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Quantitative Structure–Activity Relationship Study of 5-Iodo- and Diaryl-analogues of Tubercidin: Inhibitors of Adenosine Kinase

P. SINGH*, RAJESH KUMAR and B.K. SHARMA

Department of Chemistry, S.K. Government College, Sikar 332 001, India

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The adenosine kinase inhibitory (AKI) activity of 5-iodo and diaryl analogues of tubercidin is quantitatively analyzed using Fujita-Ban and Hansch type analyses. The Fujita-Ban analysis being a non-parametric approach assigned the highest contribution to Cl at the *X*-position, C_6H_4-4-Cl, C_6H_5, 2-furanyl and I at the *Y*-position and CH_2NH_2 and CH_3 at the *Z*-position. In addition, a OH substituent at the *C*-position also emerged as a better choice possibly due to its engagement in hydrogen bonding with some active site function. Thus a compound having Cl, C_6H_4-4-Cl, CH_2NH_2 and OH respectively at *X*-, *Y*-, *Z*- and *C*-positions is predicted to have a potency nearly 1.5 orders of magnitude higher than the most potent compound of the parent data set. The Hansch type analysis, on the other hand, is a parametric approach and is carried out on two sub-sets of original compounds. This sub-division is based on size and nature of the substituents present at the *X*- and *Y*-positions. For the compounds in the first sub-set the derived significant correlation equation suggested that the substituent at the *Y*-position exhibiting a higher field effect and a substituent such as Cl and CH_2NH_2 at *X*- and *Z*-positions, respectively, are important for a compound to show increased AKI activity. Thio/alkylthio at *X* and CH_2OCH_3 at *Z*, on the other hand, lead to a detrimental effect. Similarly for the compounds in the second sub-set, the derived significant correlation equation showed that a substituent at the *X*-position having a higher negative field effect, a substituent at the *Y*-position having bulky groups and the *C*-position occupied by a OH group are essential for enhancement of the activity of a compound.

Keywords: 5-Iodo and diaryl analogues of tubercidin; Adenosine kinase inhibition activity; QSAR analysis; Fujita-Ban and Hansch approaches; Physicochemical properties; Rationalizing the substituent selection in drug design

INTRODUCTION

Compounds that activate the adenosine receptor represent potential therapeutic agents for a variety of diseases such as hypertension, epilepsy, pain, diabetes and inflammation.[1] Such agonists, however, exhibit a relatively narrow therapeutic index due to simultaneous production of undesired pharmacological effects and have therefore failed to undergo successful clinical development. For this reason, alternative strategies were followed that would increase the potential therapeutic benefits of adenosine receptor activation and decrease the undesirable side effects.[2–9] The approach is based on the use of agents termed "adenosine regulating agents (ARAs);" these affect the production or metabolism of adenosine such that extracellular adenosine is elevated in a relatively site- and event-specific manner. The specific action is based on the principle that extracellular adenosine is produced mainly from intracellular breakdown of ATP.[10] The net ATP breakdown occurs in cells that are subjected to hypoxia or cellular stress such that repletion of ATP stores is not possible at a rate comparable with energy utilization. Compounds that divert ATP breakdown toward production of adenosine or compounds that decrease the metabolism of adenosine should therefore increase extracellular adenosine levels within hypoxic regions of tissues and have little influence in normoxic regions.

One of the important ARA targets is adenosine kinase (AK) which is a cytosolic enzyme that

*Corresponding author. E-mail: singhp1_skc_india@rediffmail.com

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catalyzes the conversion of adenosine to AMP. AK activation is mainly responsible for the clearance of adenosine and for its extremely short plasma half-life (<1 s).[11] Inhibition of AK results in increased intracellular adenosine which passes out of the cell via passive diffusion or via nucleoside transporter(s) leading to activation of nearby cell-surface adenosine receptors.[12] In this way, AK inhibition may represent an alternative mechanism for activation of adenosine receptors and production of adenosine-associated pharmacologies. Initially the study of AKIs was focused on their potential use as antiseizure agents[13–16] in a dose-dependent manner. It was also demonstrated that AKIs exhibit anticonvulsant effects against MES-induced seizures in rats and the effects are reversed by the nonspecific adenosine receptor antagonist theophylline, suggesting that the pharmacological effect is mediated by an adenosine receptor.[17] Based on the potent AKI activity reported for 5-iodotubercidin, a number of tubercidin analogues were prepared to study the structure–activity-relationship (SAR) of AK inhibition.[17] However, these compounds are reported to have weak *in vivo* potency due to their poor brain penetration or cell penetration. Consequently, a new series of compounds was reported[18] in which various structural changes were made to include large hydrophobic substituents, such as an aromatic amine and an aromatic ring, at different positions of the tubercidin molecule so as to enhance AK specificity and selectivity, as well as enhance their brain/cell penetration. Several of these compounds were found to exhibit reduced side effects when compared with adenosine receptor agonists. The SAR studies on the aforesaid series of tubercidin were mainly concerned with the alteration of substituents at different positions of the parent moiety and provided no rationale to reduce the trial-and-error factors. Hence, a quantitative SAR (QSAR) study on analogues of both series is carried out here so as to provide the rationale for drug-design and explore the possible mechanism of their action.

MATERIALS AND METHODS

The reported series of compounds consists of 5-iodo- and diaryl-tubercidins and are represented by a general structure, shown in Fig. 1. These analogues along with their biological effects and the appropriate quantifying parameters of substituents, present at different positions of the parent structure, are compiled in Tables I and II. The biological effect, measured as IC_{50}, represents the concentration of a compound required to inhibit 50% of AK activity. For a given compound, this is expressed as $-\log IC_{50}$ on a molar basis in the present study.

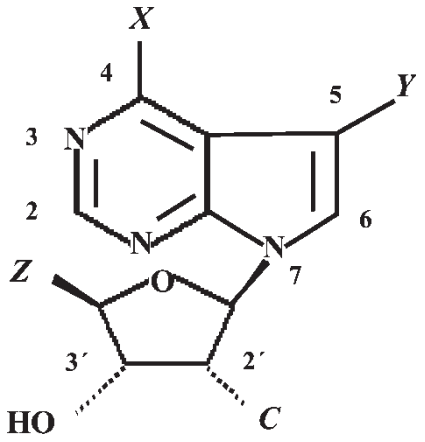

FIGURE 1 Structure of substituted analogues of tubercidin.

In order to obtain important QSAR, using the method of multiple regression analysis (MRA), both the Fujita-Ban and the Hansch type analyses were performed on these congeners. The substituents appeared especially, at the *X*- and *Y*- positions of the parent moiety of the compounds in Tables I and II to differ greatly in their size and nature and may, therefore, exhibit different modes of action at receptor sites. This prompted us to identify the congeners of both Tables separately and carry out a parameter sensitive approach such as a Hansch type analysis. The most appropriate quantifying parameters were found to be the field constant, ***F***, for the compounds in the first series and ***F*** and the van der Waals volume, *Vw*, for the compounds in the second series. For this purpose, the physicochemical parameters were taken directly from the literature[19] and the *Vw*s were calculated employing an earlier reported method.[20] Using van der Waals' radii,[20] the volume of atoms was calculated assuming a spherical shape as suggested by Bondi.[21] For calculating *Vw* of a polyatomic fragment (or substituent), a correction has to be made for the sphere overlapping in the covalent bonding between atoms, as the van der Waals' radii are larger than the covalent radii. A correction for the branching in the chain was also introduced. The usefulness of this structural parameter has already been established in the past.[22–30] In addition to these parameters the indicator variables, representing the presence or absence of certain structural characteristics, was also used in both the series. The derived QSAR equations were further subjected to a validation test[31] by the leave-one-out method (LOO). The method creates a number of modified data sets by taking away one compound from the parent data set in such a way that each observation is taken away once and once only. Then one model is

TABLE I QSAR parameters and adenosine kinase inhibition potencies of 5-iodo tubercidin analogues (see Figure 1 for structure)

										$-\log IC_{50}$ (M)			
											Calc.		
S.No.	X	Y	Z	C	F_Y	I_1	I_2	I_3	I_4	Obsd[a]	F.B.	Eq. (2)	Prctd
1	NH_2	I	CH_2OH	OH	0.40	0	0	0	0	7.59	7.75	6.81	6.77
2	NH_2	I	CH_3	OH	0.40	0	0	0	0	8.05	8.00	6.81	6.74
3	NH_2	Br	CH_3	OH	0.44	0	0	0	0	7.40	7.40	6.95	6.92
4	NH_2	Br	CH_2OH	OH	0.44	0	0	0	0	6.92	7.15	6.95	6.95
5	Cl	I	CH_2OH	OH	0.40	1	0	0	0	7.62	8.17	7.64	7.65
6	Cl	I	CH_3	OH	0.40	1	0	0	0	8.52	8.42	7.64	7.50
7	Cl	Br	CH_3	OH	0.44	1	0	0	0	7.30	7.83	7.78	7.86
8	Cl	CH_3	CH_3	OH	−0.04	1	0	0	0	6.27	5.74	6.13	6.03
9	Cl	SCH_3	CH_3	OH	0.20	1	0	0	0	7.15	6.73	6.95	6.92
10	Cl	I	CH_2N_3	OH	0.40	1	0	0	0	8.05	8.04	7.64	7.58
11	Cl	Br	CH_2N_3	OH	0.44	1	0	0	0	7.00	7.44	7.78	7.92
12	Cl	I	CH_2OCH_3	OH	0.40	1	0	0	1	6.35	6.35	6.55	6.79
13	NH_2	CH_3	CH_3	OH	−0.04	0	0	0	0	4.78	5.31	5.30	5.62
14	NH_2	SCH_3	CH_3	OH	0.20	0	0	0	0	5.87	6.30	6.12	6.15
15	NH_2	I	CH_2N_3	OH	0.40	0	0	0	0	7.46	7.61	6.81	6.77
16	NH_2	Br	CH_2N_3	OH	0.44	0	0	0	0	7.20	7.01	6.95	6.93
17	NH_2	I	CH_2OCH_3	OH	0.40	0	0	0	1	5.92	5.92	5.72	5.48
18	NH_2	I	CH_2CH_3	OH	0.40	0	0	0	0	7.22	7.06	6.81	6.79
19	NH_2	Br	CH_2CH_3	OH	0.44	0	0	0	0	6.30	6.46	6.95	6.99
20	NH_2	I	CH_2NH_2	OH	0.40	0	0	1	0	9.22	9.12	9.32	9.37
21	NH_2	Br	CH_2NH_2	OH	0.44	0	0	1	0	9.70	8.52	9.46	9.32
22	Cl	I	CH_2NH_2	OH	0.40	1	0	1	0	10.00	9.55	10.15	10.25
23	NH_2	CN	CH_2OH	OH	0.51	0	0	0	0	6.51	6.38	7.19	7.26
24	NH_2	CN	CH_3	OH	0.51	0	0	0	0	6.51	6.64	7.19	7.26
25	NH_2	$CONH_2$	CH_2OH	OH	0.24	0	0	0	0	6.33	6.21	6.26	6.26
26	NH_2	$CONH_2$	CH_3	OH	0.24	0	0	0	0	6.34	6.46	6.26	6.25
27	NH_2	H	CH_2NH_2	OH	0.00	0	0	1	0	4.89	-[a]	-[b]	-[b]
28	NH_2	Cl	CH_2OH	OH	0.41	0	0	0	0	6.68	-[c]	6.85	6.86
29	NH_2	I	$CH{=}CH_2$	OH	0.40	0	0	0	0	7.00	-[c]	6.81	6.80
30	NH_2	$COOC_2H_5$	CH_3	OH	0.33	0	0	0	0	6.00	-[c]	6.57	6.60
31	$NHCH_3$	I	CH_2OH	OH	0.40	0	0	0	0	5.92	-[c]	6.81	6.86
32	SH	I	CH_3	OH	0.40	0	1	0	0	5.19	-[c]	5.69	5.81
33	SCH_3	I	CH_3	OH	0.40	0	1	0	0	7.35	-[c]	-[b]	-[b]
34	$SCH_2CH{=}CH_2$	I	CH_3	OH	0.40	0	1	0	0	5.55	-[c]	5.69	5.72
35	S-n-C_4H_9	I	CH_3	OH	0.40	0	1	0	0	6.00	-[c]	5.69	5.61
36	$SCH_2C_6H_5$	I	CH_3	OH	0.40	0	1	0	0	6.00	-[c]	5.69	5.61
37	$SCH_2C_6H_5$-4-NO_2	I	CH_3	OH	0.40	0	1	0	0	5.70	-[c]	5.69	5.68

[a] Concentration, on molar scale, required to inhibit 50% of the AK activity; taken from Ref. [17]. [b] 'Outlier' compound in the present study. [c] Compounds ignored in formulating Fujita-Ban (F.B.) matrix.

developed for each reduced data set and the response values of the deleted observations are predicted from the model. The squared differences between predicted and actual values are added to give the predictive residual sum of squares, PRESS. In this way, PRESS will contain one contribution from each observation. The cross-validated q^2 value may further be calculated as (SSY-PRESS)/SSY, where SSY represents the variance of the observed activities of molecules around the mean value. To be a reasonable QSAR model, q^2 should be greater than 0.6, and a value of this index greater than 0.9 indicates an excellent model.

The Fujita-Ban analysis,[32] based on an additivity principle, is a non-parametric approach and requires, relatively, a larger data-set. Therefore, the compounds of both Tables were considered together to enlarge the training set in this approach. This may in turn give a better insight into the substitutional requirements for those analogues which have yet to be synthesized.

RESULTS AND DISCUSSION

In construction of the Fujita-Ban matrix, fifty three compounds of Tables I and II were initially retained and compound **4** was considered as the reference or parent congener. Thirteen compounds (**28**–**37**, **56**, **60** and **62**) from these Tables were, however, not included in the above training set as the frequency of occurrence of certain groups in these compounds was only once. To be concise, the matrix comprising of 53 compounds (rows) and 25 substituents pertaining to varying positions of the parent moiety (columns) is not documented here. The rows and columns of this matrix representing respectively the data-points and the independent

TABLE II QSAR parameters and adenosine kinase inhibition potencies of diaryl tubercidin analogues (see Figure 1 for structure)

								$-\log IC_{50}$ (M)			
									Calc.		
S. No.	*X*	*Y*	*Z*	*C*	F_X	Vw_Y	I_C	Obsd.[a]	F.B.	Eq. (3)	Prctd.
38	$NHCH_2C_6H_5$	I	CH_3	OH	–	–	–	6.10	5.86	-[b]	-[b]
39	$NHCH_2C_6H_5$	C_6H_5	CH_3	OH	–	–	–	7.52	7.76	-[b]	-[b]
40	NHC_6H_{11}	I	CH_3	OH	–	–	–	5.00	4.85	-[b]	-[b]
41	NHC_6H_{11}	C_6H_5	CH_3	OH	–	–	–	6.60	6.75	-[b]	-[b]
42	NHC_6H_5	2-Furanyl	CH_3	OH	0.00	0.608	0	8.44	8.56	7.99	7.95
43	NHC_6H_4-4-OCH_3	2-Furanyl	CH_3	OH	0.26	0.608	0	8.05	7.93	7.78	7.77
44	NHC_6H_5	H	CH_2OH	OH	0.00	0.056	0	5.90	5.90	5.57	5.41
45	NHC_6H_5	I	CH_3	OH	0.00	0.388	0	7.00	6.91	7.03	7.03
46	NHC_6H_4-4-F	I	CH_3	OH	0.43	0.388	0	6.26	6.59	6.43	6.47
47	NHC_6H_4-4-OCH_3	I	CH_3	OH	0.26	0.388	0	6.11	6.28	6.82	6.90
48	NHC_6H_4-4-OH	I	CH_3	OH	0.29	0.388	0	6.70	6.90	6.82	6.83
49	NHC_6H_4-4-CN	I	CH_3	OH	0.51	0.388	0	5.92	5.94	6.05	6.11
50	NHC_6H_5	I	CH_2OH	OH	0.00	0.388	0	6.92	6.66	7.03	7.04
51	NHC_6H_5	C_6H_5	CH_3	OH	0.00	0.785	0	9.30	8.81	8.76	8.71
52	NHC_6H_4-4-F	C_6H_5	CH_3	OH	0.43	0.785	0	8.82	8.49	8.17	8.12
53	NHC_6H_4-4-OCH_3	C_6H_5	CH_3	OH	0.26	0.785	0	8.22	8.17	8.55	8.58
54	NHC_6H_4-4-OH	C_6H_5	CH_3	OH	0.29	0.785	0	9.00	8.80	8.55	8.53
55	NHC_6H_5	C_6H_5	CH_2OH	OH	0.00	0.785	0	9.10	8.55	8.76	8.73
56	NHC_6H_4-4-F	C_6H_4-4-F	CH_3	OH	0.43	0.831	0	7.59	-[c]	8.37	8.45
57	NHC_6H_5	C_6H_4-4-Cl	CH_3	OH	0.00	0.950	0	8.92	8.86	9.49	9.60
58	NHC_6H_4-4-Cl	C_6H_4-4-Cl	CH_3	OH	0.41	0.950	0	8.57	8.63	8.84	8.88
59	NHC_6H_4-4-CN	C_6H_5	CH_3	OH	0.51	0.785	0	7.85	7.83	7.79	7.77
60	NHC_6H_4-4-CN	C_6H_4-4-OCH_3	CH_3	OH	0.51	1.020	0	9.00	-[c]	8.82	8.75
61	NHC_6H_4-4-Cl	C_6H_5	CH_3	OH	0.41	0.785	0	8.64	8.58	8.12	8.07
62	NHC_6H_4-4-CH_3	C_6H_5	CH_3	OH	−0.04	0.785	0	8.82	-[c]	8.89	8.89
63	NHC_6H_5	C_6H_5	CH_2N_3	OH	0.00	0.785	0	8.82	8.42	8.76	8.76
64	NHC_6H_5	C_6H_5	CH_2OH	H	0.00	0.785	1	6.52	6.63	6.76	7.00
65	NHC_6H_5	C_6H_5	CH_3	H	0.00	0.785	1	7.00	6.89	6.76	6.52
66	NHC_6H_5	C_6H_5	CH_2NH_2	OH	0.00	0.785	0	8.20	9.93	8.76	8.82

[a] See footnote under Table I; taken from Ref. [18]. [b] Compounds not considered in deriving Equation (3). [c] Compounds ignored in formulating Fujita-Ban (F.B) matrix.

variables while the activity values ($-\log IC_{50}$s) being considered as the dependent variable were subjected to MRA. The resulting statistical parameters of the study were:

$$n = 53, \quad r = 0.921, \quad s = 0.676, \quad F(25, 27) = 6.068$$

where *n*, *r*, *s* and *F* are respectively the number of data-points in the training set, multiple regression coefficient, standard error of estimate and *F*-ratio between the variances of calculated and observed activities. Except the *r*-value, which accounts for 85% of variance ($r^2 = 0.848$), the remaining statistical parameters of the analysis are slightly too poor to account for significant results. Possibly certain outlier compounds, present in the original training-set, are responsible for the inferiority in these parameters. The congener **27** is the only compound whose calculated $-\log IC_{50}$ value ($= 6.16$) was found to be much higher than the observed value. This compound, unsubstituted at the C-5 position was reported to be a very weak AKI, reiterating the importance of substitution at the C-5 position for the tubercidin molecules to potently inhibit AK. The data-point was, therefore, ignored further. In doing so, the corresponding row was removed from the Fujita-Ban matrix and the MRA of the new matrix lead to the results summarized in Table III. The data given within the parentheses therein are the 95% confidence intervals. The improved statistical parameters of the study are:

$$n = 52, \quad r = 0.946, \quad s = 0.551, \quad F(25, 26) = 8.914$$

The r^2-value now accounts for 89% of the variance and the *F*-value stands significant at 99% level [$F_{25,26}(0.01) = 2.58$]. The calculated values of $-\log IC_{50}$ for all the compounds in Tables I and II are also in close agreement with the observed ones. From Table III the substituents that have higher positive contributions to activity relative to substituents of the parent moiety at different positions have the following pattern:

X	*Y*	*Z*
Cl	C_6H_4-4-Cl	CH_2NH_2
	C_6H_5	CH_3
	2-Furanyl	
	I	

TABLE III Fujita-Ban contributions of substituents and parent moiety to the adenosine kinase inhibition activities of title compounds

Position	Substitution	Contribution to $-\log IC_{50}$
X	Cl	0.426(±0.52)
	NHC_6H_{11}	−3.145(±1.04)
	$NHCH_2C_6H_5$	−2.136(±1.04)
	NHC_6H_5	−1.085(±0.75)
	NHC_6H_4-4-CN	−2.058(±1.04)
	NHC_6H_4-4-Cl	−1.315(±1.20)
	NHC_6H_4-4-F	−1.404(±1.04)
	NHC_6H_4-4-OH	−1.096(±1.04)
	NHC_6H_4-4-OCH_3	−1.720(±0.97)
Y	CN	−0.766(±0.97)
	$CONH_2$	−0.942(±0.97)
	2-Furanyl	2.246(±1.14)
	H	−0.160(±1.44)
	I	0.597(±0.58)
	CH_3	−2.088(±0.98)
	C_6H_5	2.491(±0.80)
	C_6H_4-4-Cl	2.543(±1.21)
	SCH_3	−1.102(±0.98)
Z	CH_2N_3	−0.134(±0.70)
	CH_2NH_2	1.375(±0.74)
	CH_2OCH_3	−1.825(±0.97)
	C_2H_5	−0.686(±0.97)
	CH_3	0.253(±0.54)
C	H	−1.919(±0.95)
Contribution of parent compound, μ		7.148(±0.63)

The appropriate combination from the above pattern (the preference decreases downward for a given position) may be used for the future design of more active analogues of the series. The optimal activities seem to be manifested by compounds in which the *X*-, *Y*- and *Z*-positions are substituted respectively by Cl, C_6H_4-4-Cl (or C_6H_5, 2-furanyl and I) and CH_2NH_2 (or CH_3). A simple analogue (which is outside the training-set) emerging from a combination of the substituents shown in the first row above is predicted to have a potency nearly 1.5 orders of magnitude higher than that for the highest active compound of the reported compounds. Such a prediction may help in designing analogues, for synthesis in the future.

It may be recalled that the Fujita-Ban approach cannot extrapolate beyond the substituents used in the training set whereas the Hansch approach, discussed below for these compounds, can do so. However the data-set similar to Fujita-Ban study, seems to be inappropriate for this approach as the *X*- and *Y*-substituents (Tables I and II) differ both in size and nature. Also the analogues, which are present singly in these Tables and were excluded in the Fujita-Ban analysis, may now be incorporated in the training-sets of the individual Tables. Besides the earlier predicted outlier congener **27**, compounds **38**–**41** were also dropped from the training-set in the Hansch approach. In these four analogues, the *X*-substituents differ in their nature from the other compounds in Table II. In the later case, an aromatic ring (substituted/unsubstitued) is directly bonded to an NH group while in the former case it is not. Thus it becomes feasible to select various types of physicochemical parameters for the substituents of the phenyl ring attached to a basic center in a systematic manner. A number of correlative parameters pertaining to electronic, hydrophobic and steric interactions were examined for varying sites of these molecules in various possible permutations/combinations. Initially a data set comprising of substituent constants such as hydrophobicity, π, hydrogen-bond donor, *HD*, hydrogen-bond acceptor, *HA*, electronic (*meta* and *para*), σ, field, ***F***, resonance, ***R***, dipole moment, μ, Taft's steric, *Es*, molar refraction, *MR*, molecular weight, *MW* and van der Waals volume, *Vw* for each of the *X*- and *Y*-positions and π, *HD*, *HA*, *MR*, *MW* and *Vw* for the *Z*-position was considered for the compounds in Table I. A total number of 28 independent variables (11, 11 and 6 for the *X*-, *Y*- and *Z*-positions respectively) was then permuted appropriately for the three positions and subjected to MRA. This leads to a large number of QSAR equations in three independent variables, which were then subjected to various statistical tests. The correlation equation, which returned the highest *r*- and *F*-values and lowest *s*-value was finally retained for further consideration. From the generated data set for the analogues in Table II, the parameter $\boldsymbol{F}_Y$ accounting for the field effect of *Y*-substituents, emerged as the only most appropriate quantifying parameter and none for *X*- and *Z*-substituents. In addition, the indicator variables reflecting certain structural variations played an important role in developing significant correlations. The derived correlation from the data in Table II (compound **27** is still an outlier) is given by Equation (1)

$$\begin{aligned} -\log IC_{50} = {} & 3.441(\pm 1.73)\boldsymbol{F}_Y + 0.830(\pm 0.49)I_1 \\ & - 0.848(\pm 0.57)I_2 + 2.506(\pm 0.75)I_3 \\ & - 1.093(\pm 0.90)I_4 + 5.436 \\ n = 36, \quad & r = 0.884, \quad s = 0.595, \\ & F(5, 30) = 21.380 \end{aligned} \tag{1}$$

where the indicator variables I_1 and I_2 are highlighting respectively a chloro and the sulfur containing substituents at the *X*-position. A value of 1 or 0 for each of these variables indicates the presence or absence of the above-mentioned structural features of the *X*-substituents. Similarly, the indicator variables I_3 and I_4 were chosen to account for the presence or absence of certain substituents at the *Z*-position. The presence of CH_2NH_2 and CH_2OCH_3 at this position in certain compounds was arbitrarily assigned a value of 1 respectively to I_3 and I_4 while their absence was indicated by a value of 0 for each of

these variables. The statistical parameters obtained for Equation (1) denoted fairly statistically sound results and the equation as such reflected the parameteric requirement of various substitutions at different positions of the tubercidin analogues. Though the *F*-value, given above, was significant at 99% level [$F_{5,30}(0.01) = 3.70$] the r^2-value only accounted for 78% of variance in the observed activity values and the equation as such required further improvement. The only congener **33** containing an SCH_3 at the *X*-position exhibited potent inhibitory activity indicating that the active site of the enzyme may accommodate such a substituent in the same preference as other hydrophobic group such as Cl or a hydrophilic group NH_2 (held by a hydrogen bonding with the enzyme presumably through water molecules). The bulkier alkylthio groups of compounds **34**–**37**, on the other hand, are too large to fit into the presumed empty pocket thereby resulting in poor AKI activity. The unusual behavior of compound **33** is perhaps reflected in the slightly poor correlation in Equation (1). This compound was, therefore, dropped out from the data set leading the MRA into an improved statistical correlation Equation (2)

$$\begin{aligned} -\log IC_{50} &= 3.441(\pm 1.56)\boldsymbol{F}_Y + 0.830(\pm 0.44)I_1 \\ &\quad - 1.124(\pm 0.55)I_2 + 2.506(\pm 0.68)I_3 \\ &\quad - 1.093(\pm 0.82)I_4 + 5.436 \\ n &= 35, \quad r = 0.910, \quad s = 0.535, \\ F(5,29) &= 27.834, \quad q^2 = 0.773 \end{aligned} \tag{2}$$

Now both the *r*- and *F*-values were increased to account respectively for 83% of variance in the observed activities and 99% level of significance [$F_{5,29}(0.01) = 3.73$]. Also, the *s*-value and the 95% confidence intervals (± data within parentheses) associated with the regression coefficients were significantly lowered. Additionally, the higher value obtained for q^2 expressed a reasonable QSAR model. That the variables used in deriving Equation (2) had no mutual correlation is shown in Table IV. The calculated activity values, using this equation and listed in Table I, are in close agreement with the observed ones. The predicted activity values obtained from the data set of Equation (2) and various model equations, discussed earlier, were also listed in the last column of this table for comparison sake. From Equation (2), it appeared that the substituents of the *Y*-position showing a higher field effect (electronic) are advantageous in improving AKI activities. Likewise, the substituents such as Cl and CH_2NH_2 respectively at the *X*- and *Z*- positions showed an increase in activity while thio/alkylthio and CH_2OCH_3 in the same order present at these positions lead to a detrimental effect on activity.

TABLE IV The intercorrelation matrix[a] amongst the independent variables of Equation (2)

	$\boldsymbol{F}_Y$	I_1	I_2	I_3	I_4
$\boldsymbol{F}_Y$	1.000	0.140	0.111	0.117	0.067
I_1		1.000	0.240	0.053	0.137
I_2			1.000	0.125	0.101
I_3				1.000	0.075
I_4					1.000

[a] Matrix elements are the r-values.

The independent variables relating to the *X*-position in the generated data set for the compounds in Table II were considered only for the substituents of the phenyl ring and not for the substituted phenyl amino group attached to the pyrimidine ring at its 4-position. Thus position-*X*, for compounds **42**–**66** in this Table, was taken as 4-NHPh-*X* in the follow up study while ignoring compounds **38**–**41**, which had a different type of *X*-substituents. With this criterion, the generated data set again contained a large number of independent variables, which were appropriately permuted over varying positions and subjected to MRA. The large number of correlation equations obtained in this way were subjected to various statistical tests and the most significant one is given by Equation (3)

$$\begin{aligned} -\log IC_{50} &= -1.691(\pm 0.92)\boldsymbol{F}_X + 4.314(\pm 0.80)Vw_Y \\ &\quad - 2.059(\pm 0.70)I_C + 5.433 \\ n &= 25, \quad r = 0.934, \quad s = 0.431, \\ F(3,21) &= 47.854, \quad q^2 = 0.827 \end{aligned} \tag{3}$$

where $\boldsymbol{F}_X$ was the field effect of the *X*-substituents and Vw_Y was the van der Waals volume, accounting for the molecular bulk/size of the *Y*-substituents. In addition, the indicator variable, I_C was chosen to account for binary variation of *C*-substituents. A value of 1 for H and 0 for OH, present at this position, was arbitrarily assigned to it. The r^2-value accounted for 87% of variance in observed activity values and the *F*-value stood significant at the 99% level [$F_{3,21}(0.01) = 4.87$]. This showed that the statistical parameters of Equation (3) are accountable to a highly significant correlation. A high q^2 value, obtained through a cross-validation test, also indicated towards an excellent statistical model. Further, the independent variables used in deriving the above equation showed poor intercorrelations among themselves (Table V). The equation was, therefore, used to calculate the activity values of all 25 compounds of the test data set. These values, listed in Table II, were found to be in close agreement with the observed values. The predicted activity values of all the compounds, obtained through

TABLE V The intercorrelation matrix[a] amongst the independent variables of Equation (3)

	F_X	Vw_Y	I_C
F_X	1.000	0.115	0.284
Vw_Y		1.000	0.146
I_C			1.000

[a] See footnote under Table IV.

the LOO approach, were also listed in Table II for the sake of comparison. From Equation (3), it appears that the *X*-substituent that transmits a higher negative field effect, the *Y*-substituent that offers higher molecular bulk and the OH present at the *C*-position are beneficial in increasing the potency of a compound. This strategy may, therefore, be followed for designing higher potency compounds for future synthesis. The plot showing the variation of observed versus calculated activities, obtained through the Fujita-Ban and the Hansch type approaches for the compounds in Tables I and II is shown in Figure 2. Such a demonstration may help to understand the goodness of fit and to identify systematic variation of observed versus calculated activities by the two models for the compounds under present study.

Since the data set considered by the two approaches were different it may not, therefore, be appropriate to draw convergent conclusions. However, a few points on individual analysis may be outlined as follows:

1. The Fujita-Ban study, relative to parent compound, assigned the highest positive substituent contributions to Cl at the *X*-position, C_6H_4-4-Cl, C_6H_5, 2-furanyl and I at the *Y*-position and CH_2NH_2 and CH_3 at the *Z*-position. The replacement of OH with H at the *C*-position displayed a negative contribution, suggesting that a hydroxyl group was a better choice at this position. Possibly, it is engaged in hydrogen bonding with some active site group leading to enhancement in AKI potency. Based on the above findings a simple compound emerged from combination of substituents such as Cl, C_6H_4-4-Cl and CH_2NH_2 respectively at the *X*-, *Y*- and *Z*-positions with OH at the *C*-position which was predicted to have a potency nearly 1.5 orders of magnitude higher than that of the highest active reported compound.
2. For the compounds in Table I, the derived Equation (2) suggested that the substituent at the *Y*-position exhibiting a higher field effect and the substituent such as Cl and CH_2NH_2, at the *X*- and *Z*-positions, respectively were important to show an incremental effect on AKI activity. The thio/alkylthio at *X* and CH_2OCH_3 at *Z*, on the other hand, lead to decreased activity.

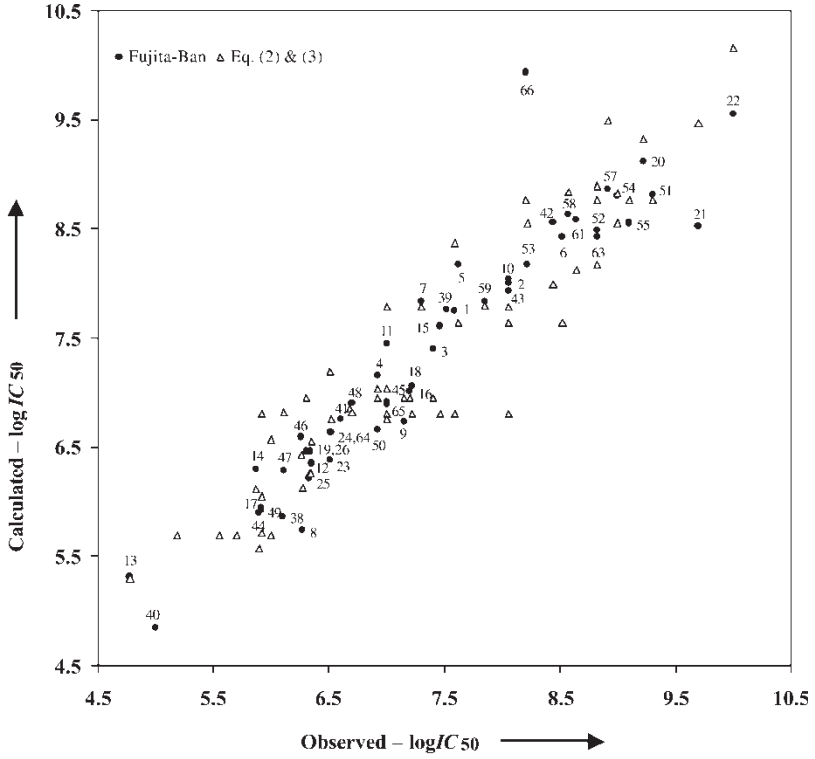

FIGURE 2 Plot of observed versus calculated $-\log IC_{50}$ values.

Similarly for the compounds in Table II, the derived significant correlation Equation (3) showed that the substituent at the *X*-position having a higher negative field effect, the substituent at the *Y*-position having a bulky group while the *C*-position was occupied by a OH group were essential for enhancement of the activity of a compound.

These guidelines may, therefore, provide a basis for rationalizing substituent selection in the future designing of effective inhibitors of adenosine kinase. Additionally, the study may also help in exploring the possible mode of action of tubercidin analogues at the molecular level.

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