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RESEARCH ARTICLE

Comparative 2D QSAR Studies of 1,8-Naphthyridine against Tumor Cell Lines

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ABSTRACT

In a persevering effort to develop better anticancer drugs, a quantitative structure activity relationship analysis using a set of 2-D descriptors was performed on a series of 1,8-naphthyridine derivatives acting by the inhibition of tubulin polymerization. QSAR models that were derived from the study were found to be statistically significant with a good predicting ability. The results obtained from the study justify the use of 2-D descriptors for exploring the requirements of binding of 1,8-naphthyridines to the heterodimer, tubulin. Attempt has been made to explore the structural and/or physicochemical requirements of the compounds, responsible for the action against tumor cells. The physicochemical descriptors and indicator variables were correlated with the biological activity

Keywords: QSAR, tubulin, Chemotherapy, antitumor, cytotoxic, naphthyridine

Introduction

The search for a highly effective anticancer agent still continues to be one of the major challenges for the scientific community worldwide.

Cancer stands the most frequent cause of deaths reported worldwide, next only to cardiovascular disorders. Despite of continual research on anticancer agents, the disease is yet to be cured. The effectiveness of anticancer chemotherapeutic agents is mainly limited due to lack of selectivity of these agents, the acquired resistance against the existing agents and the metastatic nature of the tumor cells[1]. In recent decade, with the help of tumor markers and various screening programs, detection of cancer has been possible at the early stages of the disease.

Response to chemotherapy in cancer is greatly dependent on the performance status of the patient and the disease stage[2]. Tumor response is conventionally the indication of an effective chemotherapy, but occurs late during treatment and may be obscured by diagnostic uncertainties. The primary goal of treatment should therefore be the stabilization the disease.

The research on anticancer agents has been oriented mainly to the cell cycle specific agents and more recently towards certain enzymes. The microtubule system of eukaryotic cells is an attractive target for the development of compounds useful in anticancer chemotherapeutics.[3, 4, 5]. Microtubules show highly dynamic instability and play an important role in mitosis. The cytotoxic effects of the agents interfering with the mitotic assembly functioning are due to the inhibition of polymerization of the heterodimer tubulin present in the microtubule of the mitotic spindle. Several analogues of 1,8-naphthyridine, identified as inhibitors of tubulin polymerization and having being exhibited significant anticancer activity against a number of human cancer cell lines 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 1,8-naphthyridine derivatives reported by Chen, K *et al*[6] has been performed for the prediction of the anticancer activity.

Experimental

QSAR with physicochemical descriptors and indicator variables.

In continuation with our previous work[7], a set of 23 compounds and their experimentally obtained 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 tumor cell lines. The congeneric series presented six regions of

structural variations: R_5, R_6, R_7, R'_2, R'_3 and R'_4 (Table 1). The variations at all the six regions are represented by different physicochemical descriptors (Table 3) and indicator variables (Table 4). The hydrophobic, electronic and steric constant values of the substituents were concluded from literature[8].

The efficacy data (Table2) were then subjected to multiple regression analysis with different physicochemical descriptors and indicator variables to generate QSAR equations for individual cancer cell lines and *in vitro* inhibition of tubulin polymerization.

The knowledge of the important parameters contributing to the efficacy against various cancer cell lines can be used to design new anti-tumor agents of the series.

Out of the 23 compounds in the series, 23 compounds for ITP, 19 for HL-60 (TB), NCI-H460, HCT-116, U-251, SK-MEL-5, OVCAR-3, SF-295, 0786, PC-3 and 15 for MDA-N cell lines are taken for the QSAR analysis. The remaining compounds in each cell line are rejected due to lack of discrete biological activity values.

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 statistical viewpoint, the ratio of the number of sample (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 each cell line with the physicochemical descriptors and indicator variables. The statistical quality of the equations[9] 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 (Table 6). 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.

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

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

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

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

n = no. of compounds used for cross-validation

Y_i = experimental value of the physicochemical property for the i^{th} sample

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

Results and Discussions

The relative potency of various substituted naphthyridine derivatives has been determined by inhibiting the tubulin polymerization of various human cancer cell lines and from this data the following QSAR models have been derived. The QSAR model that best defines the relative effective concentration of naphthyridine derivatives causing 50% inhibition of tubulin polymerization is shown in table 5.

The model for activity against tubulin polymerization revealed that the electronic properties, Hammett's substituent constant, along with the substitutions on position R_6 and R'_3 of the naphthyridine nucleus was important for the activity of the compound as tubulin polymerization inhibitor. On the other hand it was also justified from this model that field effect of substituent negatively correlated with the activity which suggested that the substituent that favor electron release to the nucleus are beneficial for the activity. The selected QSAR model was found to be statistically significant with and F value of 23.491 and an explained variance of 83.9%.

QSAR models were also constructed to define the relationship of the physicochemical descriptors and the indicator variables with the activity of the naphthyridine derivatives against various human cancer cell lines. These models along with their corresponding statistical parameters are depicted in table 5.

The model generated for activity against HL-60 (TB) revealed that the substitution on position R_3' with lesser field effect characteristics and higher value of the Hammett's substituent constant were required for activity. The generated equation was found to be significant with explained variance of 70%.

The same descriptors were found to be important for the 1,8-naphthyridine derivatives to be active against the cell lines NCIH 460, HCT 116, SF 295, U-251, SK-MEL-5, OVCAR-3, 7860, PC-3 and MDA-N with explained variance of 74, 76, 71, 75, 70, 76.6, 77.8, 72 and 41% respectively. All the generated QSAR models were found to be statistically significant with high F_{test} values.

To summarize, these models justified the inference drawn from the model for inhibition of tubulin polymerization. In addition, it was revealed from these models that the presence or absence of substituent at position R_6 of the naphthyridine nucleus was not so important for the anti cancer activity of the compounds but may play a vital role in the binding of the compounds to the tubulin microtubule.

It was concluded from the results of the work that for the substituted-2-Aryl-1, 8-naphthyridine-4(1*H*)-ones to be active as anti cancer agents acting by the inhibition of tubulin polymerization

in the cell, the presence of electron releasing substituent at position R_6 and R'_3 of the ring was important.

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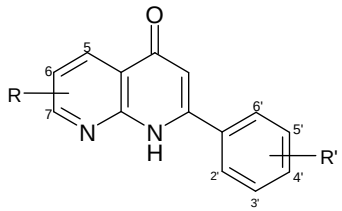

Figure 1: Parent structure of substituted-2-Aryl-1, 8-naphthyridine-4(1*H*)-ones

Table 1: Regions of structural variation in the congeneric series with substituents

Compound	R_5	R_6	R_7	R'_2	R'_3	R'_4
1	CH3	H	H	OCH3	H	H
2	H	CH3	H	H	H	H
3	H	H	H	H	OCH3	H
4	CH3	H	H	H	OCH3	H
5	H	CH3	H	H	OCH3	H
6	H	H	CH3	H	OCH3	H
7	CH3	H	CH3	H	OCH3	H
8	H	Cl	H	H	OCH3	H
9	H	Br	H	H	OCH3	H
10	CH3	H	H	H	H	OCH3
11	H	CH3	H	H	H	OCH3
12	CH3	H	H	H	H	F
13	H	CH3	H	H	H	F
14	H	Cl	H	H	H	F
15	H	H	H	H	H	Cl
16	CH3	H	H	H	H	Cl
17	H	CH3	H	H	H	Cl
18	H	H	CH3	H	H	Cl
19	CH3	H	CH3	H	H	Cl
20	CH3	H	H	H	H	CH3
21	H	CH3	H	H	H	CH3
22	H	H	CH3	H	H	CH3
23	CH3	H	CH3	H	H	CH3

Table 2: Biological activity data of the series of 1,8-naphthyridine derivatives

S. No.	-LOG IC_{50}										
	ITP	HL60(TB)	NCIH460	HCT116	SF295	U251	SKMEL5	OVCAR3	786-O	PC3	MDAN
1	-1.301	4.68	4.62	5.12	4.63	4.45	4.65	4.89	4.22	4.68	5.08
2	-1.2552	5.02	4.49	4.37	4.51	4.56	4.69	4.71	4.38	4.57	5.35
3	0.0177	7.79	7.04	7.44	7.28	7.26	7.27	7.26	7.21	7.03	
4	0.2076	7.89	7.32	7.35	7.54	7.23	7.65	7.59	7.29	7.58	
5	0.0969	7.72	7.36	7.65	7.43	7.35	7.59	7.38	7.43	7.37	
6	0.1249	7.74	7.36	7.33	7.52	7.27	7.47	7.65	7.27	7.48	7.96
7	0.0555	6.76	6.35	6.39	6.38	6.77	6.54	6.6	6.36	6.51	
8	0.1366	7.57	7.2	6.92	7.22	6.7	6.9	6.31	6.37	6.86	7.5
9	-0.176	4.89	4.58	4.75	4.19	4.27	4.46	4.52	4.25	4.5	4.68
10	-0.9444	5.36	5.21	5.19	4.71	5.2	5.41	4.86	4.6	4.74	5.68
11	-0.8864	5.64	5.39	5.38	5.28	5.38	5.43	5.53	5.29	5.27	5.77
12	-1.2552	5.32	4.49	4.79	4.1	4.4	4.79	4.25	4.47	4.29	5.45

13	-1.301	5.62	4.47	4.62	4.53	4.6	4.66	4.65	4.49	4.59	5.52
14	-1.2041	5.41	4.52	4.77	4.67	4.53	5.18	4.76	4.49	4.66	5.53
15	-1.3424	4.78	4.42	4.49	4.39	4.4	4.54	4.63	4.47	4.32	5.08
16	-0.6812	5.75	5.43	5.43	5.27	5.34	5.46	5.5	5.4	5.54	5.88
17	-0.301	5.65	5.41	5.55	5.62	5.52	5.66	5.65	5.2	5.94	6.25
18	-1.0414	5.53	5.35	5.48	5.34	5.32	5.36	5.59	5.2	5.78	6
19	-1.5051	4.6	4.65	4.83	4.79	4.52	4.85	4.74	4.67	5.2	5.44
20	-0.4623	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
21	-0.3802	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
22	-0.6902	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
23	-0.9912	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D

N.D. – not determined

HL-60 (TB), leukemia cell line; NCI-H460, non small cell lung cancer cell line; HCT-116, colon cancer cell line; SF-295, U251, CNS cancer cell lines; SK-MEL-5, melanoma cell line; OVCAR-3, ovarian cancer cell line; 786-O, renal cancer cell line; PC-3, prostate cancer cell line, MDA-N, breast cancer cell line.

Table 3: Physicochemical descriptor values of the substituents

S. No.	Pi	MR	F	R	$\sigma_{m/o}$	σ_p
1	0.54	1	16.61	0.22	-0.64	0.05
2	0.54	1	16.61	0.22	-0.64	0.05
3	-0.02	1	13.02	0.26	-0.51	0.12
4	0.54	1	17.64	0.22	-0.64	0.05
5	0.54	1	17.64	0.22	-0.64	0.05
6	0.54	1	17.64	0.22	-0.64	0.05
7	1.1	1	22.26	0.18	-0.77	-0.02
8	0.69	1	18.02	0.67	-0.66	0.49
9	0.84	1	20.87	0.7	-0.68	0.51
10	0.54	1	17.64	0.22	-0.64	0.05
11	0.54	1	17.64	0.22	-0.64	0.05
12	0.7	0	10.69	0.39	-0.47	0.27
13	0.7	0	10.69	0.39	-0.47	0.27
14	0.85	0	6.95	0.84	-0.49	0.71
15	0.71	0	10.15	0.41	-0.15	0.37
16	1.27	0	15.8	0.37	-0.28	0.3
17	1.27	0	15.8	0.37	-0.28	0.3
18	1.27	0	15.8	0.37	-0.28	0.3
19	1.83	0	14.77	0.33	-0.43	0.23
20	1.12	0	15.42	-0.08	-0.26	-0.14
21	1.12	0	15.42	-0.08	-0.26	-0.14
22	1.12	0	15.42	-0.08	-0.26	-0.14
23	1.68	0	20.04	-0.12	-0.39	-0.21

Pi – hydrophobicity, 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 4: Indicator variables representing substituents at different positions

S. No.	R_5	R_6	R_7	R'_2	R'_3	R'_4
1	1	0	0	1	0	0
2	0	1	0	1	0	0
3	0	0	0	0	1	0
4	1	0	0	0	1	0
5	0	1	0	0	1	0
6	0	0	1	0	1	0
7	1	0	1	0	1	0
8	0	1	0	0	1	0
9	0	1	0	0	1	0
10	1	0	0	0	0	1
11	0	1	0	0	0	1
12	1	0	0	0	0	1
13	0	1	0	0	0	1
14	0	1	0	0	0	1
15	0	0	0	0	0	1
16	1	0	0	0	0	1
17	0	1	0	0	0	1
18	0	0	1	0	0	1
19	1	0	1	0	0	1
20	1	0	0	0	0	1
21	0	1	0	0	0	1
22	0	0	1	0	0	1
23	1	0	1	0	0	1

1-indicates the presence of substituent at particular position

0-indicates the absence of substituent at particular position

Table 5: QSAR models and statistical parameters for various human cancer cell lines.

Human cancer cell line	QSAR MODEL	Statistical prameters			
		r	r^2	F-test	SD
IC_{50}	-log IC50 = -1.569(±0.655)f + 0.915(±0.400)σ_p + 0.256(±0.220)R_6 + 1.317(±1.067)R'_3 – 0.509	0.916	0.839	23.491	0.257
HL60 (TB)	-log IC50 = -2.535(0.404)f+1.227(±0.277)σ_p + 2.147 (±0.655) R'_3 + 6.341	0.839	0.703	11.851	0.704
NCIH 460	-log IC50 = -6..582(1.067)f+4.784(±0.828)σ_p + 2.132(±0.918) R'_3 + 6.081	0.864	0.746	14.714	0.635
HCT 116	-log IC50 = -3.215(0.534)f + 1.406(±0.333)σ_p + 2.015(±0.924)R'_3 + 6.330	0.872	0.761	15.895	0.603
SF 295	-log IC50 = -3.696(0.550)f + 1.784(±0.379)σ_p + 2.313(±0.913)R'_3 + 6.367	0.846	0.716	12.575	0.734
U-251	-log IC50 = -3.677(0.589)f + 1.426(±0.326)σ_p + 2.105(±0.895)R'_3 + 6.350	0.871	0.758	15.665	0.628
SK-MEL-5	-log IC50 = -3.036(0.4974)f + 1.261(±0.295)σ_p + 2.019(±0.878)R'_3 + 6.303	0.837	0.701	11.707	0.683
OVCAR-3	-log IC50 = -4.128(0.671)f + 1.765(±0.410)σ_p + 2.109(±0.911)R'_3 + 6.681	0.875	0.766	16.353	0.608
7860	-log IC50 = -4.211(0.671)f + 2.033(±0.462)σ_p + 2.243(±0.949)R'_3 + 6.502	0.882	0.778	17.490	0.605
PC-3	-log IC50 = -3.985(0.637)f + 2.016(±0.460)σ_p + 2.181(±0.925)R'_3 + 6.643	0.849	0.721	12.949	0.675
MDA-N	-log IC50 = -2.687(0.595)f + 1.215(±0.355)σ_p + 1.647(±0.780)R'_3 + 6.701	0.638	0.408	2.522	0.760

Table 6: PRESS statistics of the QSAR equations obtained.

S. No.	Parameters	ITP	HL60 (TB)	NCIH 460	HCT116	SF295	U251	SKMEL 5	OVCAR 3	7860	PC-3
2.	q^2	0.812	0.7075	0.6996	0.7012	0.6997	0.7201	0.7221	0.7554	0.6966	0.6064
3.	S_{PRESS}	0.296	0.734	0.499	0.501	0.789	0.564	0.601	0.665	0.606	0.796
4.	SDEP	0.319	0.608	0.667	0.650	0.561	0.701	0.765	0.741	0.795	0.790

q^2 – squared correlation coefficient of prediction

S_{press} – standard deviation of prediction

SDEP – standard error of prediction.