with two values of θ, the smaller value corresponding to the more planar conformation was used in structure–activity correlations.

Before analysing the data further, three of the data points need to be treated separately. Two of these are for acetanilide **16** and 4′-methoxyacetanilide **19**. We have already noted that one other substituted acetanilide, **2**, exhibits two conformations in its crystal structure, one having $\theta = 4.4°$ and the other $\theta = 20.7°$. 4′-Methylacetanilide **18** exhibits polymorphism and shows different conformations in the different crystals, with very similar values of θ (5.4 and 18.5°). In the case of 4′-chloroacetanilide **17** only one form with $\theta = 6.0°$ is known. It therefore seems a reasonable assumption, if we assume that the conformation with larger θ is influenced by packing forces, that in the case of **16** and **19** the crystal form showing a similarly large angle (17.1 and 19.1°) is more influenced by packing forces. These two points are therefore excluded from the analysis.

The third point is for the compound 3′,4′-dichloro-4-methoxybenzanilide **10**. This is the only benzamide derivative, and it has been suggested for similar compounds that crystal packing forces influence the conformation in the crystal.[24] For this reason, this compound too was excluded from structure–activity consideration.

The 20 compounds for which both π_{amide} and θ (degrees) values were available were subjected to multiple regression analysis with the following results.

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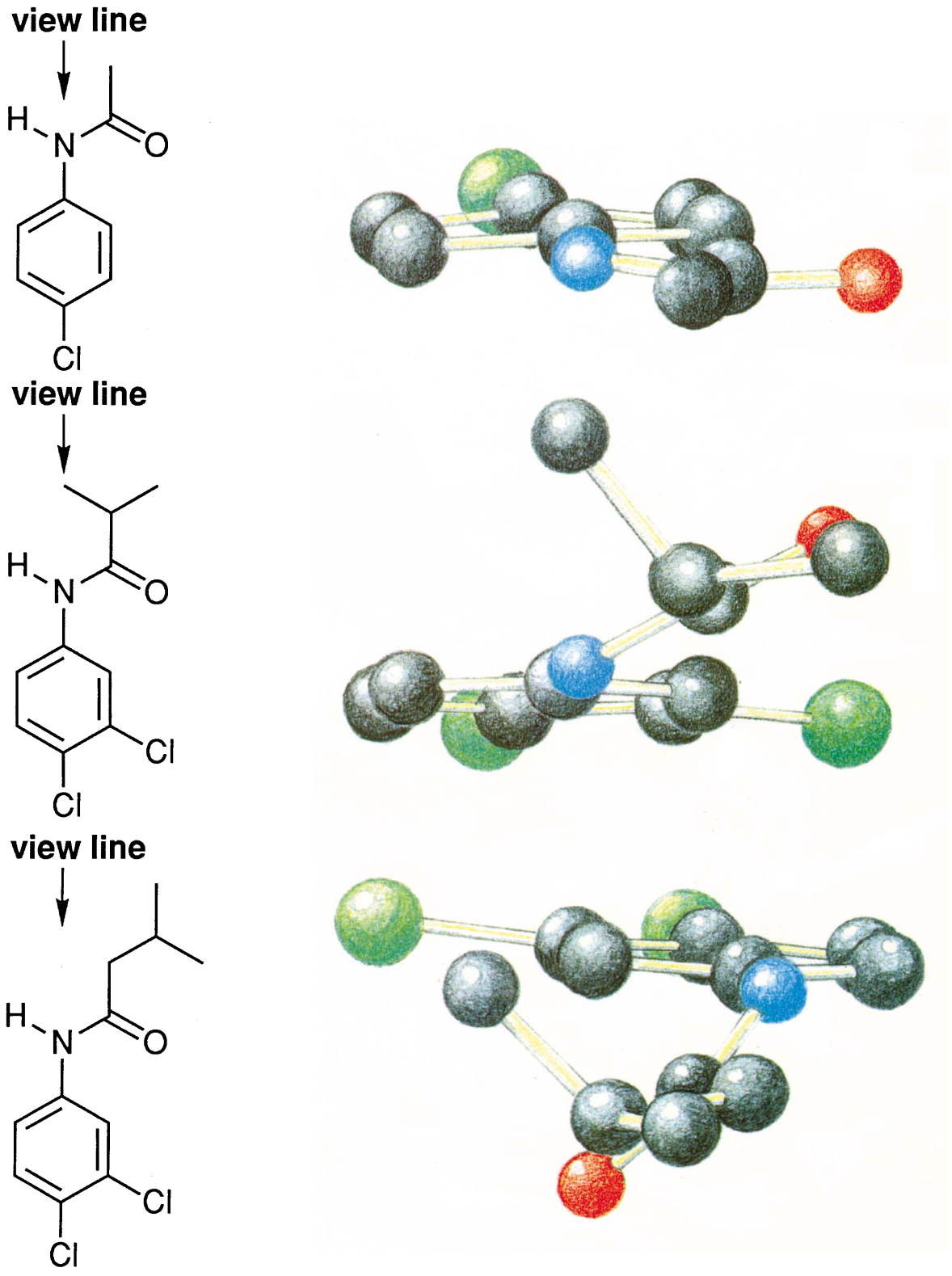

Fig. 2 Crystal-structure conformations of compounds **17**, **31** and **9** showing the angle between the phenyl and amide planes

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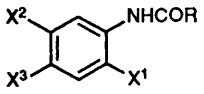
Table 3 Selected physico-chemical and *'in vitro'* data for the anilides and ureas



Compd.	X_1	X_2	X_3	R	PI_{50}	log P^c	ClogP^d	$\Sigma\pi^e$	π_{amide}	θ
2[h]	H	Cl	Cl	Me	5.43	3.01[f]	3.04	1.42	1.83	4.4, 20.7
3	H	H	H	CH_2Ph	5.51	2.70	2.93	0.00	0.00	26.9
4	H	H	NO_2	CH_2Ph	4.75	3.18	3.32	−0.28	0.48	16.5, 24.6
5	F	H	H	CH_2Ph	5.35	2.62	2.72	0.14	−0.16	41.9, 53.6
6[h]	H	Cl	Cl	c-Pr	6.76	3.83	3.39	1.42	1.83	27.5
7	H	Br	H	1-Me-c-Pr	5.97	3.14[g]	3.20	0.86	1.25	39.2
8	H	Cl	Cl	Bu	6.37	4.17	4.63	1.42	1.83	29.2
9	H	Cl	Cl	CH_2CHMe_2	5.56	4.11	4.50	1.42	1.83	47.7
10	H	Cl	Cl	*p*-$MeOC_6H_4$	4.5[a]	4.64[g]	4.64	1.42	1.83	30.4
11	H	Cl	Cl	CH_2CH_2Ph	4.91	4.85	5.14	1.42	1.83	46.2
12	H	H	H	NMe_2	5.57	0.98[g]	—	0.00	0.00	30.1
13	H	H	Pr^i	NMe_2	6.66	2.87	2.44	1.53	1.56	34.3
14	H	F	Pr^i	NMe_2	7.00	—	2.89	1.67	2.00	32.9
15	H	Cl	H	$NHCH_2CH_2CH_3$	5.22	—	3.17	0.71	1.11	36.8
16	H	H	H	Me	3.63	1.18[f]	1.16	0.00	0.00	17.1
17	H	H	Cl	Me	4.42	2.12[f]	2.18	0.71	0.94	6.0
18	H	H	Me	Me	3.61	1.70	1.81	0.56	0.22	5.4, 18.5
19	H	H	OMe	Me	3.52	1.03	1.26	−0.02	−0.15	19.1
20	H	Cl	H	CH_2Ph	5.99	3.61	3.94	0.71	1.11	—
21	H	H	Cl	CH_2Ph	6.12	3.54	3.94	0.71	0.94	—
22	H	Cl	Cl	CH_2Ph	6.80	4.47	4.81	1.42	1.83	—
23	H	H	Me	CH_2Ph	5.48	3.05	3.58	0.56	0.22	—
24	H	H	OMe	CH_2Ph	5.24	2.49	3.03	−0.02	−0.15	—
25	H	Br	H	CH_2Ph	5.79	3.77	4.09	0.86	1.25	—
26	Me	H	H	CH_2Ph	2.8[a]	—	2.77	0.56	−0.33	—
27	Cl	H	H	CH_2Ph	3.4[a]	—	3.04	0.71	0.10	—
28	H	Cl	Cl	1-Ph-c-Pr	5.81	—	5.15	1.42	1.83	—
29	H	Cl	Cl	1-Me-c-Pr	7.02	3.63	3.91	1.42	1.83	—
30	H	Cl	Cl	Et	6.59	3.07	3.57	1.42	1.83	—
31	H	Cl	Cl	$CHMe_2$	6.43	3.72	3.88	1.42	1.83	26.6
32[i]	H	Cl	Cl	Bu'	6.29	3.88	4.28	1.42	1.83	—
33	H	Cl	Cl	Ph	5.09	4.42	4.53	1.42	1.83	—
34	H	Cl	Cl	$C(Me)=CH_2$	6.87	3.49[g]	3.56	1.42	1.83	—
35	H	Cl	Cl	(*Z*)-$MeCH=CHCH_2$	6.78	—	4.09	1.42	1.83	—
36	H	Cl	Cl	(*E*)-CH=CHPh	4.33	—	6.19	1.42	1.83	—
37	H	H	Cl	NMe_2	6.59	1.94	1.88	0.71	0.94	48.9
38	H	H	Cl	NMe(OMe)	6.14	2.30	2.31	0.71	0.94	39.9
39	H	H	Cl	NMe_2	6.90	2.80	2.75	1.42	1.83	30.6
40[b]	H	Me	Pr^i	NMe_2	6.83	—	3.09	2.09	2.21	32.9
41	H	Cl	H	$NHCH_2Ph$	5.20	—	3.88	0.71	1.11	—

[a] Solubility problems — approximate values only. [b] Compound obtained from Shell Research Limited. [c] From *C* log *P* database unless otherwise stated. [d] Calculated log *P* using the CLOP3 program; [e] Ref. 10. [f] Measured as part of this study. [g] Ref 2. [h] Crystal structures published in ref. 2. [i] Crystal structure published in ref. 23.

$$PI_{50} = 0.70\,(\pm 0.46)\,\pi_{amide} + 5.0\,(\pm 0.7) \quad (2)$$
$$n = 20,\ r = 0.57,\ F = 88$$

$$PI_{50} = 0.59\,(\pm 0.34)\,\pi_{amide} - 2.2\,(\pm 1.3) \times 10^{-3}\theta^2 + 0.14\,(\pm 0.07)\,\theta + 3.2\,(\pm 0.9) \quad (3)$$
$$n = 20,\ r = 0.83,\ F = 11.8$$

As noted before, π_{amide} accounts for only a low (in this case 33%) percentage of the variance. The inclusion of the term θ and θ^2 explains a further 36%. It also appears from eqn. (3) that activity reaches a maximum when the torsion angle θ is *ca.* 34°. A plot of θ against 'corrected' activity (equal to $PI_{50} - \pi_{amide}$) is shown in Fig. 3. It is clear from Fig. 3 that too many points are clustered around $\theta = 34°$, making the correlation statistically unsound. In the present study, this cannot be avoided as we are limited by the compounds which will produce crystal structures.

Some points fall well away from the correlation, notably *N'*-(4-chlorophenyl)-*N*,*N*-dimethyl urea **37**. This has a large value of θ (48.9°) and yet still a high activity. The reason for this discrepancy is not clear, but one possibility is that the conformation in the crystal has been influenced by packing forces.

One of the points noted in the literature[7,10] is that *ortho*-substitution or *N*-methylation could influence the conformations of the amides. Unfortunately, *N*-methylation renders the compounds non-crystalline, so crystallography could not be used to investigate whether this affects the angle θ. For the *ortho*-substituted compounds, the situation is different. The structure for the *ortho*-fluorinated compound **5** shows that the angle θ is increased (41.9 or 53.6°). The effect on activity is not large (unlike the effect for *ortho*-chlorinated compounds such as **27**), probably because of the small size of the fluorine group compared with the chlorine atom; the smaller size would affect the conformation less. The crystal structure for 2,6-dichloroacetanilide is known in the literature.[25] In the two different sorts of crystal formed, the values of θ are 71.4 and 68.3° and the activity of such di-*ortho*-chlorinated species is very low.[26]

4'-Nitro(phenyl)acetanilide **4** has a small value of θ (16.5 and 24.6°), readily explained in terms of increased conjugation between the amide group and the phenyl ring, causing a more planar conformation to be preferred. This increased planarity can explain the lowered activity of this compound.

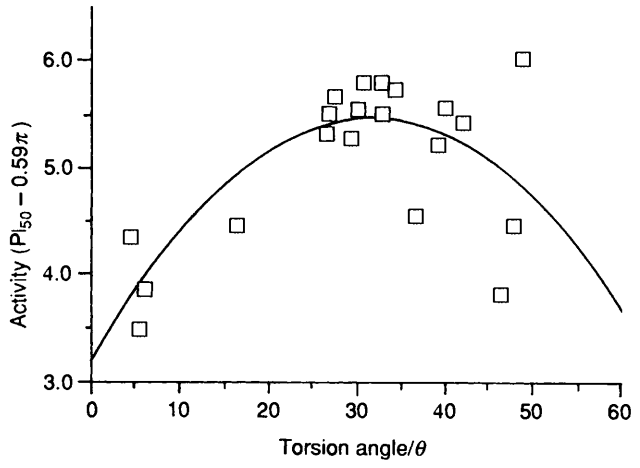


Fig. 3 Plot of 'corrected activity' *vs.* the torsion angle, θ, for selected data (see the text)

For many of the other compounds, differences in θ may be explained by steric effects within the molecule. Dunitz described[27] a correlation between the torsion angle θ and the Ar–N–CO bond angle in a large number of amides such as the ones studied here, and this correlation is also found with these compounds. The angle at nitrogen opens out as the molecule becomes more planar (*i.e.* as θ decreases), showing that there is steric interference between the *ortho* atoms attached to the ring, including hydrogens, and the carbonyl oxygen. If the amide substituent is larger, then it may place steric strain on the carbonyl and cause the aromatic ring to move out of coplanarity with the amide group. This might explain why the acetanilides **2**, **16**, **17**, **18** and **19** tend to be more planar than say the ureas **13**, **14**, **15**, **39**, **40** and **41**. There are results which cannot be explained in this way though — for instance the phenylpropananilide **11** has a larger angle θ (46.2°) than the phenylacetanilide **13**, (θ = 26.9°), even though the only difference between the amide substutents of the compounds is the addition of a methylene unit to the alkyl chain. There appears to be no obvious explanation for this difference, though it does agree with the much lower activity of compound **11** over the analogous phenylacetamide **22**. The similarly large value of θ for the 2-methylbutananilide **9** again cannot be explained.

A meaningful correlation of biological data with crystal structure must have a sound physical basis. The QSAR method is an example of a linear free-energy relationship, so that all the terms in eqn. (3) are effectively free-energy measurements. Thus, for this equation to make physical sense, the crystallographic torsion angles (as defined previously) should be related to energies. A simple explanation is as follows. If the amide or urea binds in a certain conformation only (say with $\theta = \theta'$) then any compound which exists in a minimum energy conformation different from this will have to change its conformation in order to bind. Assuming a U-shaped potential well for rotation about θ, then the further away the minimum conformation is from θ', the more energy will need to be expended in order to reach the binding conformation. To a first approximation this will produce a quadratic relationship shown in Fig. 3, with the optimum value of θ (giving highest activity) corresponding to the binding conformation. The use of these approximations may well explain why the correlation is not better than it is.

Attempts (by UV and NMR experiments) to obtain the solution conformation of the molecules or to calculated (results not shown) the energy difference between the minimum energy and assumed binding conformation in the gas phase did not produce satisfactory results, so that under the present circumstances the crystallographic method described appears to be the best available.

Conclusions

The PSII activity of acetanilides and the *N*-phenylureas considered in this study varies with the hydrophobic character of the substituents on the phenyl moiety and also appears to depend on the torsion angle θ between the planes of the amide and phenyl groups. The X-ray results indicate that optimum activity is registered when θ is *ca.* 34°. Those molecules which possess a (solid-state) minimum-energy conformation close to 34° show a high activity compared with those possessing a different minimum energy conformation. In a simple model such compounds would have a lower activity since energy would need to be expended in order to reach the binding conformation.

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