Br. J. Pharmac. (1974), 52, 87-92

THE SELECTION OF ARYLAMIDINOUREA ANTIMALARIALS BY THEIR PREDICTED PHYSICOCHEMICAL PROPERTIES

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1 A small group of arylamidinoureas for which the predicted physicochemical properties were widely spaced and uncorrelated was selected for study.

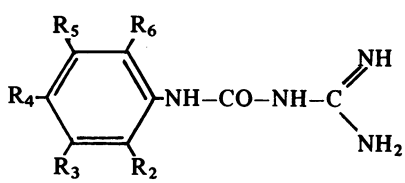
2 The antimalarial activity of each compound was measured against three species of plasmodium. Three corresponding regression equations were calculated relating the potency of each compound to its predicted physicochemical properties.

3 The potency of further test compounds was forecast for each species, by using the corresponding regression equation. These forecasts were compared with the measured activity of the test compounds, and were better than would be expected by chance.

4 It is suggested that only a few compounds in a series need be studied initially in order to derive regression equations with worthwhile predictive properties, if the compounds are selected according to appropriate criteria.

Introduction

Eighty arylamidinoureas with the general formula I:



(I)

have been synthesized and their antimalarial activities (A) studied by Richards & Walls (unpublished observations). When this information was available for 61 members of the series, it was shown by Goodford, Norrington, Richards & Walls (1973) that their activity could be forecast by the equation:

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

in which π and σ were predicted values for two of the physicochemical properties of the compounds. In the present paper an attempt is made to see whether regression equations with similar predictive properties can be derived if only a few compounds are initially available.

Methods

Physicochemical parameters

The parameter π was used to predict the effect of changing the benzenoid substituent groups R_2 to R_6 upon the lipophilicity of the arylamidinoureas (Leo, Hansch & Elkins, 1971). It 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 parameter. For multiply substituted compounds the total change in physicochemical properties was predicted by linear addition of the individual substituent parameters (Goodford, 1973).

Biological activities

Aqueous suspensions of the drugs were administered orally to mice infected with *Plasmodium vinckei* as described by Goodford *et al.*, (1973), in order to measure antimalarial activity *in vivo*. The observations were interpreted by logistic curves determined by a non-linear fitting procedure (Powell, 1965) in order to eliminate any subjective bias. This provided estimates of the individual doses (i.e. the ED_{50} expressed in mmol/kg) needed to reduce parasitaemia to half the control value, after

allowing for variation between experiments. It also gave estimates of the standard deviations. Both the doses and the deviations were expressed relative to one compound chosen as standard. Antimalarial activities A were then calculated as the logarithms of the reciprocals of the relative individual molar doses.

Rhesus monkeys were infected with *P. knowlesi* and blood was collected when approximately 2% of their erythrocytes were parasitized. The blood was diluted 1 : 6 with sterile culture medium and passed through a column of cellulose powder to remove leucocytes. [^{3}H]-leucine was added to the treated blood and aliquots were distributed into culture flasks. Drugs were dissolved in ethanol solutions and known aliquots were dried onto filter-paper discs which were added to the flasks. Measurements were made of [^{3}H]-leucine incorporated by drug-treated and control cultures after 18 h incubation. A further series of experiments was carried out in exactly the same way, but with blood from rats infected with *P. berghei*.

Relative biological activities *in vitro* were calculated as described above from the millimolar concentrations in the culture flasks (Richards & Williams, 1973; Williams & Richards, 1973) and regression equations were derived as before (Goodford *et al.*, 1973) with the general form:

$$A = a\pi + b\sigma + c \qquad (2)$$

Selection of compounds

It was first necessary to select a small group of arylamidinoureas for study. In order to ensure that the derived regression equation would be fairly comparable with that of Goodford *et al.* (1973), the choice was restricted to the 61 compounds available to those authors. However, reliable values of π and σ were not known for all of these, and some of the compounds had *ortho* substituent groups for which no generally applicable σ constants could be assigned (Barlin & Perrin, 1966). Such limitations reduced the number of compounds available to the 41 whose $\pi : \sigma$ coordinate positions are plotted in Figure 1.

Results

The group of compounds

It was decided that between seven and ten arylamidinoureas should be studied in order to derive the coefficients a, b and c of equation (2), since these would provide between four and seven degrees of freedom. The number finally chosen

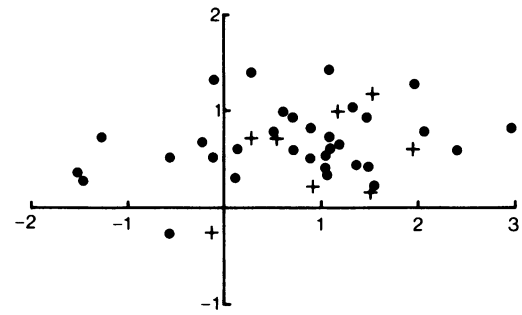


Fig. 1 Abscissae: the predicted lipophilicity parameter (π). Ordinates: the predicted electronic parameter (σ). The points represent the $\pi : \sigma$ coordinate positions of the compounds considered at the start of the present study. The eight crosses represent the eight compounds selected.

was eight compounds, and this was a compromise between the overall objective of using as few as possible, and the need for a meaningful statistical analysis. The individual compounds were selected in order to have as wide a spread of π and σ values as was practicable, whilst ensuring that these predicted parameters were not significantly correlated with each other (Goodford, 1973).

Several groups of compounds were rejected because of difficulty in synthesizing large quantities for extensive testing, and the compounds of the final group are shown in Fig. 1 and Table 1 together with their predicted π and σ values. These eight compounds provide five degrees of freedom for establishing the coefficients in equation (2). They cover the range of σ from -0.27 to 1.15, which is 80% of the full range for the 41 points shown in Fig. 1, and they also cover 45% of the full π range. Their $\pi : \sigma$ coordinate positions are not closely clustered and their mean $\bar{\pi} = 0.97$; $\bar{\sigma} = 0.54$ is not far from the overall mean $\bar{\pi} = 0.75$; $\bar{\sigma} = 0.64$. Finally their π and σ values are not significantly correlated ($r = 0.42$; $P > 0.1$), and although this choice of compounds is not ideal it was accepted as a working compromise.

Antimalarial activity in vivo

The observations on *P. vinckei* in mice *in vivo* for the eight chosen compounds were used to calculate a regression equation in π and σ:

$$A = 0.35\pi + 0.78\sigma - 0.06 \qquad (3)$$

which may be compared with the original equation (1) derived from the previous observations on more compounds by Goodford *et al.* (1973). The equations are clearly similar and the coefficients of

π and σ are not appreciably changed. Moreover, the multiple correlation coefficient of equation (3) is 0.85, which is statistically significant at the 5% level of probability and greater than the value of 0.68 for the previous equation (1). However, the new coefficients are not significantly different from zero when tested by a t test at the 5% level:

Coefficient of $\pi = 0.35$, s.e. = 0.23, $t = 1.54$,
$0.2 > P > 0.1$, d.f. = 5
Coefficient of $\sigma = 0.78$, s.e. = 0.34, $t = 2.29$,
$0.1 > P > 0.05$, d.f. = 5

and on a conventional statistical appraisal the new regression might therefore be rejected (Tute, 1971; Goodford, 1973).

An alternative method of appraising equation (3) is to assess how well it forecasts the antimalarial activities of further compounds, and the new equation was therefore used to forecast the activities of more arylamidinoureas directly. These forecasts, based on the present results and equation (3), were then compared with the actual antimalarial observations made previously by Goodford *et al.* (1973) on the same compounds *in vivo*.

Of the 31 compounds whose activities were tabulated by Goodford *et al.*, four had to be omitted because they were used to establish the present equation (3). When the forecasts and measured activities of the remaining 27 were plotted against each other, it was seen that a substantial proportion of the forecasts were in the right direction (Figure 2). The validity of the forecasts compared with the measured activities was tested in two ways. Firstly a rank correlation test was performed by ranking the compounds, both in the order of the forecasts and in the order of the measurements, and calculating the correlation coefficient of the two sets of ranks. The resulting value of 0.51 from 26 ranks was significant at the 1% level and accordingly the hypothesis that the ranks of the measurements were independent of the ranks of the forecasts was rejected. This test was used since it requires no assumptions about the statistical distribution of

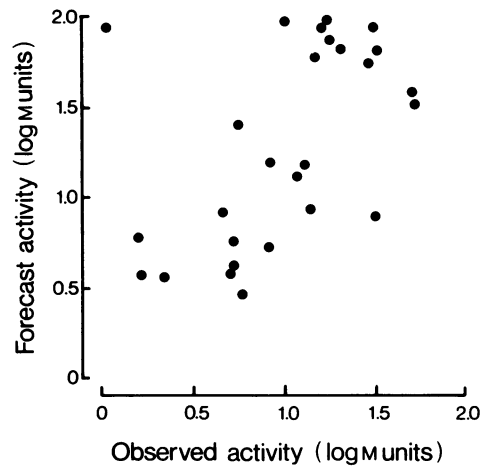


Fig. 2 Abscissae: the observed antimalarial activity (Log M units) of the 27 compounds used to assess the forecasting properties of equation (3). Ordinates: the forecast antimalarial activities of the same compounds according to equation (3). The correlation of the points from bottom left to top right is significant at the 1% level, but individual points deviate seriously from this trend. It is therefore suggested that forecasts should be assessed on a statistical basis.

Table 1 The eight arylamidinoureas studied to derive the regression equations

Substituent groups		*Predicted parameters*		*Measured antimalarial activity A (Log molar units)*		
R_3	R_4	π	σ	P. vinckei *in mice* in vivo	P. knowlesi *in monkey erythrocytes* in vitro	P. berghei *in rat erythrocytes* in vitro
H	OCH_3	−0.12	−0.27	0	0	0
H	Cl	0.93	0.23	0.47 ± 0.04	1.63 ± 0.09	0.41 ± 0.08
NO_2	H	0.54	0.71	0.18 ± 0.06	1.55 ± 0.14	−0.35 ± 0.10
Cl	CN	1.18	1.00	1.67 ± 0.07	1.81 ± 0.02	0.18 ± 0.02
Cl	Cl	1.97	0.60	1.03 ± 0.06	2.42 ± 0.04	0.62 ± 0.10
Cl	NO_2	1.54	1.15	1.47 ± 0.02	1.73 ± 0.03	0.14 ± 0.11
OCH_3	CN	0.26	0.74	0.41 ± 0.04	1.20 ± 0.05	0.00 ± 0.04
CH_3	Cl	1.49	0.16	0.36 ± 0.04	2.21 ± 0.16	0.89 ± 0.07

Molar activities are expressed on a logarithmic scale relative to the reference compound, together with their estimated standard deviations. (See text.)

the values involved. Secondly an F test was used to test the hypothesis that the 26 measurements were normally distributed about the same linear function of π and σ, apart from a constant, as the eight measurements from which the regression equation was calculated, and with the same variance. The resulting value of the F variate was 1.21 with 25 and 5 degrees of freedom and was not significant at the 5% level. Accordingly the hypothesis was not rejected. Once again as found by Goodford *et al.* (1973), the 3,5 di-nitro, 4-chloro compound showed the most serious deviation, and when this compound was omitted the rank correlation coefficient rose to 0.65 and the value of the F variate declined to 0.66. Thus individual forecasts out of many may be seriously wrong, but a group of forecasts can be shown to be more related to new measurements than would be expected at random and to be as near to the new measurements as would be expected from the derivation of the forecasting equation.

In vitro *activity against* P. knowlesi

When the antimalarial activity of the eight chosen compounds was first measured against *P. knowlesi* in monkey blood cultures, it was immediately apparent that the results did not parallel the observations with the same compounds on *P. vinckei* in mice (Table 1). For example the most potent compound in mice *in vivo* was only third best against *P. knowlesi.* However the calculated regression showed the same trends as equations (1) and (3), with increasing potency as π and σ increased:

$$A = 0.88\pi + 0.21\sigma + 0.60 \qquad (4)$$

although the relative importance of π and σ is now reversed. Moreover the larger coefficient of 0.88 for π explains the changed order of potency against *P. knowlesi,* and the two most active compounds against this parasite have high π values as might be expected.

Equation (4) has a multiple correlation coefficient of 0.91 which is statistically significant at the 2% level, but the conventional statistics for the coefficient of σ are unsatisfactory:

Coefficient of $\pi = 0.88$, s.e. $= 0.21$, $t = 4.12$, $0.01 > P > 0.005$, d.f. $= 5$

Coefficient of $\sigma = 0.21$, s.e. $= 0.32$, $t = 0.64$, $0.6 > P > 0.5$, d.f. $= 5$

A further set of *in vitro* experiments were therefore carried out on a newly selected group of nine compounds in order to assess the forecasting abilities of equation (4). These compounds were again chosen to provide a wide uncorrelated spread of π and σ. When the forecasts according to that equation were compared with the new observations, they had a rank correlation coefficient of 0.88 which from nine observations is significant at the 1% level. Furthermore the test of the sameness of the linear relation in the two sets of data gave a value for F of 1.67 with eight and five degrees of freedom which was not significant. It was concluded that equation (4) provides worthwhile forecasts, notwithstanding its inadequate statistics for σ.

In vitro *activity against* P. berghei

The antimalarial activities of the eight compounds were also measured against *P. berghei* in rat blood cultures (Table 1). When the regression equation was established, it showed that potency increased with π but decreased with σ:

$$A = 0.53\pi - 0.54\sigma + 0.01 \qquad (5)$$

which was surprising because the potency against the first two parasites had increased with both physicochemical parameters. However, the multiple correlation coefficient of 0.90 for equation (5) was statistically significant at the 2% level, and the statistics of the individual coefficients were also satisfactory.

Coefficient of $\pi = 0.53$, s.e. $= 0.12$, $t = 4.54$, $0.01 > P > 0.005$, d.f. $= 5$

Coefficient of $\sigma = -0.54$, s.e. $= 0.18$, $t = 3.09$, $0.05 > P > 0.02$, d.f. $= 5$

The difference between the coefficient of σ in this equation and the coefficient of σ in equation (3) is 1.32 with a standard error of 0.38. The ratio of these is 3.47 which is significant at the 2% level by Behrens' test, contrary to the hypothesis that the two coefficients are equal, and so the new findings were interpreted as showing that there was a genuine difference between the biological test systems.

Another equation was calculated when a further series of observations on *P. berghei* had been made on 16 compounds, and this confirmed the negative coefficient for sigma:

$$A = 0.32\pi - 0.56\sigma + 0.00 \qquad (6)$$

with still more significant statistics. Moreover, when the nine observations on new compounds were compared with their forecasts from equation (5), they showed a rank correlation coefficient of 0.83 which with nine observations is significant at the 1% level and the F test gave a value of 1.53 which was not significant and thus it was concluded that equation (5) gives worthwhile forecasts. It was therefore difficult to avoid the conclusion that the *P. berghei* test system *in vitro* really did show different properties from the *P.*

vinckei parasites in living mice, and from *P. knowlesi* in monkey blood.

Discussion

The present observations demonstrate that it was not necessary to synthesize and measure the biological activities of over 60 arylamidinoureas, in order to establish a regression equation in π and σ which could be used to forecast the potency of further compounds against *P. vinckei in vivo*. Measurements on only eight carefully selected members of the series yielded a regression equation (3) which gave significantly better forecasts than would be expected by chance. Moreover, further measurements on two other *in vitro* biological tests also gave equations (4 and 5) which forecast adequately in their respective test systems. The activities of the new individual compounds of course deviated more or less from forecast, but the overall forecasting trends were clear and statistically significant. To some extent the deviations were due to experimental variation, and the remaining variance may be ascribed to a genuine failure of the two-parameter equations to forecast perfectly. The rest of the 53 compounds, out of those originally synthesized, may well provide information on other matters, but they are not needed to define those aspects of biological activity which depend on π and σ.

Five criteria were used when the initial compounds were selected for the present study. It was necessary to have as wide and uniform a spread of values for each predicted physicochemical parameter as was practicable. The predicted parameter values were not significantly correlated with each other. Sufficient compounds were chosen to provide enough degrees of freedom to carry out statistical tests. Subject to this requirement the number of compounds was kept as small as possible, and the individual compounds were chosen to be easy for synthesis. One final point should be emphasized. The preliminary study of Goodford *et al.* (1973) had already shown that π and σ were of relevance to the antimalarial activity of the arylamidinoureas, and the present investigation was aimed specifically at those two parameters. In an open ended investigation in which more parameters were to be considered, it would have been necessary to study more compounds and apply more criteria.

The unexpected results with *P. berghei in vitro* must be considered. Since regression (5) does not point to high activity in the same $\pi : \sigma$ direction as the others, it should be possible to forecast arylamidinoureas that had little effect on *P. berghei*, but were potent against *P. knowlesi in vitro*. They would need positive π and σ values, and one may conclude that the present method is capable of designing drugs with selective activities as well as high potency. Moreover, the greater the number of biological test systems in which a series of compounds is examined, the greater are the benefits of optimizing the choice of compounds, and the greater the chance of designing a selectively potent drug.

One serious problem remains. The present findings show convincingly that the three antimalarial test systems do not give mutually compatible results with the arylamidinoureas. However, the findings do not show which is the most appropriate system for testing antimalarial drugs to be used in man.

We are grateful to Dr M.J. Davey Mr O. Fanimo, Dr D. Gilbert, Miss J.A. Matthews, Mrs N. Trist, Dr L.P. Walls and Dr B.C. Weatherley for much valued help and discussion in the course of this work.

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(Received February 1, 1974)