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QSAR Studies of Anti-malarial Agents: 2,4-Diamino Pyrimidine Derivatives

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The discovery and development of a novel target site to treat malaria is still continued worldwide. The bifunctional enzyme dihydrofolate reductase-thymidylate synthetase (DHFR-TS) is found in malarial parasite. The DHFR domain of the enzyme plays an important role in nucleic acid synthesis, which acts as an attractive target for designing antimalarial compounds. The 2,4-diaminopyrimidine derivatives have been reported to be antimalarial agents by virtue of their inhibition of DHFR.

The physico-chemical descriptors, indicator variables and biological activity (TM_4, K_1CB_1) of 2,4-diaminopyrimidine derivatives were considered for quantitative structure activity analysis. Highly correlated significant equations were obtained from the multiple regression analysis and are taken for further analysis as represented below.

TM_4:

$$-\log IC_{50} = 0.523751(\pm 0.493089)\pi - 0.0391451(\pm 0.0372016)MR - 1.64545(\pm 1.32314)I_{R_1} + 1.20497(\pm 0.887005)I_{R_5} - 0.735828(\pm 0.755899)$$

$$n = 28,\ r = 0.668,\ r^2 = 0.447,\ F_{test} = 4.6562,\ s = 0.642,\ Q^2 = 0.314$$

K_1CB_1:

$$-\log IC_{50} = -0.677(\pm 0.613)\sigma_m - 0.595(\pm 0.434)\sigma_p + 0.478(\pm 0.317)I_{R_2} + 0.628(\pm 0.791)I_{R_1} - 1.058(\pm 0.247)$$

$$n = 26,\ r = 0.763,\ r^2 = 0.583,\ F_{test} = 7.355,\ s = 0.362$$

The results show that for activity against wild type (TM_4) *pf* DHFR, hydrophobicity of the substituents (π) and substituent at position R_5 was important (positive correlation) whereas substituent at R_1 position and a higher electron polarizability, the value of the substituent (MR) would be detrimental to the activity (negative correlation). On the other hand, the activity against S108N mutant form (K_1CB_1) of *pf* DHFR, substituents at R_1 and R_2 would be beneficial whereas the electronic property of the molecule was found to be detrimental to the activity.

Key Words: QSAR, 2,4-Diamino pyrimidine, Malaria, Dihydrofolate reductase, Polarizability, Hydrophobicity.

INTRODUCTION

Even after continuous efforts, malaria is still one of the most deadly diseases affecting the third world countries of Africa, Asia and Latin America[1]. The problem is compounded by the occurrence of resistance to various anti-malarial drugs. The bifunctional enzyme DHFR-TS is found in the malarial parasite. The DHFR domain of this enzyme plays an important role in the nucleic acid synthesis

in plasmodium. In plasmodium, the synthesis of purines and pyrimidines, which are necessary for the formation of nucleic acid, occurs by the salvage pathway and the *de novo* pathway respectively which are not present in mammals[2].

The enzyme DHFR-TS is involved in the synthesis of pyrimidine. DHFR catalyzes the NADPH dependent reduction of the 5,6 double bond of dihydrofolate (FH_2) to produce tetrahydrofolate (FH_4). The FH_4 is converted by an enzyme (serine hydroxyl methyl transferase) to an active cofactor 5,10-methylene FH_4 and the enzyme TS uses this cofactor for the conversion of dUMP to dTMP[3].

Two anti-malarial agents acting by inhibiting the DHFR domain of the enzyme DHFR-TS have been reported till date. 2,4-Diaminopyrimidine derivatives have been reported for inhibition of enzyme DHFR and could be better anti-malarial agents against the resistant forms of the parasite.

Several analogs of 2,4-diamino pyrimidine, identified as potential inhibitors of dihydrofolate reductase enzyme and having exhibited significant anti-malarial activity, are subjected to QSAR analysis. QSAR analysis describes how a given biological activity varies as a function of molecular descriptors describing the chemical structure of the molecule. Thus QSAR studies have good predictive ability and simultaneously provide deeper insight into the mechanism of drug-receptor interactions. Here, the QSAR study of a series of 2,4-diamino pyrimidine derivatives reported by Tarnchompoo *et al.*[4] has been performed for the prediction of the anti-malarial activity.

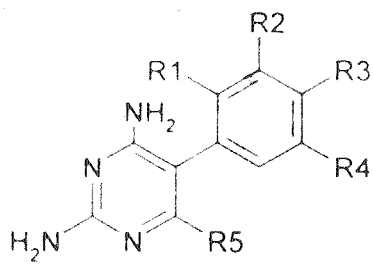

Fig. 1. Parent structure of 2,4-diamino pyrimidine derivatives

EXPERIMENTAL

QSAR with physico-chemical descriptors and indicator variables

In this work, a set of 28 compounds and their biological activity values are gathered from literature. The activity parameters are given in terms of log ($1/IC_{50}$), where IC_{50} refers to the concentration of the compound required to inhibit 50% of the parasites. The congeneric series presented five regions of structural variations: R_1, R_2, R_3, R_4 and R_5 (Table-1). The variations at all the five regions are represented by different physico-chemical descriptors (Table-2) and indicator variables (Table-3). The hydrophobic, electronic and steric constant values of the substituents were concluded from literature.

The efficacy data (Table-1) were then subjected to multiple regression analysis with different physico-chemical descriptors and indicator variables to generate QSAR equations for two types of parasites, *viz.*, Mutant type (K_1CB_1) and the

wild type (TM_4). The knowledge of the important parameters contributing to the efficacy against the different types of parasites can be used to design new anti-malarial agents of the series.

Of the 28 compounds in the series, 26 compounds were taken for the QSAR analysis. The remaining 2 compounds were rejected due to lack of discrete biological activity data.

TABLE-1
REGIONS OF STRUCTURAL VARIATION OF VARIOUS 2,4-DIAMINOPYRIMIDINE DERIVATIVES AND THE EFFICACY DATA

Compd. name	R1	R2	R3	R4	R5	TM4	K1CB1
DAP 1	H	H	Cl	H	Et	1.0969	–1.490
DAP 2	H	H	Br	H	Et	1.3010	–1.398
DAP 3	H	H	Me	H	Et	1.0000	–1.104
DAP 4	H	H	*tert*-But	H	Et	0.2218	–0.591
DAP 5	H	H	Ph	H	Et	–1.117	–0.644
DAP 6	H	H	OMe	H	Et	0.5228	—
DAP 7	H	H	H	H	Et	0.1549	–-0.058
DAP 8	H	Cl	H	H	Et	0.3979	–0.114
DAP 9	H	Cl	Cl	H	Et	1.2218	–1.241
DAP 10	H	-	OCH_2O	H	Et	0.3979	–1.332
DAP 11	Cl	H	Cl	H	Et	–1.0530	–1.352
DAP 12	H	OMe	$(2,4\text{-}Cl_2Ph)CH_2O$	H	Et	–0.5680	—
DAP 13	H	Br	OEt	Br	Et	–0.5680	–1.241
DAP 14	H	H	Cl	H	H	–0.3620	–1.418
DAP 15	H	H	Cl	H	Me	0.3010	–1.779
DAP 16	H	H	Cl	H	$(CH_2)_3COOMe$	1.3979	–1.348
DAP 17	H	H	Cl	H	$(CH_2)_3Ph$	1.3010	–1.025
DAP 18	H	H	H	H	H	–1.3200	–1.288
DAP 19	H	H	H	H	Me	–0.8690	–1.489
DAP 20	H	H	H	H	$(CH_2)_3COOMe$	0.3979	–1.496
DAP 21	H	H	H	H	*n*-hex	1.2218	–0.114
DAP 22	H	H	H	H	$(CH_2)_3Ph$	0.6989	–0.362
DAP 23	H	Cl	H	H	H	–0.7780	–0.945
DAP 24	H	Cl	H	H	Me	0.3802	–0.681
DAP 25	H	Cl	H	H	$(CH_2)_3COOMe$	0.0969	–1.560
DAP 26	H	Cl	H	H	$(CH_2)_3Ph$	0.1549	–0.477
DAP 27	H	Cl	H	H	$(CH_2)_3Ph(MeO\text{-}p)$	0.3979	–0.431
DAP 28	H	Cl	H	H	$(CH_2)_2O(CH_3)OPh$	0.3979	–0.519

TM_4: Wild type of plasmodium falciparum species.
K_1CB_1: S108N mutant type of *Plasmodium falciparum* species.

TABLE-2
PHYSICO-CHEMICAL DESCRIPTOR VALUES OF THE SUBSTITUENT

Compd name	π	H_A	H_D	MR	F	R	$\sigma_{m/o}$	σ_p
DAP 1	1.73	0	0	19.42	0.36	–0.25	0.30	0.08
DAP 2	1.88	0	0	22.27	0.39	–0.27	0.32	0.08
DAP 3	1.58	0	0	19.04	–0.09	–0.23	–0.14	–0.32
DAP 4	3.00	0	0	33.01	–0.12	–0.23	–0.17	–0.35
DAP 5	1.98	0	0	38.75	–0.13	–0.18	–0.01	–0.16
DAP 6	1.00	0	0	21.26	0.21	–0.61	0.05	–0.42
DAP 7	1.02	0	0	14.42	–0.05	–0.10	–0.07	–0.15
DAP 8	1.73	0	0	19.42	0.36	–0.25	0.30	0.08
DAP 9	2.44	0	0	24.42	0.77	–0.40	0.67	0.31
DAP 10	0.33	2	1	21.02	0.50	–1.25	0.17	–0.79
DAP 11	2.44	0	0	29.42	0.77	–0.40	0.67	0.31
DAP 12	4.27	2	1	66.15	1.36	–1.76	0.90	–0.51
DAP 13	3.12	1	0	41.56	1.05	–0.88	0.81	–0.07
DAP 14	0.71	0	0	10.15	0.41	–0.15	0.37	0.23
DAP 15	1.27	0	0	14.77	0.37	–0.28	0.30	0.06
DAP 16	2.38	1	0	38.94	0.62	–0.39	0.53	0.17
DAP 17	4.35	0	0	51.43	0.37	–0.62	0.22	–0.29
DAP 18	0	0	0	5.15	0	–0.15	0.37	0.23
DAP 19	0.56	0	0	9.77	–0.04	–0.13	–0.07	–0.17
DAP 20	1.67	1	0	33.94	0.21	–0.24	0.16	–0.06
DAP 21	3.36	0	0	38.02	–0.04	–0.78	–0.42	–1.02
DAP 22	3.64	0	0	46.43	–0.24	–0.47	–0.15	–0.52
DAP 23	0.71	0	0	10.15	0.41	–0.15	0.37	0
DAP 24	1.27	0	0	14.77	0.37	–0.28	0.30	0.06
DAP 25	2.38	1	0	38.94	0.62	–0.39	0.53	0.17
DAP 26	4.35	0	0	51.43	0.37	–0.62	0.22	–0.29
DAP 27	4.33	1	0	59.30	0.63	–1.13	0.34	–0.56
DAP 28	4.13	2	2	68.43	0.87	–2.10	0.32	–1.37

π: hydrophobicity, H_A: Hydrogen acceptor index, H_D: Hydrogen donor index
MR: molar refractivity, F: field effect, R: resonance effect,
σ_p: Hammett's substituent constant for the *para* substituent,
$\sigma_{m,o}$: Hammett's substituent constant for the *meta* and *ortho* substituents.

TABLE-3
INDICATOR VARIABLES REPRESENTING SUBSTITUENT AT DIFFERENT POSITIONS

Compd. name	I_{R1}	I_{R2}	I_{R3}	I_{R4}	I_{RS}
DAP 1	0	0	1	0	1
DAP 2	0	0	1	0	1
DAP 3	0	0	1	0	1
DAP 4	0	0	1	0	1
DAP 5	0	0	1	0	1
DAP 6	0	0	1	0	1
DAP 7	0	0	0	0	1
DAP 8	0	1	0	0	1
DAP 9	0	1	1	0	1
DAP 10	0		1	0	1
DAP 11	1	0	1	0	1
DAP 12	0	1	1	0	1
DAP 13	0	1	1	1	1
DAP 14	0	0	1	0	0
DAP 15	0	0	1	0	1
DAP 16	0	0	1	0	1
DAP 17	0	0	1	0	1
DAP 18	0	0	0	0	0
DAP 19	0	0	0	0	1
DAP 20	0	0	0	0	1
DAP 21	0	0	0	0	1
DAP 22	0	0	0	0	1
DAP 23	0	1	0	0	0
DAP 24	0	1	0	0	1
DAP 25	0	1	0	0	1
DAP 26	0	1	0	0	1
DAP 27	0	1	0	0	1
DAP 28	0	1	0	0	1

1 indicates the presence of substituent at particular position.
0 indicates the absence of substituent at particular position.

Multiple Linear Regression Analysis

The stepwise multiple regression analyses were carried out using the statistical software Openstat2, version 6.5.1, designed and standardized by Bill Miller and Stat Val. Correlation matrix was obtained to justify the use of more than one variable in the study. The variables used were with maximum correlation to activity and minimum inter-correlation with each other. From the statistical viewpoint, the ratio of the number of samples (N) to the number of variables used (M) should not be very low; usually it is recommended that $N/M \geq 5$.

The QSAR equations were constructed for efficacy data of both the species of malarial parasite with the physico-chemical descriptors and indicator variables. The statistical quality of the equations[5] was judged by the parameters like correlation coefficient (r), explained variance (r^2), standard error of estimate (s) and the variance ratio or overall significance value (F).

The accepted equations are validated for stability and predictive ability using "leave-one-out" and cross validation technique. The statistical parameters used to access the quality of the models are the predictive sum of squares (PRESS) of validation. Finally, the standard cross-validated correlation coefficients r^2 and q^2 are also calculated.

$$PRESS = (Y_{pred} - Y_{obs})^2$$

$$S_{press} = \sqrt{PRESS/(n - k - 1)}$$

$$Q^2 = 1 - PRESS/[\Sigma(Y_{pred} - Y_{obs})^2]$$

$$SDEP = \sqrt{PRESS/n}$$

n = no. of compounds used for cross-validation

Y_i = experimental value of the physico-chemical property for the ith sample

Y = value predicted by the model built without the sample i.

The relative potency of various substituted 2,4-diamino pyrimidine derivatives has been determined by inhibiting the growth of two different species of the malarial parasite and from this data the following QSAR models have been derived. The QSAR model that best defines the relative effective concentration of 2,4-diamino pyrimidine derivatives causing 50% inhibition of the malaria parasite is:

TM_4

$$-\log IC_{50} = 0.523(\pm 0.493)\pi - 0.039(\pm 0.037)MR - 1.64545(\pm 1.323)I_{R_1} + 1.204(\pm 0.887)I_{R_2} - 0.735(\pm 0.755)$$

$$n = 28,\ r = 0.668,\ r^2 = 0.447,\ F_{test} = 4.6562,\ s = 0.642,\ Q^2 = 0.314$$

K_1CB_1:

$$-\log IC_{50} = -0.677(\pm 0.613)\sigma_m - 0.595(\pm 0.434)\sigma_p + 0.478(\pm 0.317)I_{R_2} + 0.628(\pm 0.791)I_{R_1} - 1.058(\pm 0.247)$$

$$n = 26,\ r = 0.763,\ r^2 = 0.583,\ F_{test} = 7.355,\ s = 0.362,\ Q^2 = 0.491$$

The result obtained from QSAR analysis indicates that different physico-

chemical descriptors were responsible for variation in wild type (TM_4) and S108N mutant forms (K_1CB_1) of *pf* DHFR inhibitory activity. The results show that for the activity against TM_4, hydrophobicity of the substituent (π) and the substituent at R_5 position are important (positive correlation) whereas substituent at R_1 position and a higher electron polarizability value of the substituent (MR) would be detrimental to the activity (negative correlation). On the other hand, for the activity against K_1CB_1 the substituent at R_1 and R_2 positions would be beneficial whereas the electronic property of the substituent is found to be detrimental to the activity.

Conclusion

It was concluded from the study that mutation in the active site of *pf* DHFR has led to the changes in the binding pattern of the enzyme. Due to these changes in the binding pattern, the conventional drugs used in the treatment of malaria have been inefficient for the cure of the disease in recent years. The study explored some of the changes that would be required in the conventional prototype molecule 2,4-diamino pyrimidine, widely used in the treatment of malaria, for its effective use as an anti-malarial agent, in the fast growing cases of resistant strains of *Plasmodium falciparum*.

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