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FORECASTING THE ANTIMALARIAL ACTIVITIES OF ARYLAMIDINOUREAS FROM THEIR MEASURED PHYSICOCHEMICAL PROPERTIES

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1 The octanol : water partition coefficient (PC), protein binding coefficients to albumin (*Ba*) and haemoglobin (*Bh*), proton magnetic resonance (PMR) chemical shifts and pK_a of 8 arylamidinoureas have been measured.

2 There were statistically significant correlations for 7 of the 8 compounds between the predicted lipophilicity parameter π and log PC, *Ba* and *Bh*, and also between Hammett's electronic parameter σ and the PMR and pK_a observations.

3 3-Chloro,4-nitrophenylamidinourea showed a larger deviation from these correlations than any other compound. Geometric calculations based on the covalent radii and bond angles of the molecule suggested that the neighbouring chloro and nitro substituents were interacting sterically.

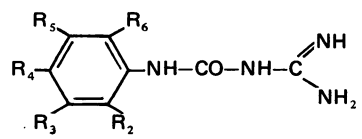
4 Molecular orbital calculations and the observed ultra-violet spectrum provided further evidence for such an interaction, and suggested that the nitro group is twisted out of the plane of the benzene ring in this molecule.

5 Such steric interactions can introduce an additional source of error when attempts are made to correlate the biological and predicted physicochemical properties of compounds.

6 Generalized parameters such as π and σ cannot be used with confidence to predict the properties of compounds in which such steric interactions occur.

Introduction

It was found by Goodford, Norrington, Richards & Walls (1973) that the antimalarial activity A of an arylamidinourea in the series with general formula:



(I)

could be forecast by the equation:

$$A = 0.28\pi + 0.86\sigma - 0.09 \qquad (1)$$

where π and σ were predicted values for two of the physicochemical properties of the compound. The equation gave adequate forecasts for the activities of 12 out of 13 new arylamidinoureas, although it had a multiple correlation coefficient of only 0.68. The possibility that a better correlation would be obtained by the use of measured properties has now been examined.

Methods

Physicochemical parameters

The effect of changing the benzenoid substituent groups R_3 to R_5 upon the lipophilicity of the arylamidinoureas was predicted with the parameter π (Leo, Hansch & Elkins, 1971). This was estimated from substituted phenols in view of the electron-donating nature of the amidinourea side-chain. Hammett's (1940) constant σ which measures the electron-withdrawing properties of the substituents was the second predicted parameter. When more than one benzenoid substituent group was present, the total change in physicochemical properties was predicted by linear addition of the individual substituent parameters.

Protein binding

(a) *Binding to albumin* All solutions were made in 0.1 M sodium phosphate buffer pH 7.4. Binding was determined after equilibrium dialysis for 18 h at 37° C of 5 ml drug solution against a sac containing 5 ml bovine serum albumin solution

(fraction V. Armour Chemical Co.). Albumin concentrations up to 3×10^{-4} M were used, depending on the tightness of binding. After dialysis the concentration of unbound drug was measured spectrophotometrically or by ^{14}C counting. The dialysis sac was prepared from Visking tubing which was washed in 10^{-2} M disodium edetate (EDTA) and then soaked for 24 h in each of two changes of distilled water.

(b) *Binding to haemoglobin* Horse haemoglobin was prepared by the method of Garby, Gerber & de Verdier (1969). Solutions of drug and protein were prepared in a buffered balanced salt solution containing (mM) KCl 110, NaCl 20, MgCl 3, potassium phosphate 1, pH 7.4. Binding was determined by a modification of the ultracentrifugal procedure of Garby *et al.* (1969). Solutions of drug and haemoglobin (3×10^{-4} M) were centrifuged for 16 h at 75,000 *g* in the 6 x 15 ml swing-out rotor of the MSE Superspeed 65 Centrifuge. The upper, protein-free layer was aspirated and the concentration of free drug determined spectrophotometrically.

(c) *Expression of results* For a protein with n binding sites each of binding constant K, in equilibrium with unbound drug at concentration D

$$K = \frac{R}{D(n-R)}$$

where R is the number of drug molecules bound per molecule of protein. For the present series of compounds n was not known and could not be determined experimentally because of the low solubility of the test compounds. Over a range of concentrations of each drug the ratio R/D did not change significantly and we conclude that $n \gg R$ over the range of concentrations available to us. Results were therefore calculated as R/D ($\simeq nk$) which should be proportional to K on the not unreasonable assumption that n is constant for a congeneric series such as that studied in the present investigation.

For comparison with the other parameters the binding results are stated on logarithmic scales:

$B_a = \log (R/D)$ for albumin
$B_h = \log (R/D)$ for haemoglobin

Partition coefficient

Each compound was dissolved in 6 mM $NaOH/Na_2B_4O_7$ buffer at pH 9.95 and a concentration giving an absorbance of 0.2-0.8 at a suitable wavelength in the range 230-290 nm. Serial dilution measurements demonstrated the absence of phenomena which could lead to apparent deviations from Beer's Law. The absorbance A of the initial solution was determined (A initial) and 100 ml aliquots were then added to 5.0 ml octanol (Koch-Light 'Puriss') in 250 ml conical flasks, and the mixture equilibrated with gentle intermittent shaking for 1 week. The absorbance of the aqueous phase was redetermined (A final) after 30 min centrifugation at 600 *g*, and the partition coefficient (PC) calculated as:

$$PC = \frac{\text{A initial} - \text{A final}}{\text{A final}} \times 20 \qquad (2)$$

Proton magnetic resonance

The proton magnetic resonance spectrum for each compound was determined by Mr A.G. Ferrige at 100 MHz on a Varian HA-100 using a probe temperature of 27°C. Hexadeuterodimethylsulphoxide was used as the solvent and a concentration of approximately 100 mg/ml was employed. A capillary containing 10% hexamethyldisiloxane dissolved in carbon tetrachloride was used to establish a field-frequency lock. Chemical shifts of the aromatic protons were measured in parts/10^6 from the lock signal.

Apparent pK_a

Each compound was dissolved at a concentration of approximately 1 mM in 20 mM HCl, and 0.1 or 0.2 ml pipetted into 3 ml phosphate buffer in a 1 cm path-length silicon cuvette at room temperature. Ultra-violet absorption spectra were recorded on a Unicam SP800 spectrophotometer for a range of 7 or 8 buffers between pH 6.2 and 7.5 at ionic strength of 0.1. The pH of each solution was verified with Radiometer Blood pH equipment. Values of absorbance A^{λ} were measured from the recorded spectra for several different wavelengths λ.

Values of pK_a $A^{\lambda}_{BH^+}$ and A^{λ}_{B} were fitted for each wavelength λ from equation (3):

$$pH - pK_a = \log_{10}\left(\frac{A^{\lambda} - A^{\lambda}_{BH^+}}{A^{\lambda}_{B} - A^{\lambda}}\right) \qquad (3)$$

using an iterative calculation. This method avoids the necessity of measuring the absorbance of the base in dilute acid and dilute alkali, when second order medium effects may perturb the spectrum. Each final pK_a value is derived from observations at four different wavelengths.

Results

Protein binding

Observations were made on a group of eight arylamidinoureas (Table 1) chosen to have ranges

of π and σ as wide and uncorrelated as practicable. These were the same compounds as were used by Cranfield, Goodford, Norrington, Richards, Sheppey & Williams (1974). It was found that haemoglobin binding B_h was related to π according to the equation:

$$B_h = 0.36\pi + 2.78 \quad (5)$$

$t = 4.35$; $F = 18.9$;

$d.f. = 6$; $0.005 > P$; $M.C.C. = 0.87$;

which shows that lipophilic arylamidinoureas tended to bind more strongly than hydrophilic compounds.

By contrast, there was a less satisfactory relationship between albumin binding B_a and π:

$$B_a = 0.65\pi + 3.46 \quad (6)$$

$t = 2.53$; $F = 6.39$; $d.f. = 6$;

$0.05 > P > 0.02$; $M.C.C. = 0.72$

and when the results were plotted it was found that the poorer statistics were particularly due to the deviation of the 3-chloro,4-nitroarylamidinourea from the regression line. The residual for this compound was more than double that of any other, and the regression for the remaining seven arylamidinoureas was more acceptable:

$$B_a = 0.45\pi + 3.50 \quad (7)$$

$t = 4.14$; $F = 17.14$; $d.f. = 5$;

$0.01 > P > 0.005$; $M.C.C. = 0.88$

Equation (7) is plotted in Figure 1a, which demonstrates the deviation of the 3-chloro, 4-nitro compound from the regression for the other seven, and it was this finding which first suggested the possibility that π might be an inadequate prediction of lipophilicity in this series of compounds.

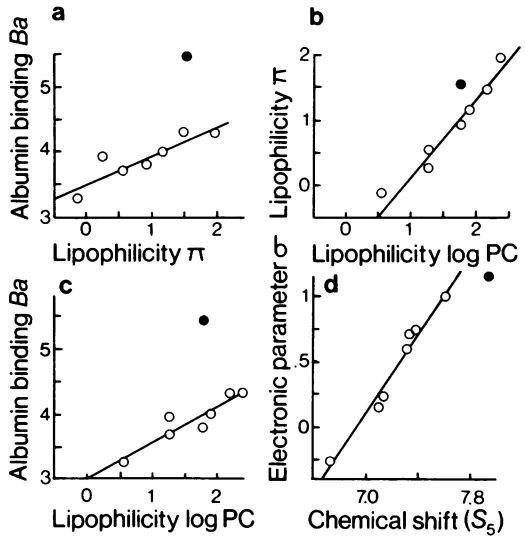


Figure 1 The relationships between predicted physicochemical properties (π and σ) and those observed (B_a, log PC, S_5). Logarithmic units. Each circle represents one of the eight compounds. The black circle represents the anomalous 3-chloro, 4-nitro compound, and the linear regressions are least-squares fits to the other seven points. See text.

Partition coefficient

The partition coefficients (PC) for the same eight compounds were determined at high pH in order to suppress ionization, and were related to π according to the equation

$$\pi = 1.15 \log PC - 0.92 \quad (8)$$

$t = 7.38$; $F = 54.52$; $d.f. = 6$;

$0.001 > P$; $M.C.C. = 0.95$

Table 1 The benzenoid substituent groups, predicted parameters and measured properties of the eight arylamidinoureas

Substituent groups		*Predicted parameters*		*Observed protein binding*		*Observed physicochemical properties*		
R_3	R_4	π	σ	B_h	B_a	log PC	S_5	pK_a
meta	para	*lipophilicity*	*electronic*	*Haemoglobin*	*Albumin*	*log partition coefficient*	*Proton 5 chemical shift*	*Acidity*
H	OCH_3	−0.12	−0.27	2,53	3.26	0.56	6.71	7.73
H	Cl	0.93	0.23	2.98	3.79	1.78	7.14	7.36
NO_2	H	0.54	0.71	3.11	3.68	1.28	7.34	7.23
Cl	CN	1.18	1.00	3.32	4.01	1.90	7.62	6.95
Cl	Cl	1.97	0.60	3.51	4.31	2.39	7.32	7.21
Cl	NO_2	1.54	1.15	3.34	5.43	1.79	7.94	7.17
OCH_3	CN	0.26	0.74	3.09	3.94	1.28	7.39	6.96
CH_3	Cl	1.49	0.16	3.22	4.31	2.19	7.10	7.58

which was statistically acceptable with a slope that did not differ significantly from unity. Once again the 3-chloro,4-nitroarylamidinourea had the largest residual error (Figure 1b), and when it was omitted the equation became

$$\pi = 1.12\,\log PC - 0.93 \qquad (9)$$

$t = 9.57$; $F = 91.6$; $d.f. = 5$;

$0.001 > P$; $M.C.C. = 0.97$

However, the π value for this compound deviated from the π: log PC regression (equation (9) in the wrong direction to explain the discrepant point in Figure 1a, and the 3-chloro,4-nitroarylamino-urea therefore showed an even larger residual error when albumin binding B_a was regressed directly against log PC for all eight compounds:

$$B_a = 0.63\,\log PC + 3.05 \qquad (10)$$

$t = 1.74$; $F = 3.04$; $d.f. = 6$;

$0.2 > P > 0.1$; $M.C.C. = 0.58$

When the individual points are examined (Figure 1c) the anomalous behaviour of this one compound is still more clearly displayed, particularly in contrast to the satisfactory regression of B_a against log PC for the remaining seven arylamidinoureas:

$$B_a = 0.55\,\log PC + 3.01 \qquad (11)$$

$t = 5.79$; $F = 33.52$; $d.f. = 5$;

$0.005 > P$; $M.C.C. = 0.93$

Proton chemical shift

In view of the anomalies of the 3-chloro, 4-nitro compound in the previous observations, it was of interest to establish whether σ provided a reliable prediction of the electron-withdrawing properties of these two substituent groups in adjacent positions on the arylamidinourea benzenoid ring. In the absence of shift measurements for the C-1 carbon atom, the proton bonded to the nitrogen atom at position 1 of the ring would best reflect changes in substituent σ values, but for the complications arising from proton exchange. However, substituents at position 3 perturb the electrons at positions 1 and 5 equally via a field effect. Moreover, substituents at position 4 perturb the electrons at positions 1 and 5 by an essentially similar resonance effect. It was therefore not unreasonable to study the chemical shift S_5 of proton 5, and this was found to have a close correlation with σ:

$$\sigma = 1.25\,S_5 - 8.62 \qquad (12)$$

$t = 9.74$; $F = 95.01$; $d.f. = 6$;

$0.001 > P$; $M.C.C. = 0.97$

As previously, the same compound had the largest residual error (Figure 1d) and when it was omitted the regression equation became:

$$\sigma = 1.49\,S_5 - 10.32 \qquad (13)$$

$t = 12.92$; $F = 167.04$; $d.f. = 5$;

$0.001 > P$; $M.C.C. = 0.99$

which is plotted on the figure.

pK_a

Although the chemical shift S_5 correlated well with σ it is not possible to justify rigorously the arguments in the previous section in favour of studying position 5. An attempt was therefore made to study the effect of the benzenoid substituent groups upon the main side-chain directly. Since the amidinourea moiety is partly ionized in the physiological range, it seemed possible that pK_a observations might not only show the relevant electron- distribution in the side-chain, but might also be directly related to the biological activities of the compounds. However, the relationship between σ and pK_a:

$$\sigma = -1.51\,pK_a + 11.52 \qquad (14)$$

$t = 4.56$; $F = 20.83$; $d.f. = 6$;

$0.005 > P > 0.001$; $M.C.C. = 0.88$

was not as close as the relationship between σ and S_5. The 3-chloro,4-nitro compound once again had the largest residual error, and the t-value increased when it was omitted:

$$\sigma = -1.41\,pK_a + 10.69 \qquad (15)$$

$t = 6.92$; $F = 47.88$; $d.f. = 5$;

$0.001 > P$; $M.C.C. = 0.95$

The anomalies of this one compound therefore account for the poorer results to some extent. However, a further factor may be that the observed ionization occurs at the terminal amidine group. This is isolated by the rest of the side-chain from the resonance effects of the benzenoid substituents, and hence the pK_a observations may only be indirectly related to sigma.

Steric interactions

It seemed possible that some of the anomalies of the 3-chloro, 4-nitro compound might be due to steric interactions between the chlorine and its adjacent oxygen atom. The distance between their atomic centres was therefore calculated, taking reasonable values (Table 2) for covalent radii and bond angles, and was found to be 2.5 Å assuming that all the atoms were in the plane of the benzene

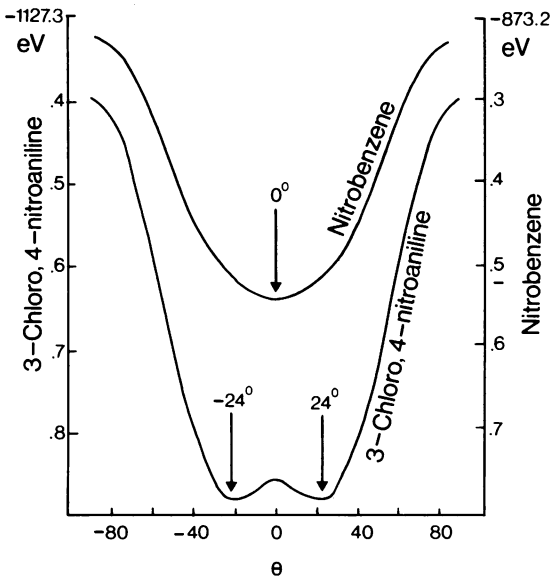


Figure 2 Results of molecular orbital calculations by the extended Hückel Treatment on 3-chloro, 4-nitroaniline and nitrobenzene. The nitrogen and chlorine atoms were constrained to lie in the plane of the benzene ring. Abscissae; the angle of rotation θ between this plane and the plane of the $-NO_2$ substituent group. Ordinates, the total energy calculated in eV. For nitro benzene there is a single minimum when the planes are parallel ($\theta = 0°$), but for 3-chloro, 4-nitroaniline there is a potential energy barrier at coplanarity and double minima at $\theta = \pm 24°$. See text.

ring. It is generally accepted that steric interference may occur in such situations if atoms approach nearer than twice the sum of their covalent radii (Braude & Sondheimer, 1955), which are approximately 0.99 Å and 0.55 Å for chlorine and doubly bonded oxygen respectively. Hence the interference spheres would touch at a centre-to-centre distance of 3.08 Å, and the calculated value of 2.5 Å indicates an unacceptable proximity unless the nitro group were rotated out of the plane of the benzene ring, and/or the chlorine atom displaced.

In a further attempt to predict this interaction, molecular orbital calculations were performed on nitrobenzene and 3-chloro,4-nitroaniline according to the extended Hückel treatment (Hoffman, 1963) using the bond lengths and angles shown in Table 2. The hydrogen, chlorine and nitrogen atoms were constrained to stay in the plane of the benzene ring, and the total binding energy E was calculated for each molecule as a function of the angle θ between the plane of the nitro group and the plane of the benzene ring. This energy was calculated for 10° increments of θ, and a single minimum at $\theta = 0°$ was found for nitrobenzene (Figure 2) whereas for 3-chloro, 4-nitroaniline a potential energy barrier was found for this planar conformation above a double minimum at ± 24°.

Such a calculation can only be approximate, because it is subject to the limitations of the extended Hückel method and the choice of atomic parameters. Nevertheless, it again shows that there may be a steric interaction between neighbouring chloro and nitro benzenoid substituents which can be relieved if the nitro group rotates out of the plane of the benzene ring.

For benzenoid substituents which have π electrons or lone pairs overlapping the π orbitals of the benzene ring, it is well established that steric perturbations can lead to changes in mesomeric interaction and absorption intensity of the ultra-violet absorption band usually seen in the 260-280 nm region (Braude & Sondheimer, 1955; Baddeley, Smith & Vickars, 1956; Frolen & Goodman, 1961). In the present case a single chloro substituent has little effect on the ultra-violet absorption of benzene (Table 3), but a single nitro group enhances the absorption intensity greatly. When both are present and unhindered as in the *para* isomer 1-chloro, 4-nitro benzene, the substituents interact and actually give a still higher intensity. However, positioning the nitro substituent *ortho* to the chloro in 1-chloro, 2-nitrobenzene has the reverse effect, and this is again compatible with a rotation of the nitro group about the C–N bond axis. There may also be a simultaneous bending of the Cl atom out of the plane of the benzene ring (Forbes, 1960), but on either interpretation one must conclude that the adjacent benzenoid chloro and nitro groups interact sterically.

Table 2 The covalent bond radii and angles used to calculate the chlorine-oxygen distance in 3-chloro, 4-nitro phenylamidinourea and 3-chloro, 4-nitroaniline

For benzene:	CC = 1.40 Å	CCl = 1.70 Å	CCC = 120°
For nitro:	CN = 1.48 Å	NO = 1.21 Å	ONO = 124°
For amino:	CN = 1.43 Å	NH = 1.01 Å	HNH = 120°

Discussion

The protein binding studies were initiated in order to ascertain whether the antimalarial activity of arylamidinoureas was associated with a selective binding to haemoglobin. This might either facilitate the uptake of drug by the parasite, or make the haemoglobin less available as a source of nutrient. However, the results lend no support to either hypothesis, as the ratio *Bh/Ba* for arylamidinoureas is similar to that of other small aromatic molecules (Hansch, Kiehs & Lawrence, 1965; Kiehs, Hansch & Moore, 1966). Any correlation between antimalarial activity and protein binding is therefore probably a reflection of their mutual correlation with lipophilicity.

The present results show little evidence against the use of π to predict lipophilicity and σ to predict electron-withdrawal in seven out of the eight arylamidinoureas studied. However, the 3-chloro,4-nitrophenylamidinourea shows a number of anomalies, and it is reasonable to interpret these in terms of the intramolecular steric hindrance in this compound. Twisting the nitro group out of the plane of the benzene ring disrupts the mesomeric interaction between the two, and thereby invalidates σ calculated from tabulated values in the literature. The 'corrected' σ is lower and is therefore more compatible with the compound's observed pK_a. Moreover, Fujita, Iwasa & Hansch (1964) have shown that the π value for any substituent is related to its σ value. Hence the 'corrected' σ for 3-chloro, 4-nitro phenylamidinourea leads to a 'corrected' π value which similarly reduces the discrepancy between log PC and π for this compound.

If the 4-nitro group is forced out of the plane of the benzene ring by the influence of the neighbouring 3-chloro substituent, it will itself have an anomalous effect upon the other substituent positions in the benzene ring. Hence it is not surprising that this compound has the largest residual error in the $\sigma : S_5$ correlation, and the proton chemical shift may in fact be a useful experimental probe for investigating such steric effects.

Table 3 Ultra-violet spectral characteristics of substituted benzenes. The depression of extinction coefficient from 8,900 for nitrobenzene to 4,000 in 1-chloro,2-nitrobenzene demonstrates a steric interaction between these *ortho* substituent groups.

Compound	λ_{max}	ϵ
Chlorobenzene	253	400
Nitrobenzene	253	8,900
1-Chloro, 4-nitro benzene	265	12,000
1-Chloro, 2-nitro benzene	244	4,000

Cranfield *et al.* (1974) observed the antimalarial activities of the present eight compounds in three test systems: *Plasmodium vinckei* in mice *in vivo*; *P. knowlesi* in monkey erythrocytes *in vitro*; and *P. berghei* in rat erythrocytes *in vitro*. They then calculated regression equations against π and σ for each activity, and used their equations to forecast the activities of further compounds. However, the present findings throw doubt on any equations which rely on predicted values of π and σ for the 3-chloro,4-nitrophenylamidinourea, and we have therefore tested alternative strategies in order to get over the difficulties raised by such a compound.

The findings of Cranfield *et al.* (1974) are summarized in Table 4 (method 1) for comparison with three alternative methods of calculation. With each of these alternatives it is accepted that the predicted π and σ for the 3-chloro,4-nitro compound are anomalous, but different methods are adopted for dealing with the anomalies. In method 2 (Table 4) the anomalous compound is ignored altogether, and the regressions calculated for the other seven. In method 3 the regressions are calculated for all eight compounds using the observed physicochemical measurements log PC and S_5. Finally, in method 4 'corrected' values of π and σ are calculated for the one compound from its measured log PC and S_5 values and from equations (9) and (13). These 'corrected' values are then used with the predicted π and σ parameters for the other seven compounds in order to derive regression equations.

No one method is consistently superior to the other three, when the conventional statistics (variance ratio F and multiple correlation coefficient R) of the equations are compared. For example with *P. vinckei* in mice the first method gives an F ratio of 6.4 with 5 and 2 degrees of freedom which is significant at the 5% probability level, while the second method only gives 3.4 with 4 and 2 d.f. which is not even significant at 10%. However, an opposite trend is observed when the same methods are compared for *P. knowlesi* where the F statistic (21.9) for method 2 is significant at the 1% probability level while method 1 fails to satisfy the same criterion. In fact neither method 1 nor method 2 demonstrates a clear superiority, and one must conclude after further study of the F and R values that these statistics do not afford any reliable guide for choosing between methods on the basis of the present observations. However, it will be observed in Table 4 that the coefficient of the lipophilicity term is always positive for the *P. vinckei* observations, and that it is always much less than the coefficient of the electronic term

Table 4 A comparison of regression equations derived by four different methods (see text)

Parasite	Host	Method of deriving equation															
		Method 1				Method 2				Method 3				Method 4			
		8 Compounds predicted π and σ				7 Compounds predicted π and σ				8 Compounds observed log PC and S_5				8 Compounds, 7 predicted 1 from observations			
		F	R	π	σ	F	R	π	σ	F	R	log PC	S_5	F	R	π	σ
P. vinckei	Mice *in vivo*	6.4	0.85	0.35	0.78	3.4	0.79	0.34	0.73	6.5	0.85	0.28	1.18	6.6	0.85	0.34	0.75
P. knowlesi	Monkey *in vitro*	11.9	0.91	0.88	0.21	21.9	0.96	0.93	0.47	38.3	0.97	1.16	0.21	20.2	0.94	0.96	0.25
P. berghei	Rat *in vitro*	11.1	0.90	0.53	−0.54	9.2	0.91	0.54	−0.51	11.0	0.90	0.66	−0.61	8.7	0.88	0.52	−0.35

Under each method is given the variance ratio *F*, the multiple correlation coefficient *R*, the coefficient of the lipophilicity term and the coefficient of the electronic term in the equation. The first method is that of Cranfield *et al.* (1974).

(row 1). This precedence is reversed for *P. knowlesi* where the electronic term plays a minor role, while for *P. berghei* its sign is consistently reversed. Hence each test system yields a characteristic type of equation, irrespective of the method of calculation.

The consistency of the equations is further demonstrated when they are used to forecast the antimalarial activities of more arylamidinoureas. Cranfield *et al.* (1974) studied a total of 44 forecasts, all of which were made on fresh compounds that had not been used to derive the regression equations. They assessed the forecasting ability of an equation by comparing the ranking order of forecast potency with the ranking order of measured antimalarial activity of the compounds, and this same method has been used to test the equations from methods 2 and 4 for comparison with method 1 which was that used by Cranfield *et al.* themselves. Method 3 cannot be used for forecasting at all, since the equations use the measured properties log PC and S_5 which would not be known for any novel compound until it had actually been synthesized.

The ranking order of 9 forecasts for *P. knowlesi* activity was the same for methods 1, 2 and 4, and there was only one small change in the 9 forecasts for *P. berghei* (ranks 4 and 5 were reversed for method 4). All 26 forecasts for *P. vinckei* were the same for all methods, except that ranks 3 and 4 were reversed for method 1, and there is very little to choose between the forecasting properties of the equations derived by methods 1, 2 and 4. However, it would be unwise to generalize from these findings, and in future investigation it would be much more straightforward to use compounds for which steric interactions do not occur. The probability of bulky groups interacting can be quickly assessed by geometric calculations using published covalent atomic radii, and compounds can be selected for study where this pitfall will be avoided. If this is done at the early stages of a physicochemical-activity relationships investigation, when the regression equations are first being calculated, it should be possible to retain all the advantages of the approach and eliminate one potential source of error.

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