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Predictions of the antimalarial activity of arylamidinoureas

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Summary

1. Regression equations were calculated relating the observed antimalarial potencies of arylamidinoureas to their predicted physicochemical properties.

2. A two-parameter equation was used to forecast the antimalarial activity of more arylamidinoureas.

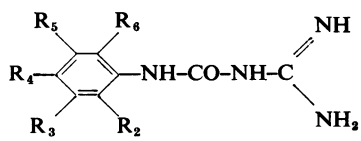
3. Thirteen compounds of the predicted type were synthesized, and their observed antimalarial potencies compared with the forecasts.

Introduction

Attempts to correlate biological activity quantitatively with physicochemical properties (reviewed by Tute, 1971) have usually been restricted to studies on completed series of compounds. In the present paper we describe an attempt to use regression equations based on approximate observations of the antimalarial activity of arylamidinoureas (Richards & Walls, unpublished observations) in order to forecast which new compounds in this series should be highly active. Thirteen of the new compounds were then synthesized and their observed biological activities compared with the forecasts.

Methods

Values of the parameter π were used to predict the effects of substituents in the benzene ring upon the lipophilicity of arylamidinoureas with the general formula I:



In view of the electron-donating nature of the amidinourea side-chain, the values of π measured in substituted phenols were appropriate (Fujita, Iwasa & Hansch, 1964; Leo, Hansch & Elkins, 1971). Hammett's (1940) constant σ was the second parameter (Jaffé, 1953), since Richards & Walls considered that the electron-withdrawing properties of the substituents were important. The third predicted parameter was Taft's (1956) constant E_s which relates to the steric interactions between an *ortho* substituent and the side-chain. The additivity rule was used to predict π, σ and E_s values for each compound studied (Goodford, 1973).

Aqueous suspensions of the drugs were administered orally to groups of five mice previously inoculated with *Plasmodium vinckei*. The mice received 7 individual doses of drug, first on the afternoon of the day of infection and then

twice daily for 3 days. Parasitaemias in treated and untreated control mice were estimated microscopically in Giemsa stained blood films made four days after infection. Compounds were not normally tested at individual doses higher than 100 mg/kg, i.e. total doses of 700 mg/kg. Biological activity (A) was recalculated from Richards & Walls original observations and expressed as the logarithm of the reciprocal of the dose (expressed in millimoles/kg) which reduced parasitaemia to half the control value.

The regression equations had the general form:

$$A = a\pi + b\sigma + cE_s + d$$

and values of a, b, c and d were calculated by the method of least squares (Seal, 1964) so that the variations in A from compound to compound were related to the corresponding variations in π, σ and E_s. The actual combinations of independent variables π, σ and E_s were chosen by physicochemical rather than statistical criteria.

Results

Establishing regression equations

Sixty-one of the arylamidinoureas tested by Richards & Walls were available for consideration when the present study began, but 28 did not have measurable activity and so could not be included in the analysis. Values of π, σ and E_s were available for the substituents in 26 of the 33 remaining compounds, and the first regression equation was calculated for these 26:

$$A = 0{\cdot}17\ \pi + 0{\cdot}58\ \sigma + 0{\cdot}23\ E_s + 0{\cdot}19 \qquad (1)$$

The regression coefficient for E_s is positive in equation (1), and since E_s values are themselves negative when there is an *ortho* substituent group in the molecule (Taft, 1956), the E_s term would predispose *ortho* substituted compounds to low antimalarial activity. This fact, coupled with the well known unreliability of *ortho* σ values, led to the decision that the forecasting phase of the present study should be restricted to molecules with *meta* and *para* substituents only.

TABLE 1. *The arylamidinoureas synthesized by Richards & Walls which were used to derive equation* (2) *(see text).*

Substituent groups		Predicted parameters		Measured antimalarial activity A (log units)
R_3	R_4	π	σ	
H	CN	0·14	0·63	0·777
H	NO_2	0·49	0·78	0·908
NO_2	H	0·54	0·71	0·168
Cl	CN	1·18	1·00	1·445
CH_3	CN	0·70	0·56	0·716
CN	Cl	0·69	0·91	1·495
Cl	Cl	1·97	0·60	1·113
CN	CN	–0·10	1·31	0·658
NO_2	Cl	1·47	0·94	1·120
CH_3	Cl	1·49	0·16	0·335
Cl	NO_2	1·54	1·15	1·200
Br	CN	1·31	1·02	0·930
F	CN	0·61	1·00	1·144
Cl	H	1·04	0·37	0·697
OCH_3	Cl	1·05	0·34	0·214
C_2H_5	CN	1·08	0·59	0·204
CN	NO_2	0·26	1·39	1·074
Cl	F	1·35	0·44	0·732

The second regression was run on the data in Table 1 for the remaining 18 non-*ortho* substituted arylamidinoureas, and E_s was omitted because it did not relate to the *meta* and *para* positions. The best-fitting equation was:

$$A=0{\cdot}28\ \pi+0{\cdot}86\ \sigma-0{\cdot}09 \qquad (2)$$

and it seemed likely from the relatively high coefficient of σ in this equation that high antimalarial activity should be found in compounds containing electron-withdrawing groups, as already observed by Richards & Walls. However, the coefficient of π in equation (2) was also appreciable, and since the range of possible π values exceeds the range of σ, it seemed probable that the activity might be increased still further if the substituent was also lipophilic.

In an attempt to check the general validity of equation (2) the 61 arylamidinoureas were next divided into 3 sub-classes:

(a) compounds of high activity (A>1·0);

(b) compounds with some activity (1·0>A>0);

(c) compounds with no detectable activity (0>A).

It was observed that the mean π and mean σ values both increased on going

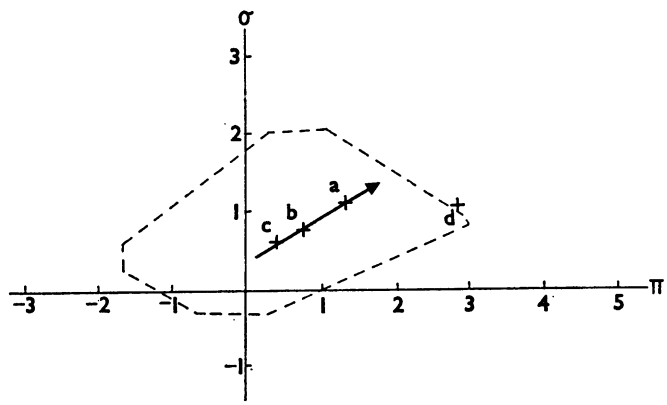


FIG. 1. The logarithms of the relative partition coefficients (π) and relative electron densities in the benzene ring (σ) of the arylamidinoureas studied. All the originial 61 compounds have π and σ co-ordinates within the divided line. a, b and c show the mean co-ordinate positions of potent, less potent and weak antimalarials. The arrow indicates the direction of increasing activity, and d shows the mean co-ordinate position of all the 3,4,5-trisubstituted arylamidinureas which were synthesized after equation (2) had been derived (see text).

from the inactive to the active compounds (Fig. 1), showing that the trends for 18 compounds shown by equation (2) were also detectable in the series as a whole.

Forecasting

The multiple correlation coefficient for equation (2) is 0.68. A low value was expected, because the biological measurements were only preliminary observations, but 0·68 is still statistically significant at the 0·01 probability level, and the equation shows a clear overall trend for antimalarial activity to increase with both π and σ. However, numerous factors other than those represented by π and σ must obviously contribute to the biological activity of an arylamidinourea, and so any predictions made solely on the basis of equation (2) might have only a modest chance of success. Furthermore, the variation of biological activity

with π is frequently quadratic rather than linear (Hansch & Fujita, 1964), and deviations from linearity might become increasingly significant as π increases along the trend line.

The range covered by the 18 compounds which were used to derive equation (2) was not great enough to define a quadratic relationship, so it would have been rash to make too large an extrapolation when using the equation in an attempt to predict new compounds of high antimalarial activity. The compounds selected for further synthesis were therefore designed with ring substituents conferring high positive π and σ values to the molecule, so that they lay in the top right hand quadrant of Fig. 1, but only just outside the dashed line surrounding the cluster of compounds previously made. Table 2 shows the predicted π and σ values for these new compounds, and their forecast biological activities compared with their actual activities measured experimentally.

TABLE 2. *The 3,4,5-trisubstituted arylamidinoureas which were predicted or synthesized for the present study*

Substituents			Parameters		Antimalarial activities A (log units)		Deviation from
R_3	R_4	R_5	π	σ	forecast	observed	forecast
Br	Br	Br	3·47	1·05	1·78	1·02	0·76
Cl	Cl	Cl	3·01	0·97	1·59	1·46	0·13
Cl	CN	Cl	2·22	1·37	1·71	1·17	0·54
NO_2	Cl	NO_2	2·01	1·65	1·89	0	1·89
Cl	Br	Cl	3·21	0·98	1·65	1·33	0·32
Br	Cl	Br	3·27	1·01	1·69	1·24	0·45
Br	CN	Br	2·48	1·46	1·86	1·21	0·65
Br	Cl	Cl	3·14	0·99	1·64	1·52	0·12
Br	Cl	F	2·57	0·95	1·45	1·70	–0·25
Cl	Cl	F	2·44	0·94	1·40	1·72	–0·32
Cl	Cl	CF_3	3·46	1·02	1·76	1·49	0·27
Cl	F	Cl	2·39	0·81	1·28	0·75	0·53
Br	Cl	CF_3	3·59	1·03	1·80	1·23	0·57

Discussion

The new compounds were all tri-substituted arylamidinoureas with no *ortho* groups, and their mean π and mean σ co-ordinate values are shown in Fig. 1 (cross d). It will be observed that they have a high mean π value which is favourable to high antimalarial potency according to regression equation (2). However, their mean σ value is actually less than the mean σ for sub-class (a) which included all the previously-known compounds of high activity, and one may conclude that the new compounds stand to gain little on average from σ in comparison with the best compounds already known. The specification for high potency, which is implicit in equation (2), has been well-followed for π in the present study, but less well-followed for σ on average.

Eleven of the thirteen new compounds had logarithmic activities $A>1{\cdot}0$, and the new compounds were relatively potent taken as a group, as was forecast. Moreover the forecasting ability of regression equation (2) may be tested in another way, by applying it to the 28 original compounds whose biological activities were too weak for measurement. π and σ values were available for ten of these which had only *meta* and *para* substituents, and their mean forecast antimalarial activity was 0·37 according to equation (2). This is close to the threshold of detectable activity which may be calculated as $A=0{\cdot}4$ for a typical

arylamidinourea of molecular weight 250 tested at the maximum individual dose level of 100 mg/kg. Thus the mean forecast for the group of compounds too weak for measurement is close to the threshold of measurement.

The present results show that a regression equation relating physicochemical to biological activities can be used to predict new potent compounds. Furthermore, it was only necessary to use approximate biological observations in order to derive the equation, and it was not necessary to have a very high multiple correlation coefficient. It is hardly surprising that two of the compounds were much weaker than forecast because it is inconceivable that the detailed properties of a whole series of compounds acting on intracellular parasites in an intact animal could be predicted on the basis of only two parameters. Furthermore, two of the new compounds were more potent than any arylamidinoureas previously known, and although the activity of individual compounds deviated above or below those forecast, it appears that the method can predict compounds with relatively high potency when averaged over the group as a whole.

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