QSAR Studies on Substituted Bis-(acridine-4-carboxamides) as Potent DNA Intercalators

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A series of bis-(acridine-4-carboxamide) analogues possessing significant cytotoxicity against P_{388} leukemia cell cultures, which were designed as bis-DNA intercalating agents, was subjected to the quantitative structure activity relationship analysis. Quantitative structure activity relationship studies by Fujita-Ban model gave excellent correlations (correlation coefficient, r=0.997) for the 24 compounds included in the final regression analysis. It was observed that substitutions on position 5 favor the activity while any group on position 6 decreases the activity strongly. These results will be useful in designing 'lead' compounds to be useful as bis-DNA intercalators for the therapy of leukemia.

Effective treatment of cancer results from destruction of the cancer cells, which is directly accompanied by cytotoxicity of a drug against proliferating cells. Most of the clinically available anticancer drugs interfere with DNA function to exert their cytotoxicity. DNA intercalators represent an important class of anticancer drugs[1]. It is reported that a direct correlation exists between cytotoxic potency of a drug and their strength of reversible DNA binding potential[2]. In the past decade, bis-intercalators have emerged as a novel class of potent anticancer drugs. These agents possess better DNA binding potential than the classical monomeric intercalators[3]. They are capable of intercalating both the strands in a double helical DNA. Several dimeric compounds have been developed in the recent years as bis-intercalator[3-5] and a few of those are in clinical trails[5].

Recently, a series of substituted N,N'-bis-[(substituted acridine-4-carboxamido) propyl] methylamines linked by $(CH_2)_3N(CH_3)(CH_2)_3$ chain was reported to possess significant *in vitro* cytotoxicity against P_{388} (murine leukemia) cell lines (Table 1)[6]. As a part of our rational drug design program on novel antitumor agents, this theoretical study is aimed at determining the quantitative relationships between various substitutions on bis-(acridine-4-carboxamides) and their antileukemic activity. In the present investigation, the quantitative structure activity relationship (QSAR) study was performed by Fujita-Ban model[7] as the data set was found to be suitable for the purpose.

Fujita-Ban model is best represented by log 1/C=Σai.xi+μ..1, where, C is the concentration of test compound, xi is group contribution of i^{th} substituents, ai is coefficient of xi at i^{th} position (1 if substituted/0 if no substituents are present, i.e. for H) and μ is log 1/C of the parent nucleus i.e. unsubstituted compound. Alternatively, Eqn. 1 is represented by log BA=Σai.xi+μ ..2, where, BA is biological activity and μ represents log BA calculated for the parent compound.

Using Eqn. 1, 23 simultaneous linear equations (Eqns. 3-25) were constructed to explore the relationship between the structures of title compounds with antileukemic activity. Representative sample Eqns are as follows: $Z5_1+Z6_1+Z7_1+\mu$=-1.3618..3, $Z5_2+Z6_1+Z7_1+\mu$ =-2.2306..4 and $Z5_1+Z6_1+Z7_{11}+\mu$ =-2.9867... 25.

All these simultaneous linear Eqns were subjected for multiple linear regressions to determine contribution of vari-

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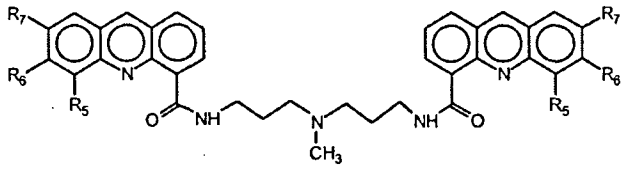
TABLE 1: ANTILEUKEMIC ACTIVITY OF SUBSTITUTED BIS-(ACRIDINE-4-CARBOXAMIDES) AGAINST P_{388} CELL CULTURE.



Compound	Substitutions			IC_{50} (nmol)[a]	Log $1/IC_{50}$
	R_5	R_6	R_7		
1	H	H	H	130	-2.1140
2	Me	H	H	23	-1.3617
3	Et	H	H	170	-2.2304
4	Ph	H	H	1230	-3.0900
5	OMe	H	H	430	-2.6345
6	F	H	H	16	-1.2041
7	Cl	H	H	46	-1.6627
8	Br	H	H	15	-1.1761
9	CF_3	H	H	240	-2.3802
10	NMe_2	H	H	2130	-3.3283
11	H	Me	H	345	-2.5378
12	H	OMe	H	76	-1.8808
13	H	F	H	24	-1.3802
14	H	Cl	H	66	-1.8195
15	H	Br	H	49	-1.6902
16	H	NO_2	H	760	-2.8808
17	H	H	Me	270	-2.4314
18	H	H	Et	1080	-3.0334
19	H	H	i-Pr	1450	-3.1614
20	H	H	Ph	710	-2.8512
21	H	H	OMe	132	-2.1206
22	H	H	F	140	-2.1461
23	H	H	Cl	170	-2.2304
24	H	H	Br	225	-2.3522
25	H	H	NMe_2	970	-2.9868

a denotes the concentration required to inhibit 50% growth in cell culture.

ous substituents and parent moiety towards the activity. Multiple linear regressions and other statistical analysis were performed using Sci-QSAR software (Tripos Inc., USA). Acceptability of regression equation was judged by examining the significance of regression constants by t test, correlation coefficient (r) and squared correlation coefficient (r^2). A compound was considered outlier when residual (absolute differences between the observed and calculated values) exceeded twice the standard error of estimate of the equation. Regression constants were considered at 95% confidence intervals[8,9].

When all the compounds were included in the matrix, a regression Eqn. 26 was obtained which was statistically not significant. $Log1/IC_{50}=0.712(\pm0.511)[Br]_{R5}-0.0168(\pm0.357)[OMe]_{R7}-5.755(\pm1.612)[NO_2]_{R6}+3.718(\pm 2.25)$, n=25, r=0.721, r^2=0.5187, F=16.03..26.

By excluding the outlier compound (10), which possessed low cytotoxicity, QSAR of data set for the 24 compounds having substitutions on positions 5, 6 and 7 yielded the best Eqn. 27, which is $Log1/IC_{50}=0.938(\pm0.254)[Br]_{R5}-0.0065(\pm0.168)[OMe]_{R7}- 6.95(\pm0.562)\ [NO_2]_{R6}-2.114\ (\pm 0.028)$, n=24, r=0.997, r^2=0.995 and F=37.34 ...27.

Using this Eqn. the cytotoxicity of 24 compounds was calculated. Results of the analysis and calculated antileukemic activity are presented in Table 2. Fujita-Ban analysis of the data set could not give satisfactory correlations with all of the compounds taken together. Hence, the regressions were repeated by excluding outlier compound (10) having very low activity. This resulted in excellent correlations (r >0.8) in each set of substituents at positions (5,7), (6,7) and (5,6) taken together. By excluding compound 10, the regression analysis yielded the best equation (27) with high statistical acceptance. Using this equation, activities of 24 compounds were calculated, which were in accordance to the observed activities showing minimum residual activity (Table 2). The results clearly showed that a substitution on position 6 or 7 has a negative effect of varying degree on activity, which is mostly at position 6. A positive effect is observed with Br, F and CH_3 substitutions on position 5. It could also be observed that increase in the bulk or size of a group (Et, i-Pr, Ph) decreased the activity; whereas presence of smaller groups favored activity. In this series, compound having 5-Br substitution was the most potent. Thus, 5-bromo-7-methoxy analogue can serve as a lead in improving DNA binding potential of this series of bis-(acridine-4-carboxamide) derivatives.

TABLE 2: CALCULATED ACTIVITY AND QSAR DATA OF SUBSTITUTED. BIS-(ACRIDINE-4-CARBOXAMIDE) AGAINST P_{388} CELLS.

Compound	Log $1/IC_{50}$ Observed	Log $1/IC_{50}$ Calculated[a]	Residual Activity	Group Contribution
1	-2.1140	-2.1140	0.0	-
2	-1.3617	-1.4825	-0.1208	0.7522
3	-2.2304	-2.2301	0.0003	-0.1166
4	-3.0900	-3.0212	0.0688	-0.9759
5	-2.6345	-2.6355	-0.0010	-0.5205
6	-1.2041	-1.2059	-0.0018	0.9099
7	-1.6627	-1.6832	-0.0205	0.4513
8	-1.1761	-1.1752	0.0009	0.9380
9	-2.3802	-2.3781	0.0021	-0.2662
11	-2.5378	-2.5395	-0.0017	-6.8330
12	-1.8808	-1.8911	-0.0103	-5.6127
13	-1.3802	-1.3606	0.0196	-6.4815
14	-1.8195	-1.8208	-0.0013	-7.3408
15	-1.6902	-1.6915	-0.0013	-6.8854
16	-2.8808	-2.8795	0.0013	-5.4550
17	-2.4314	-2.4332	-0.0018	-0.3173
18	-3.0334	-3.0336	-0.0002	-0.9194
19	-3.1614	-3.1810	-0.0196	-1.0470
20	-3.2455	-3.5548	-0.3093	-1.1315
21	-2.1206	-2.1216	-0.0010	-0.0065
22	-2.1461	-2.1485	-0.0024	-0.0321
23	-2.2304	-2.2300	0.0004	-0.1164
24	-2.3522	-2.3609	-0.0087	-0.2381
25	-2.9868	-3.5156	-0.5429	-0.8510

a denotes that the values were calculated using equation 27; compound 10 was not included in the regression equation. Mean Residual=0.0116; SE=0.0219; % Variance<20%

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Intestinal Permeation Mediated Absorption Interactions between Atenolol and Furosemide

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Peroral multi-drug administration is often associated with severe drug-drug interactions. These interactions can occur either in the gut lumen, at absorptive site or after absorption. Present work was undertaken to study the interaction between atenolol and furosemide at the absorption site. Alteration in the intestinal permeability was studied using rat single-pass intestinal perfusion technique, to assess the changes on administration of single and combination drugs. Permeability coefficient (P_{eff}) of atenolol reduced to a statistically significant level ($P<0.05$) when co-perfused with furosemide, with P_{eff} values (x10^{-4} cm/sec) of 0.0686±0.0433 and 0.0154±0.0326 for drug perfused individually and in combination, respectively, indicating the possibility of drug-drug interaction occurring at the absorption site.

Oral route of drug administration is the most preferred one, both by physicians and patients, due to ease of delivery and better patient compliance[1], and the treatment may be accomplished using single drug or a combination of two or more drugs. The latter may be either fixed dose combinations or concomitant administration of individual dosage units. This multi-drug administration can be a potential cause for a variety of drug-drug interactions, the consequence of which can be significant, especially in the case of narrow therapeutic index drugs. Most of the drug interactions have been studied at the pharmacokinetic and pharmacodynamic levels[2-4]. However, drug-drug interactions may also occur in the (i) pre-absorption stage due to changes in stability, dissolution characteristics, and/or (ii) absorption stage due to altered intestinal permeability induced by biochemical or physiological changes[4], leading to altered bioavailability.

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The present work aims to identify absorption interactions between atenolol (ATN) and furosemide (FRD) using *in situ* rat single-pass intestinal perfusion (SPIP) model. The combination of ATN, a β blocker, and FRD, a diuretic, was selected as it is one of the most widely prescribed combinations in cardiovascular disorders. Both these drugs are poorly permeable from gastrointestinal tract[5], and hence, their oral absorption would be more critically affected by any changes in the intestinal permeability. The permeability coefficient (P_{eff}) values of ATN and FRD, when perfused alone and in combination, were calculated after suitable correction for water flux, using gravimetric method after density correction for exiting intestinal perfusate.