

AD 742239

Unclassified

AD

Report No. 6

PROGNOSES OF ANTIMALARIAL EFFECTIVENESS FROM STUDIES OF
RELATIONSHIPS BETWEEN MOLECULAR CONSTITUTION AND
CHEMOTHERAPEUTIC RESPONSE

Annual Progress Report

by

William Paul Purcell, Ph.D., Principal Investigator
Professor and Chairman
Department of Molecular and Quantum Biology

June 1971

Supported by

U.S. Army Medical Research and Development Command
Washington, D. C. 20314

Research Contract No. DA-49-193-MD-2779

REPORT # 8 - AD 711875

Department of Molecular and Quantum Biology

College of Pharmacy

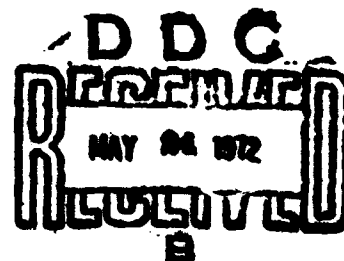
University of Tennessee Medical Units

Memphis, Tennessee 38103

Reproduced by
NATIONAL TECHNICAL
INFORMATION SERVICE
Springfield, Va 22151

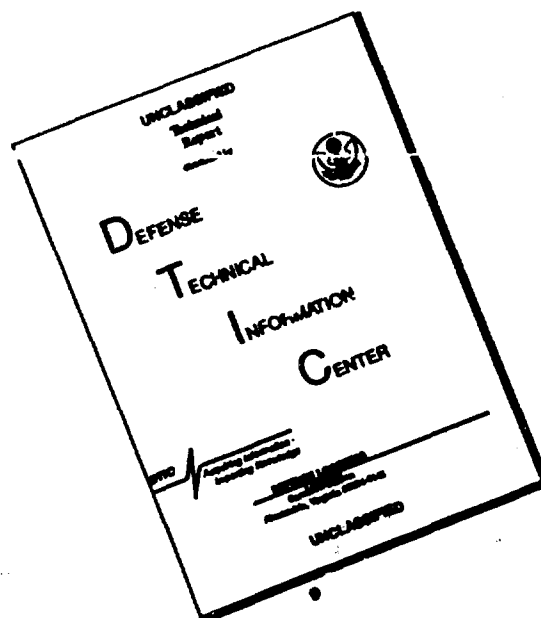
Approved for public release;

distribution unlimited.



15

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST
QUALITY AVAILABLE. THE COPY
FURNISHED TO DTIC CONTAINED
A SIGNIFICANT NUMBER OF
PAGES WHICH DO NOT
REPRODUCE LEGIBLY.

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the report is classified)

1. ORIGINATING ACTIVITY (Corporate author)

Department of Molecular and Quantum Biology
College of Pharmacy
University of Tennessee Medical Units
Memphis, Tennessee 38103

2. REPORT SECURITY CLASSIFICATION

Unclassified

3. GROUP

4. REPORT TITLE

Prognoses of Antimalarial Effectiveness from Studies of Relationships
Between Molecular Constitution and Chemotherapeutic Response

5. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Annual Progress Report No. 6 (1 July 1970 through 30 June 1971)

6. AUTHOR(S) (First name, middle initial, last name)

William P. Purcell, Ph.D., Principal Investigator

7. REPORT DATE

June 1971

8. TOTAL NO OF PAGES

9. NO OF REFS

10. CONTRACT OR GRANT NO

DA-49-193-MD-2779

11. ORIGINATOR'S REPORT NUMBER(S)

12. PROJECT NO.

13. OTHER REPORT NUMBER(S) (Any other number(s) which may be used to identify this report)

14. DISTRIBUTION STATEMENT

This document has been approved for public release and sale; its distribution is unlimited.

15. SUPPLEMENTARY NOTES

16. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and
Development Command
Washington, D.C. 20315

17. ABSTRACT

The purpose of this research is to eradicate chemotherapeutically malignant in the human host; drug resistant strains of *P. falciparum* constitute the primary target. Our aim is to suggest molecules for synthesis and testing which have a high probability of success as effective antimalarials.

Results for this report period include a rather thorough fundamental study on drug-DNA interactions following the work reported by O'Brien and Hammett. This study consists of Free-Wilson and Hansch analyses on a series of compounds to arrive at a consistent model describing the mechanism of interaction. The second major activity during this report period consists of molecular orbital calculations of molecules of interest as known or potential antimalarials. Work along these lines includes parameterization of selected molecules and investigation of various levels of approximation (HMO, Del Re, Pariser-Parr-Pople, CNDO/2). We have also undertaken rather fundamental studies in quantitative structure-activity relationships to bring our software to a higher level of sophistication. The decoding of the structure/sub-structure searching programs provided by Walter Reed has been accomplished during this report period. We have successfully translated these programs into language acceptable by the IBM 360/40 system.

DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

Security Classification

Security Classification

KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
drug design Free-Wilson analyses Hansch analyses malaria medicinal chemistry molecular orbital calculations quantitative structure-activity relationships regression analyses						

Security Classification

Unclassified

AD

Report No. 6

PROGNOSES OF ANTIMALARIAL EFFECTIVENESS FROM STUDIES OF
RELATIONSHIPS BETWEEN MOLECULAR CONSTITUTION AND
CHEMOTHERAPEUTIC RESPONSE

Annual Progress Report

by

William Paul Purcell, Ph.D., Principal Investigator
Professor and Chairman
Department of Molecular and Quantum Biology

June 1971

Supported by

U.S. Army Medical Research and Development Command
Washington, D. C. 20314

Research Contract No. DA-49-193-MD-2779

Department of Molecular and Quantum Biology
College of Pharmacy
University of Tennessee Medical Units
Memphis, Tennessee 38103

Approved for public release;
distribution unlimited.

SUMMARY

The purpose of this research is to eradicate chemotherapeutically malarias in the human host; drug resistant strains of P. falciparum constitute the primary target. Our aim is to suggest molecules for synthesis and testing which have a high probability of success as effective antimalarials.

Results for this report period include a rather thorough fundamental study on drug-DNA interactions following the work reported by O'Brien and Hahn.² This study consists of Free-Wilson and Hansch analyses on a series of compounds to arrive at a consistent model describing the mechanism of interaction. The second major activity during this report period consists of molecular orbital calculations of molecules of interest as known or potential antimalarials. Work along these lines includes parameterization of selected molecules and investigation of various levels of approximation (Hückel, Del Re, Pariser-Parr-Pople, CNDO/2). We have also undertaken rather fundamental studies in quantitative structure-activity relationships to bring our software to a higher level of sophistication. The decoding of the structure/sub-structure searching programs provided by Walter Reed has been accomplished during this report period. We have successfully translated these programs into language acceptable by the IBM 360/40 system.

FORWARD

This project PROGNOSIS OF ANTIMALARIAL EFFECTIVENESS FROM STUDIES OF RELATIONSHIPS BETWEEN MOLECULAR CONSTITUTION AND CHEMOTHERAPEUTIC RESPONSE was authorized under Contract No. DA-49-193-MD-2779 and was activated 1 July 1965.

This report covers the period 1 July 1970 through 30 June 1971 and constitutes the sixth annual progress report.

The Principal Investigator would like to express his appreciation for the contributions made by members of the Department of Molecular and Quantum Biology. In particular he would like to thank Dr. George E. Bass, Assistant Professor, Miss Jane E. Parker, Research Assistant, Mr. Ozra E. Millner, Jr., Graduate Research Assistant, Miss Ann Ray, Departmental Secretary, and Miss Sherry Hoskins, Special Assistant.

Computer programming and computational services were provided in part by Dr. David M. Vaught, Director, the Computer Center, Memphis State University, and Visiting Assistant Professor, Department of Molecular and Quantum Biology and Mr. James C. Ziegler of Data Communications Corporation. Computer facilities were supplied by the University of Tennessee Computer Project and the Biometric Computer Center. We are most grateful to the fine staff at both of these centers for their able assistance.

TABLE OF CONTENTS

	Page Number
Summary	2
Forward	3
Table of Contents	4
Statement of the Problem	5
Background and Approach to the Problem	5
Results and Discussion of Results	5
I. Drug-DNA Interaction Studies	5
A. Free-Wilson Analysis	6
B. Hansch Analysis	6
II. Molecular Orbital Calculations	9
III. Quantitative Structure-Activity Relationships	9
A. Fundamental Studies	9
B. Software Development	10
IV. Decoding Structure/Sub structure Searching Program Provided by Walter Reed	10
Literature Cited	11
Distribution List	13
Abstract (DD Form 1473, Document Control Data-R and D)	14

STATEMENT OF THE PROBLEM

The malaria problem has been defined precisely by Col. William D. Tigertt¹ and we will only state here that we are dedicated to the chemotherapeutic eradication of malarias in the human host with drug resistant strains of P. falciparum as the main target. Our primary objective is to predict the antimalarial activity of a compound before it is even synthesized and to suggest molecules for synthesis and evaluation having high probability of success.

BACKGROUND AND APPROACH TO THE PROBLEM

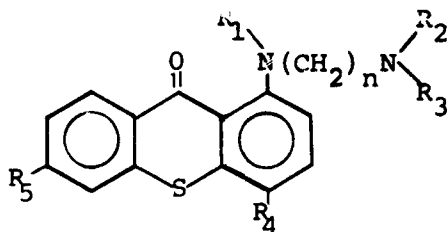
The reader is referred to Progress Reports Numbers 1, 2, 3, 4, and 5 submitted June 1966, June 1967, June 1968, June 1969, and June 1970, respectively, for the background and previous work on this problem.

RESULTS AND DISCUSSION OF RESULTS

I. Drug-DNA Interaction Studies

The work by O'Brien and Hahn² precipitated our interest in fundamental studies associated with the mechanism of antimalarial action. In particular, it has been suggested that chloroquine and chloroquine analogs intercalate selectively with the parasite DNA and, thereby, block the enzymatic synthesis of DNA and RNA. We have conducted several studies to investigate the validity of this proposed mechanism. The results of these studies during the past year are summarized below.

It has been suggested^{2,3} that the inhibition of Bacillus subtilis growth by Miracil D (1-diethyl amino ethyl amino-4-methyl-10 thiaxanthene 1) and its analogs is a consequence of drug-DNA interaction. In order to gain a better understanding of the nature of this phenomenon and how it may be related to the mechanism of antimalarial activity, we have subjected the data presented by these authors to regression analyses using the Free-Wilson Model and the Hansch Model.



A. Free-Wilson Analysis

The quantitative results found from the Free-Wilson analysis support the qualitative observations by Hirschberg, *et al.*³ High bacteriostatic activity apparently is favored by a hydrogen on the proximal nitrogen of the 1-side chain, a methyl group attached at ring position 4, sulfur at position 10, and Cl at position 6. For those analogs with a diamino side chain, activity increases significantly with separation (at least up to four carbons) of the nitrogens. While no trend is apparent for the groups attached to the terminal nitrogen on the diamino side chain, the most active groups are predicted to be C_4H_9 or $CH_2C(OH)(CH_3)_2$ and hydrogen.

B. Hansch Analysis

The Hansch Model has also been applied to the data of Hirschberg, *et al.* As in the Free-Wilson Analysis, the activity parameter chosen was the minimum inhibitory concentration of *Bacillus subtilis* and the analogs are the same as those given above (I).

Physicochemical parameters chosen for this study were octanol-water partition coefficients, π , selected from the literature⁴ and the quantum mechanical charges (Hückel pi-electron and Del Re sigma-electron contributions) for the proximal nitrogen, Q_{N1} , the terminal nitrogen, Q_{N2} , and the charge on the atom at R_5 , Q_X . The generalized structure-activity equation that was used is:

$$\text{Log } (25 \times 10^{-5}/C) = a^2 + b + cO_{N1} + dO_{N2} + eO_Y + f \quad (1)$$

(C = concentration effecting 50-100% growth inhibition without lysis)

In the series studied, structural variations involved either -H or -Cl at R_5 , -H or -CH₃ at R_1 , -H, -CH₃ or -OC₂H₅ at R_4 , -H, alkyl, and hydroxy groups at R_2 and R_3 , and N-N separations of 2, 3, and 4 carbons (n = 2,3,4) in the diamino side chain. Multiple regression analyses using all combinations of parameters in Equation 1 were carried out for the entire series of compounds and for variously constituted subseries. The highest correlations for each series (or subseries) are listed in Table 1. The results in Table 1 suggest that the relationship between structural variations and bacterial growth inhibition is complex indeed for these compounds. The broadest conclusion one might draw is that structural variation in one portion of the molecule (at R_1 or R_5 for example) has a strong influence on interactions associated with other portions (such as the diamino side chain) of the molecule. We are continuing these studies to try to clarify this matter.

Table 1: Hansch Analyses on Miracil D
Analogues. Highest Correlation Results

	No. of Compounds	Parameters for Optimum Correlation	Explained Variances
All compounds	23	Q_{N1}, Q_x	0.45
R_1 H	14	$-, Q_{N1}$	0.30
R_1 CH_3	9	$-2, -, Q_x$	0.55
R_4 CH_3	14	Q_{N1}, Q_x	0.46
R_5 Cl	9	$-2, \pi, Q_{N1}$	0.53
R_5 H	14	$-, Q_{N1}$	0.80
		$-2, -$	0.80
		Q_{N1}, Q_{N2}^+	0.78
N-N sepn 2C atoms	15	Q_{N1}, Q_x	0.52
N-N sepn 3C atoms	6	Q_{N1}, Q_{N2}^+, Q_x	0.46
N-N sepn 2C atoms	8	$-2, \pi, Q_x$	0.81
and R_1 R_4 CH_3			
N-N sepn 2C atoms	7	Q_{N1}, Q_{N2}^+, Q_x	0.61
and R_1 H			
N-N sepn 2C atoms	8	Q_{N1}	0.68
and R_5 H			
N-N sepn 2C atoms	7	π^2, π, Q_{N1}	0.62
and R_5 Cl			
N-N sepn 2C atoms	5	π^2, π, Q_{N1}	0.85
and R_1 H, R_4 CH_3			
N-N sepn 3C atoms	5	π^2, π, Q_{N2}^+	0.98
and R_1 H, R_4 CH_3			

II. Molecular Orbital Calculations

We have continued fundamental studies in quantum mechanics with the ultimate objective of applying molecular orbital calculations to molecules of interest as known or potential antimalarials. Once the electronic indices of these molecules are determined from molecular orbital calculations, it is intended that certain correlations will be attempted between the antimalarial activity and these indices.

Our work along these lines during this report period include parameterization of selected molecules through the use of electric dipole moment measurements and the investigation of various levels of approximation (Hückel, Del Re, Pariser-Parr-Pople, CNDO/2) to determine various factors which one should consider in selecting a particular method for a specific purpose. These results are detailed in our Quarterly Report dated April 26, 1971, for the period January, February, March, 1971, and summarized in two abstracts associated with National American Chemical Society Meetings.^{4,5}

III. Quantitative Structure-Activity Relationships

A. Fundamental Studies

With increasing activity in the area of quantitative structure-activity models⁶⁻⁸ it has come to our attention that investigators have been misled from time to time by inappropriate applications of these models and, in particular, the statistical interpretation. Therefore, we have undertaken rather fundamental studies of these methods with the idea of removing the mystery from the algebraic considerations and suggesting guidelines for application and statistical interpretation.⁹

The results of our findings may be summarized by stating that 1) the de novo or Free-Wilson model may be applied with and without statistical interpretation. If it is with statistical interpretation one needs to be very careful that there are at least two independent observations of a specific functional group on two molecules before statistics may be applied and 2) statistical interpretation should be used with great

care. We feel rather strongly that the parameter explained variance, which includes the number of degrees of freedom, should be evaluated along with such common terms of correlation coefficient t-test, etc.¹⁰

B. Software Development

Over the past years we have continued to upgrade and refine our software for quantitative structure-activity relationships. We are now capable of conducting these regression analyses using many parameters, evaluating all combinations and permutations of these parameters automatically, and computing the correlation coefficient, t-test, F-test, and explained variance automatically with the t-test giving significance on a term-by-term basis.

IV. Decoding Structure/Sub-structure Searching Program Provided by Walter Reed

Approximately three years ago we were given the Structure/Sub-structure Searching Programs that Dr. Jacobus and his associates developed for the malaria program. We have successfully translated these programs into language acceptable by the IBM 360/40 System. When the documentation is completed, these programs will be made available to the U.S. Army Medical Research and Development Command. We are keeping in close contact with Captain Robert O. Pick and Mrs. June Schaeffer in this connection.

LITERATURE CITED

1. "Present and Potential Malaria Problem," Tigertt, W. B. (1966) Mil. Med. Suppl., 131, 853-856.
2. "Chloroquine Structural Requirements for Binding to Deoxyribonucleic Acid and Antimalarial Activity," O'Brien, R. L. and Hahn, F. E. (1965) Antimicrob. Agents Chemother., 315-320.
3. "Structure-Activity Studies on the Mechanism of Action of Miracil D," Hirschberg, E., Weinstein, I. B., Gersten, N., Marner, E., Finkelstein, T., and Carchman, R. (1968) Cancer Research, 28, 601-607.
4. "Experimental Electric Dipole Moments of Selected N,N-Dimethylaniline Derivatives and Comparison with Values Calculated from Molecular Orbital Approximations," Clayton, J. M., and Purcell, W. P., September, 1970, 160th National Meeting of the American Chemical Society, Chicago, Illinois, Physical Chemistry No. 196.
5. "A Comparison Between the Theoretical and Experimental Electric Dipole Moments of Selected N,N-Dimethylaniline Derivatives," Clayton, J. M., Bass, G. E., and Purcell, W. P., Abstracts of Papers, March 1971, 161st National Meeting of the American Chemical Society, Los Angeles, California, Physical Chemistry No. 15.
6. "Physicochemical Parameters in Drug Design," Purcell, W. P., and Clayton, J. M., (1969) Annual Reports in Medicinal Chemistry, 1968, edited by C. K. Cain, Academic Press, New York, New York, Chapter 28, pp. 314-321.
7. "Physicochemical Parameters in Drug Design," Clayton, J. M., Millner, O. E., Jr., and Purcell, W. P. (1970) Annual Reports in Medicinal Chemistry, 1969, edited by C. K. Cain, Academic Press, New York, New York, Chapter 26, pp. 285-295.

8. "Quantitated Structure-Activity Relationships," Cammarata, A. (1971) Annual Reports of Medicinal Chemistry, 1970, edited by C. K. Cain, Academic Press, New York, New York, Chapter 24, pp. 245-253.
9. "Quantitative Structure-Activity Models. Some Conditions for Application and Statistical Interpretation," Hudson, D., Bass, G. E., and Purcell, W. P. (1970) Journal of Medicinal Chemistry, 13, p. 1184.
10. "Minimal Statistical Data for Structure-Function Correlations," Craig, P. N., Hansch, C. H., McFarland, J. W., Martin, Y. C., Purcell, W. P., and Zahradnik, R. (1971) Journal of Medicinal Chemistry, 14, 447.