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A QSAR Study on the Antileishmanial Activity of Some Substituted Pyrimidines and Pyrazolo [1,5-a] pyrimidines

Y.S. PRABHAKAR* AND V.J. RAM
Medicinal Chemistry Divn., Central Drug Research Institute, Lucknow - 226 001.

A quantitative structure activity relationship (QSAR) study was made on the antileishmanial activity of substituted pyrimidine, and pyrazolo [1,5-a] pyrimidine analogues using physicochemical and steric descriptors of the varying substituents. The study of pyrimidine analogues indicated the necessity of having unsubstituted pyrimidine N(3) for Antileishmanial activity. Also, the C(2) substituent, which is adjacent to N(3), of pyrimidine imposed steric restrictions for a compound to become leishmanicide. In case of pyrazolo[1,5-a] pyrimidine analogues, the study suggested the role of hydrophobicity and polarity of substituents on antileishmanial activity.

LEISHMANIASIS is one of the most dreaded parasitic diseases with devastating effects on the host. The availability of very limited drugs for its chemotherapy, and their high toxicity and undesirable side effects on the host prompted investigators to look for alternative biochemicals paths for its clinical control.[1] The leishmanial parasites are devoid of machinery of purine biosynthesis and solely depend on salvage mechanism for these nucleosides[2-5]. Considering this biochemical difference in the purine metabolism of the leishmanial parasite and the host, our laboratory explored a number of purine, pyrimidine and related analogues as potential chemotherapeutic agents.[6-10] To further these studies we investigated the quantitative structure-activity relationships (QSAR) among some substituted pyrimidine,[7,8] and pyrazolo [1,5-a] pyrimidine[9,10] analogues and the results are presented here.

MATERIALS AND METHODS

The *in vivo* (golden hamster, *Mesocricetus auretus)* leishmanicidal activity of 3,4-dihydro-5-cyano-2, 3,6-trisubstituted pyrimidin- 4-ones[7] (Table 1) against amastigotes of *Leishmania donovani* (HOM/IN/80/DDS) at a fixed dose of 10 mg/kg/day x 5, and the *in vitro* leishmanicidal activity of 3,4-dihydro-5-cyano-6-(N- ethylcarbazolo-3-yl)-2-substitutedthiopyrimidin-4-ones[8] (Table 2) and 3-amido-2-methylthio-5, 6,7-trisubstituted pyrazolo[1,5-a] pyrimidines[9,10] (Table 3) against macrophages of *L. donovani*, isolated from peritoneal cavity of cotton rats infected with amastigotes of *L. donovani*, at a fixed dose of 100 μg/ml (corresponding approximate molar doses have been given in the footnotes of respective Tables) were considered here for QSAR study. The activity data has been subjected to logit transformation i.e., log{P/(100-P)}, where P is the per cent response at the given dose, and expressed as $\log BR_{(vivo)}$ and $\log BR_{(vitro)}$ to represent *in vivo* and *in vitro* activities, respectively, and are listed in Tables 1-3. The hydrophobicity(π), molar refractivity (MR), Swain and Luptons resonance (R) and polar (F) constants from Hansch and Leo's monograph[11] and Verloop's steric parameters, substituent length (L) and four widths (B1, B2, B3, and B4)[12], have been considered as the physicochemical and steric descriptors of the

*For correspondence

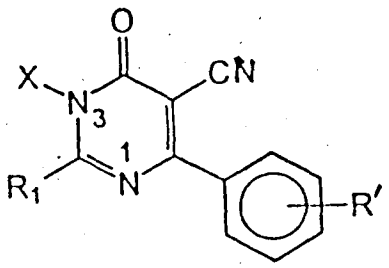

Table 1: Physicochemical parameters and antileishmanial Activity of 3,4-Dihydro-5-cyano-2,3,6-Trisubstituted Pyrimidin-4-ones

Comp No	X	R1	R'	VwX ($10^2Å^3$)	LXa (Å)	VwR1 ($10^2Å^3$)	πR1[a]	LR1 (Å)	logBR(vivo) (Obs)[b]	(Cal)[c]
1	H	SH	3-OMe	0.018	2.06	0.194	0.39	3.47	-	0.53
2	H	SH	4-OMe	0.018	2.06	0.194	0.39	3.47	0.54	0.53
3	H	SMe	3-OMe	0.018	2.06	0.357	0.61	4.30	-	0.55
4	H	SCH2Ph	3-OMe	0.018	2.06	1.051	2.06	8.50	-	0.69
5	H	SCH2Ph	4-Me	0.018	2.06	1.051	2.06	8.50	0.68	0.69
6	H	SCH2Ph-pCl	3-OMe	0.018	2.06	1.216	2.77	-	-	0.76
7	H	SCH2CHCH2	3-OMe	0.018	2.06	0.623	1.04	5.40	0.55	0.59
8	H	SPh-2, 4(NO2)2	3-OMe	0.018	2.06	1.293	1.80	-	-	0.67
9	Me	SMe	3-OMe	0.180	3.00	0.357	0.61	4.30	-	-0.08
10	Et	SEt	3-OMe	0.334	4.11	0.511	1.07	5.24	-	-0.03
11	Et	SEt	4-Me	0.334	4.11	0.511	1.07	5.24	-0.01	-0.03
12	CH2Ph	SCH2Ph	3-OMe	0.874	3.63	1.051	2.06	8.50	-0.01	0.06
13	CH2Ph	SCH2Ph	4-Me	0.874	3.63	1.051	2.06	8.50	0.12	0.06
14	Me	NHPh	4-Me	0.180	3.00	0.814	1.37	4.53	0.30	-
15	H	SCH2Ph	3-Me	0.018	2.06	1.051	2.06	8.50	0.74	0.69

[a]substituent lengths, L, from ref. 12 and π from ref. 11;

[b]taken from ref. 7; % inhibition dose in moles is 30.416 μM/Kg/dayx5

[c]calculated using equation (5).

substituent groups of the compounds under study. Also, van der Waals volumes of the substituent groups were computed[13] as a descriptor of gross steric effect. A linear multiple regression analysis with least-square method was applied in deriving correlations. Only those parameters found significant in the regression equations are listed in the corresponding Tables.

RESULTS AND DISCUSSION

For compounds listed in Table 1, there exists good correlation between the steric parameters of substituent (X) on N(3) and antileishmanial activity as shown.

$$\log BR_{(vivo)} = 0.628 - 0.528\ (\pm 0.218) IX$$
$$n=8,\ r=0.925,\ s=0.125,\ F=35.55 \qquad (1)$$

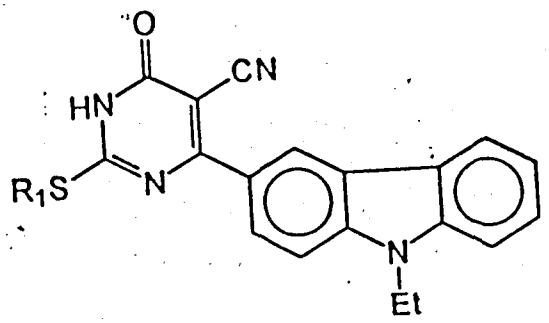

Table 2: Substituent length and antilesishmanial activity or 3,4-dihydro-5-cyano-6-(N-ethylcarbazolo-3-yl)-2-substituted thio pyrimidin-4-ones.

Comp No.	R1	LR1[a] (Å)	logBR(vitro) (Obs)[b]	(Cal)[c]
16	H	2.06	-1.06	-1.06
17	Et	4.11	toxic	-
18	CH2CHCH2	5.11	0.16	0.02
19	CH2CO2Et	7.09	-0.60	-0.54[d]
20	CH2CONH2	5.00	0.16	-0.02
21	CH2CH2OH	4.79	toxic	-
22	CH2-c-C3H5	5.25	-0.14	0.06
23	CH2Ph	3.63	-0.45	-0.50
24	CH2Ph-p-Cl	4.42	-0.39	-0.23.

[a]Taken from ref. 12;
[b]taken from ref. 8; % inhibition dose in moles is 0.2454 μm/ml
[c]calculated using equation (8);
[d]calculated using equation (7).

$$\log BR_{(vivo)} = 0.558 - 0.665\ (\pm 0.465)\ VwX$$
$$n=8,\ r=0.819,\ s=0.189\ F=12.30\ (F.95=5.99) \qquad (2)$$

$$\log BR_{(vivo)} = 1.320 - 0.338\ (\pm 0.090)\ LX$$
$$n=8,\ r=0.966,\ s=0.085,\ F=83.75 \qquad (3)$$

Here, n is the number of data points, r is the correlation coefficient, s is the standard error of the estimate, and F is the F-ratio between the calculated and observed activities. All the equations, excepting those where F.95 values are indicated, are significant at more than 99% level. The data in the parenthesis is the 95% confidence intervals of the regression coefficients. In equation (1), IX is an indicator parameter defined as zero when X is H and as unity when X is alkyl/ aryl group. In equations (1) - (3), the negative regression coefficients of IX, VwX and Verloop's parameter, LX, indicate the steric restrictions at N(3) in determining the activity of these analogues. Also, among the Verloop's parameters, length, L, of the substituent is best correlated with the activity indicating the maximum restrictions in specific direction. The following equations provide an opportunity to examine how the C(2) substituent (R1) contribute towards the activity of these compounds.

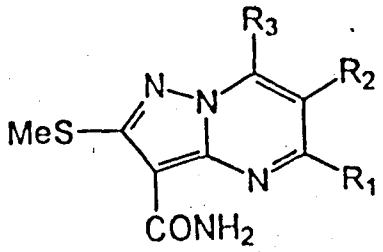

Table 3 Physicochemical parameters and antileishmanial activity of 3-Amido-2-methylthio-5,6,7-substituted pyrazolo[1,5-a] pyrimidines

Comp No	R1	R2	R3	⊼R1[a]	⊼R2	⊼R3	FR1[a]	logBR(vitro) (Obs)[b]	(Cal)[c]
25	Me	H	OH	0.56	0.0	-0.67	-0.04	-0.36	-0.27
26	Me	Cl	OH	0.56	0.71	-0.67	-0.04	-0.21	-0.27
27	Me	H	Me	0.56	0.0	0.56	-0.04	toxic	-
28	Ph	H	Ph	1.96	0.0	1.96	0.08	-0.11	-0.12
29	H	CO2Et	NH2	0.0	0.51	-1.23	0.0	-0.50	-0.57
30	SMe	CN	NH2	0.61	-0.57	-1.23	0.20	-0.95	-0.92
31	SMe	H	Ph	0.61	0.0	1.96	0.20	-0.87	-0.92
32	SMe	CN	OH	0.61	-0.57	-0.67	0.20	-0.95	-0.92
33	H	CO2Et	OH	0.0	0.51	-0.67	0.0	-0.61	-0.57

[a] taken from ref.11;
[b] taken from ref. 9,10; % inhibition dose in moles is 0.3548 μM/ml
[c] calculated using equation (9).

$$\log BR_{(vivo)} = 0.692 - 0.622(\pm 0.136)\, IX + 0.186(\pm 0.205) VwR1$$
$$n=7,\ r=0.988,\ s=0.062,\ F=81.64 \qquad (4)$$

$$\log BR_{(vivo)} = 0.490 - 0.630(\pm 0.128)\, IX + 0.099(\pm 0.077)\, \overset{\delta}{\pi} R1$$
$$n=7,\ r=0.989,\ s=0.058,\ F=95.16 \qquad (5)$$

$$\log BR_{(vivo)} = 0.422 - 0.626\ (\pm 0.128)\, IX + 0.032\ (\pm 0.033)\, LR1$$
$$n=7,\ r=0.989,\ s=0.058,\ F=92.54 \qquad (6)$$

In deriving these equations compound 14, with non - thioalkyl/ thioaryl group as R1, is not included as it is found to be an outlier in the bivariate equations and its inclusion reduces their significance. Among the equations (4) - (6), the regression coefficient of R1 is statistically significant in equation (5), and in equations (4) and (6) they are borderline cases. However, as Vw,π and L are highly intercorrelated here, one can broadly suggest the steric/ hydrophobic requirement of R1 substituent. No meaningful role could be assigned to R' substituent of C(6) aryl moiety of the analogues in the regression equations.

In vitro Antileishmanial activity is available for 3,4-dihydro-5- cyano-6-(N-ethylcarbazolo-3-yl)-2-substitutedthiopyrimidin-4-ones (Table 2), structurally related analogues of compounds listed in Table 1. In these compounds the only variation is in R1 substituent and the correlation of the activity with the length of this substituent is as shown.

$$\log BR_{(vitro)} = -3.072 + 1.180\ (\pm 0.822)\ LR1 - 0.116\ (\pm 0.089)\ LR_{12}$$

n=7, r=0.903, s=0.229, F-8.90 (F.95=6.94) (7)

$$\log BR_{(vitro)} = -1.782 + 0.352\ (\pm 0.178)\ LR1$$

n=6, r=0.939, s=0.176, F=30.05 (8)

The equation (7) indicates the optimum length of R1 as 5.086A° which is attained in compounds 18, 20 and 22. Here, compound 19 is responsible for the parabolic nature of equation (7) and its exclusion has resulted in linear equation (8). The signs of regression coefficients of R1 substituent between equations (6) and (8) are comparable. Also, the regression equations of compounds of Tables 1 and 2 jointly suggest the restrictions imposed on variations of the X and R1 substituents of these analogues. Hence among these compounds variations in the X and R1 substituent offer limited scope in the discovery of an analogue with marked improvement in activity compared to the one presented in the study.

Coming to the 3-amido-2-methylthio-5,6,7-trisubstituted pyrazolo [1,5-a] pyrimidines of Table3, their *in vitro* antileishmanial activity is best correlated as a function of hydrophobicity and polarity of substituent groups as follows.

$$\log BR_{(vitro)} = -0.573 + 0.343\ (\pm 0.113)\ \pi R1 - 2.794\ (\pm 0.622)\ FR1$$

n=8, r=0.985, s=0.068, F=80.37 (9)

$$\log BR_{(vitro)} = -0.438 + 0.098\ (\pm 0.074)\ \Sigma\pi - 2.491\ (\pm 1.237)\ FRI$$

n = 8, r=0.935, s=0.139, F=17.45 (10)

In equation (10), $\Sigma\pi$ is sum of πR1 π R2 and π R3. The above two equations highlight the importance of substituents with negative polarity (R1) and hydrophobicity in determining the inhibitory activity of these compounds. Here, the choice of substituent group for R1 is directed by equation (9), wherein the balance of polarity and hydrophobicity of R1 are important. And at the same time, equation (10) while accounting for the polarity of R1, dictates the collective hydrophobicity of R1, R2 and R3 groups in deciding inhibitory activity of these compounds. These equations suggest that within the vicinity of substituent space studied there exists scope for more potent analogues compared to those incorporated here.

In conclusion, the chemotherapeutic potential of nucleosides and their bases is due to the fact that they are the components of nucleic acids which are involved at the molecular levels of regulation of the parasites. Among the pyrimidine analogues studied here, the most active one is compound 15. According to the regression equations 1-6, the antileishmanial activity of pyrimidines indicate the necessity of having unsubstituted N(3) for activity, that is, the best choice for X is hydrogen. Probably this may be one of the important sites of pyrimidine system which plays a role in its uptake by the parasite. Interestingly, the C(2) substituent (R1), which is adjacent to N(3) position, of pyrimidine also imposes steric restrictions for compounds to become active. This further suggests that for the uptake of pyrimidine analogues by the parasite a relatively sterically free N(3) and its neighbourhood is necessary. As a result, in the enhancement of the activity of these analogues, the variations in R1 may offer only a limited scope. Coming to the R' on the aryl moiety of C(6) of Pyrimidines, we could not assign any role in regression equations to account for the variations in the activity. In the above background any further congeneric variations of compound 15 may result in limited improvement of the activity. The parasite may be salvaging pyrazolo[1,5-a]-pyrimidines with different identity compared to that of pyrimidines. According to equations 9 and 10 the activity of these compounds can be further optimised by appropriately changing the polarity of R1 and hydrophobicity of R1, R2 and R3 groups. Here, the study offers scope for further expansion of this series for more potent analogues than the ones already presented here.

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