CODEN IEJMAT *Internet Electronic Journal of Molecular Design* **2005**, *4*, 793–802 ISSN 1538–6414
BioChem Press http://www.biochempress.com

Inter*net* Electronic Journal of Molecular Design

November 2005, Volume 4, Number 11, Pages 793–802

Editor: Ovidiu Ivanciuc

Special issue dedicated to Professor Danail Bonchev on the occasion of the 65th birthday

QSAR Analysis of Some Cytotoxic Thiadiazinoacridines

Garvita Choudhary,[1] C. Karthikeyan,[1] N. S. Hari Narayana Moorthy,[2] S. K. Sharma,[3] and Piyush Trivedi [1]

[1] Drug Design Laboratory, Department of Pharmacy, SGSITS, Indore, India
[2] School of Pharmaceutical Sciences, RGPV, Bhopal, India
[3] Maharaja Surajmal Institute of Pharmacy, New Delhi, India

Received: February 23, 2005; Revised: May 12, 2005; Accepted: June 28, 2005; Published: November 30, 2005

Citation of the article:
G. Choudhary, C. Karthikeyan, N. S. H. N. Moorthy, S. K. Sharma, and P. Trivedi, QSAR Analysis of Some Cytotoxic Thiadiazinoacridines, *Internet Electron. J. Mol. Des.* **2005**, *4*, 793–802, http://www.biochempress.com.

Inter*net* Electronic Journal of Molecular Design

BioChem Press
http://www.biochempress.com

QSAR Analysis of Some Cytotoxic Thiadiazinoacridines

Garvita Choudhary,[1] C. Karthikeyan,[1] N. S. Hari Narayana Moorthy,[2] S. K. Sharma,[3] and Piyush Trivedi [1,*]

[1] Drug Design Laboratory, Department of Pharmacy, SGSITS, Indore, India
[2] School of Pharmaceutical Sciences, RGPV, Bhopal, India
[3] Maharaja Surajmal Institute of Pharmacy, New Delhi, India

Received: February 23, 2005; Revised: May 12, 2005; Accepted: June 28, 2005; Published: November 30, 2005

Internet Electron. J. Mol. Des. **2005**, *4* (11), 793–802

Abstract

Motivation. Cancer is one of the major causes of death worldwide. DNA intercalators are important class of therapeutic agents used in chemotherapy of cancer. The effectiveness of current cancer chemotherpeutic agents is seriously limited due to development of intrinsic resistance and toxicity problems. Acridine derivatives have received much attention in the present scenario owing to their ability to intercalate DNA and inhibit the enzyme Topoisomerase II. As a part of ongoing efforts to develop new anticancer agents, a series of acridine derivatives fused with heterocyclic ring as DNA intercalating agents were subjected to quantitative structure–activity relationships analysis.

Method. QSAR analysis was performed on the series employing Hansch approach. Various physicochemical and steric parameters were calculated for the molecules reported in the series using Chem 3D package of molecular modeling Software Chemoffice 2001. QSAR models were generated employing Sequential multiple regression method using in–house statistical program VALSTAT.

Results. Statistically significant models with *R*–values 0.89 and 0.81 were obtained. Models were validated using leave one out and bootstrapping methods. Results obtained show that dipole–dipole energy, Van der Waals energy, Connolly accessible area, ovality and stretch bend energy are contributing to biological activity. among these, dipole–dipole energy, Van der Waals energy, stretch bend energy plays an important role as positive contribution is seen in the models.

Conclusions. Findings of present study reveal π–π stacking interactions between the planar tricyclic aromatic rings of the acridine moiety and the nucleotide bases of the DNA and necessity of less bulky substituents. Substituents those increase the flexibility of thiadiazinoacridines will be conducive to the cytotoxic activity. Also the orientation of atoms in the substituents (R) on the nitrogen atoms at the positions 1 and 3 influences the activity of thiadiazinoacridines significantly.

Keywords. Thiadiazinoacridines; DNA intercalation; QSAR; quantitative structure–property relationships.

Dedicated on the occasion of the 65th birthday to Danail Bonchev.

* Correspondence author; phone: +91–0731–2368582; fax: +91–0731–2368582; E–mail: drtrivedislab@yahoo.co.in.

1 INTRODUCTION

Among the first agent used to treat cancer were member of mustard family, the first clinical trial with nitrogen mustard began in 1942, initiating the era of cancer chemotherapy. During the next 20 years many cancer chemotherapeutics were synthesized and tested [1]. The effectiveness of cancer chemotherapy is mostly limited due to two major problems which are still to be overcome, the lack of selectivity of anticancer agents and the occurrence of intrinsic or acquired resistance leading to side effects and sometimes failure of treatment [2]. Drugs used for treatment act over various enzymes, compete with metabolites or act on a specific growth phase of the cell cycle. DNA intercalation represents an important category of anticancer agents. Intercalating agents wedge between bases along the DNA, intercalated drug molecules affect the structure of DNA preventing polymerase and other DNA binding protein needed for functioning properly. The result is prevention of DNA synthesis; inhibition of transcription and induction of mutation [1]. DNA intercalation also involves inhibition of cell growth. Acridine derivatives are important member of such kind of inhibitors. The acridine derivative fused with 5 or 6 membered heterocyclic rings yields polycyclic derivatives, which play, an important role in DNA intercalation. They are structurally characterized by the presence of a planar or semi planar chromophore portion, possibly capable of intercalation with DNA. A noticeable example in phase II clinical trial is 5–nitropyrazo–le[3,4,5–*kl*]acridines [3,4]. A number of polycyclic derivatives with antitumour properties were studied including the pyrimido[5,6,1–*de*]acridines [5,6], pyrazolo[3,4,5–*kl*]acridine–5–carboxamides [7], pyrazolo[3,4,5–*mn*]pyrimido[5,6,1–*de*]acridines [8]. Considering the relevance of acridines as anticancer agents, a QSAR analysis was performed on a series of thiadiazinoacridines reported by Antonini *et. al*. [9]. A Quantitative structure–activity relationship (QSAR) enables the investigators to establish a reliable quantitative structure–activity and structure–property relationships to derive an *in silico* QSAR model to predict the activity of novel molecules prior to their synthesis. The overall process of QSAR model development can be divided into three stages namely, the data preparation, data analysis, and model validation, representing a standard practice of any QSAR modeling.

In this research, an attempt has been made to describe the Quantitative structure–activity relationship (QSAR) analysis of thiadiazinoacridines to study and deduce a correlation between structure and antiproliferative activity of these derivatives.

2 MATERIALS AND METHODS

A training set of 21 thiadiazinoacridines exhibiting potent antiproliferative activity was taken from the reported work of Antonini *et al*. [9] The activity data have been given as IC_{50} values, where IC_{50} refers to the experimentally determined molar concentration of the thiadiazinoacridines required to inhibit growth of HT29 human colon adenocarcinoma cell lines by 50%.

The biological activity values [IC_{50} (μM)] reported in the literature were converted to molar units and then further to –log scale and subsequently used as the response variable for the QSAR analysis. The –log values of IC_{50} along with the structure of compounds in the series is presented in Table 1.

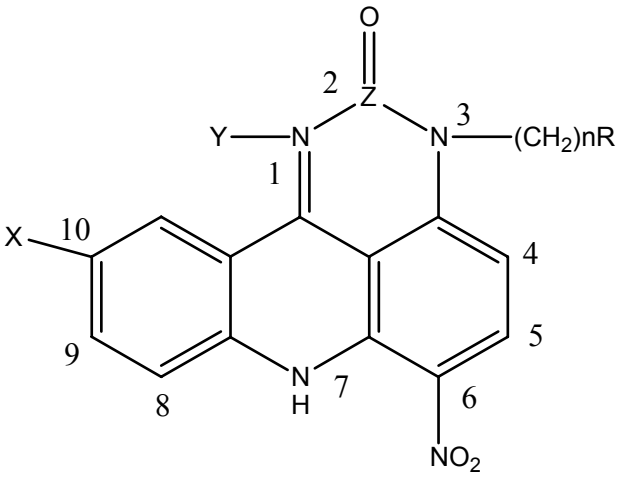

Figure 1. General structure of thiadiazinoacridines.

Table 1. Biological activity data and structures of the compounds in the series

Compd.	X	Y	n	R	Z	IC_{50} (μm)	–log IC_{50}
1	OCH_3	H	2	$N(CH_3)_2$	–S–	0.087	7.06
2	OCH_3	H	2	$N(CH_3)_2$	–CH–	0.62	6.20
3	OCH_3	H	3	$N(CH_3)_2$	–S–	0.60	6.22
4	OCH_3	H	3	$N(CH_3)_2$	–CH–	0.26	6.58
5	OCH_3	H	2	$N(CH_2CH_3)_2$	–S–	0.13	6.88
6	OCH_3	H	2	$N(CH_2CH_3)_2$	–CH–	0.29	6.53
7	H	H	2	$N(CH_3)_2$	–S–	0.027	7.56
8	H	H	2	$N(CH_3)_2$	–CH–	0.76	6.11
9	H	H	3	$N(CH_3)_2$	–S–	0.56	6.25
10	H	H	3	$N(CH_3)_2$	–CH–	0.77	6.11
11	H	H	2	$N(CH_2CH_3)_2$	–S–	0.25	6.60
12	H	H	2	$N(CH_2CH_3)_2$	–CH–	0.86	6.06
13	H	H	2	Piperidino	–S–	0.28	6.55
14	CH_3	H	2	$N(CH_3)_2$	–S–	0.24	6.62
15	CH_3	H	3	$N(CH_3)_2$	–S–	0.50	6.30
16	CH_3	H	2	$N(CH_2CH_3)_2$	–S–	0.64	6.19
17	CH_3	H	2	Piperidino	–S–	0.28	6.55
18	OCH_3	$(CH_3)_2N(CH_3)_2$	2	$N(CH_3)_2$	–S–	0.033	7.48
19	OCH_3	$(CH_3)_2N(CH_3)_2$	2	$N(CH_3)_2$	–CH–	5.6	5.25
20	OCH_3	$(CH_3)_2N(CH_3)_3$	3	$N(CH_3)_2$	–S–	2.1	5.67
21	OCH_3	$(CH_3)_2N(CH_3)_3$	3	$N(CH_3)_2$	–CH–	4.8	5.31

2.1 Computer Software

All the computations in the present study were performed on PIV workstation. The molecular structures of the training set were sketched using ChemDraw Ultra module of CS ChemOffice 2001 molecular modeling software ver. 6.0, supplied by Cambridge Software Company. The

sketched structures were exported to Chem3D module in order to create its 3–D model. Each model was "cleaned up" and energy minimization was performed using Allinger's MM2 force field by fixing Root Mean Square Gradient (RMS) to 0.1 kcal/mol Å. Further geometry optimization was done using semiemperical AM1 (Austin Model) Hamiltonian method, closed shell restricted wave function available in the MOPAC module until the RMS value becomes smaller than 0.001 kcal/mol Å.

Table 2. Descriptors calculated for the QSAR study

S. No.	Descriptor	Type
1	Heat of Formation (HF)	Thermodynamic
2	Boiling Point (BP)	Thermodynamic
3	Critical Pressure (CP)	Thermodynamic
4	Critical Temperature (CT)	Thermodynamic
5	Critical Volume (CV)	Thermodynamic
7	Henry's Law Constant (HLC)	Thermodynamic
8	Ideal Gas Thermal Capacity (IGTC)	Thermodynamic
9	LogP	Thermodynamic
10	Melting Point (MP)	Thermodynamic
11	Molar Refractivity (MR)	Thermodynamic
12	Standard Gibbs Free Energy (SGFE)	Thermodynamic
13	Connolly Accessible Area (CAA)	Steric
14	Connolly Molecular Area (CMA)	Steric
15	Connolly Solvent–Excluded Volume (CSEV)	Steric
16	Ovality (OVA)	Steric
17	Principal Moment of Inertia – X (PMI–X)	Steric
18	Principal Moment of Inertia – Y (PMI–Y)	Steric
19	Principal Moment of Inertia – Z (PMI–Z)	Steric
20	Dipole Moment (D)	Electronic
21	Dipole Moment –X Axis (DX)	Electronic
22	Dipole Moment –Y Axis (DY)	Electronic
23	Dipole Moment –Y Axis (DZ)	Electronic
24	Electronic Energy (EE)	Electronic
25	HOMO Energy (HOMO)	Electronic
26	LUMO Energy (LUMO)	Electronic
27	Repulsion Energy (RE)	Electronic
28	Bend Energy (E_b)	Thermodynamic
29	Charge–Charge Energy (CCE)	Thermodynamic
30	Charge–Dipole Energy (CDE)	Thermodynamic
31	Dipole–Dipole Energy (DDE)	Thermodynamic
32	Non–1, 4 VDW Energy (E_v)	Thermodynamic
33	Stretch Energy (SE)	Thermodynamic
34	Stretch–Bend Energy (SBE)	Thermodynamic
35	Torsion Energy (E_t)	Thermodynamic
36	Total Energy (E)	Thermodynamic
37	Van der Waals e 1,4 Energy (VDWE)	Thermodynamic
38	VDW 1,4 Energy (VDWE)	Thermodynamic

The low energy conformers obtained from the aforementioned procedure was used for the calculation of the ChemSAR descriptors. The ChemSAR descriptors include physicochemical, thermodynamic, electronic and spatial descriptors available in the 'Analyze' option of the Chem3D package (Table 2). The descriptors calculated for the present study accounts four important

properties of the molecules: physicochemical, thermodynamic, electronic and steric, as they represent the possible molecular interactions between the receptor and thiadiazinoacridines.

Sequential multiple regression analysis was performed using in–house program VALSTAT [10] in order to generate QSAR models. In this technique, the program search for all permutation and combination sequentially for the given data set and provides best model based on squared correlation coefficient (R^2). The program also computes the correlation coefficient (R), standard deviation (SD), variance and Fischer ratio values (F–values) for each parameter in the QSAR. Each of the statistical parameters mentioned above were used for assessing the statistical significance of a QSAR. Additionally the developed QSAR models were also checked for significance of the regression coefficients in the model and for multicolinearity problem by the calculation of Student's t–test values (t–value) and variance inflation factor values (VIF) respectively using statistical software SYSTAT [11]. VIF values greater than five indicates that information of descriptors can be hidden by correlation of descriptors [12].

The generated QSAR models were validated for predictive ability inside the model (Leave one out method) using VALSTAT. The statistical program which is tailored specifically for QSAR statistics estimates the predictive potential of model by calculating the validation parameters squared cross–correlation coefficient (q^2), standard deviation of sum of square of difference between predicted and observed values (S_{PRESS}) and standard deviation of error of prediction (S_{DEP}).

Table 3. Descriptors contributing to the cytotoxic activity of thiadiazinoacridines.

Comp. No.	CAA	DDE	VDWE	OVA	SBE
1	607.554	–3.620	19.791	1.556	–0.365
2	596.616	–5.212	19.406	1.55	–0.151
3	643.178	–3.575	20.072	1.588	–2.151
4	628.458	–5.208	20.162	1.577	–0.165
5	643.178	–3.575	20.072	1.583	–2.067
6	644.008	–5.187	21.414	1.571	–0.055
7	596.436	–3.308	19.491	1.508	–0.678
8	583.603	–5.025	19.170	1.497	–0.490
9	642.357	–3.674	20.296	1.570	–0.515
10	629.973	–5.476	20.178	1.550	–10.342
11	612.031	–3.310	19.216	1.542	–0.538
12	599.168	–5.036	18.823	1.529	–0.336
13	624.925	–3.834	18.816	1.523	–19.926
14	588.831	–3.389	17.559	1.536	–0.562
15	628.332	–3.772	18.288	1.575	–2.312
16	642.827	–3.694	17.520	1.534	–19.254
17	650.239	–3.921	21.302	1.572	–0.424
18	726.492	–2.516	27.116	1.600	–0.911
19	713.088	–6.469	19.922	1.586	–16.603
20	803.748	–2.542	22.876	1.649	–20.392
21	771.312	–6.164	26.542	1.625	0.197

3 RESULTS AND DISCUSSION

Biological activity data and various physicochemical parameters were taken as dependent and independent variables respectively and correlations were established using sequential multiple regression analysis. The descriptors selected for modeling cytotoxic activity of acridine derivatives are summarized in Table 3.

Among the many correlations generated, two best triparametric models were selected on the basis of statistical significance. The best models obtained are given below along with their statistical measures.

Model 1

$$\begin{gathered} pIC_{50} = 10.583\ (\pm 1.594) - 0.010\ (\pm 0.003)\ CAA + 0.326\ (\pm 0.121)\ DDE \\ + 0.174\ (\pm 0.085)\ VDWE \\ n = 21 \quad R = 0.890 \quad R^2 = 0.793 \quad F_{(3,17)} = 21.795 \quad SD = 0.287 \quad variance = 0.082 \\ DW = 1.762 \quad r^2_{bs} = 0.753 \quad q^2 = 0.637 \quad S_{PRESS} = 0.381 \quad S_{DEP} = 0.343 \quad Chance < 0.01 \end{gathered} \tag{1}$$

Model 2

$$\begin{gathered} pIC_{50} = 16.263\ (\pm 7.531) - 5.31\ (\pm 4.784)\ OVA + 0.337\ (\pm 0.156)\ DDE \\ + 0.029\ (\pm 0.023)\ SBE \\ n=21\ R=0.812\ R^2=0.660\ F_{(3,17)}=11.0385\ SD=0.368\ variance=0.13\ 5 \\ DW = 1.803\ r^2_{bs} =0.652\ q^2=0.424\ S_{PRESS}=0.481\ \ S_{DEP}=0.432 \end{gathered} \tag{2}$$

Model 1 has good correlation between biological activity and parameters as R = 0.89 and explains 79% variance in cytotoxic activity. Low standard deviation of the model demonstrates accuracy of the model. The model showed overall significance level better than 99%, with the $F_{(3,17)}$ = 21.776 against values of 99%significance $F_{(3,17,\alpha)}$ = 5.38. Value of chance is less than 0.01, which shows there is significant relationship between Connolly accessible area (CAA) Van der waals energy (VDWE) Dipole–dipole Energy (DDE), and biological activity. Validation parameters bootstrapping r^2, r^2_{bs} = 0.77 and cross validated r^2 (q^2 = 0.557) reflects the good predictive power of the model.

The descriptors CAA and VDWE were found to be moderately correlated to each other as shown in the correlation matrix (Table 4).

Table 4. Correlation matrix for model 1

	CAA	DDE	VDWE
CAA	1.000		
DDE	0.022	1.000	
VDWE	0.764	0.012	1.000

Although the intercorrelation between the two descriptors is within the acceptable range (< 0.8) [13], absence of any serious multicolinearity problem is further confirmed by the calculation of VIF values for both the descriptors (Table 6), which are less than 5. Table 6 also presents the t statistics

at 95% significance level for the regression coefficients in the Model 1 and Model 2.

Furthermore, the models were tested for autocorrelation problem by evaluation of Durbin Watson Statistics, which is 1.762 and 1.803 for model 1 and 2 respectively. D–W statistics affirm absence of autocorrelation among residuals as their values are between the acceptable ranges from 1.5 to 2.5 [14,15] (Table 6).

Table 5. Correlation matrix for model 2

	OVA	DDE	SBE
OVA	1.000		
DDE	0.056	1.000	
SBE	0.129	0.044	1.000

Table 6. t–Statistics, VI F and D–W statistic values for the descriptors in selected models

Model No.	Constant/Descriptor	t–value	VIF	D–W Statistics
Model 1	Constant	14.058		
	CAA	–5.845	2.403	
	DDE	5.698	1	1.762
	VDWE	4.333	2.403	
Model 2	Constant	4.567		
	OVA	–2.35	1.019	
	DDE	4.592	1.022	1.803
	SBE	2.162	1.043	

Table 7. Observed calculated and predicted activities of mono and disubstituted thiadiazinoacridines

Comp	$pIC_{50\ exp}$	Calculated activities		Predicted activities	
		Model 1	Model 2	Model 1	Model 2
1	7.06	6.85	6.74	6.83	6.71
2	6.20	6.37	6.24	6.42	6.25
3	6.22	6.56	6.53	6.59	6.57
4	6.58	6.19	6.10	6.15	6.04
5	6.88	6.69	6.63	6.67	6.61
6	6.53	6.26	6.14	6.23	6.09
7	7.56	7.01	7.09	6.93	6.97
8	6.11	6.52	6.57	6.59	6.72
9	6.25	6.58	6.64	6.6	6.67
10	6.11	6.09	5.85	6.09	5.81
11	6.60	6.81	6.91	6.83	6.95
12	6.06	6.34	6.40	6.33	6.45
13	6.55	6.44	6.27	6.43	6.12
14	6.62	6.72	6.92	6.74	6.96
15	6.30	6.33	6.53	6.34	6.55
16	6.19	6.08	6.28	6.05	6.32
17	6.55	6.59	6.55	6.6	6.55
18	7.48	7.31	6.86	7.09	6.68
19	5.25	4.9	5.15	4.61	5.08
20	5.67	5.8	6.03	6.04	6.5
21	5.31	5.58	5.53	6.83	5.67

Model 2 accounts for 66% variation in cytotoxic activity of thiadiazinoacridines. F–statistics of the model proves it to be statistically significant (> 99%), as the calculated $F_{(3,17)} = 11.038$ exceed tabulated value $F_{(3,17,\alpha)} = 5.38$ of 99% significance. Low standard error of

estimation (<0.4) suggests a high degree of confidence in the analysis. Moreover the descriptors used to construct the model are not correlated with each other as suggested by their correlation matrix and VIF values respectively (Table5, Table 6). However, the model manifest moderate predictive potential as indicated by cross–validated correlation coefficient value.

Bootstrapping method and leave one out method were used for the validation of the QSAR models. The observed, calculated and predicted activities shown by these methods are listed in Table 7.

The descriptor DDE in the models represents the sum of electrostatic terms resulting from the interaction of two dipoles. The descriptor bears a positive coefficient, which suggests significance of dipole–dipole interactions for the cytotoxic activity of thiadiazinoacridines. The observation possibly highlights π–π stacking interactions between the planar tricyclic aromatic rings of the acridine moiety and the nucleotide bases of the DNA.

The Van der waals energy is a thermodynamic parameter which can be defined as the sum of pair wise Vander waals interaction energy terms for atoms separated by exactly 3 chemical bonds, related to the structure of the molecule itself. The coefficient of the descriptor VDWE bears a positive sign in the model 1 which indicates that increase in the VDWE between atoms separated by 3 chemical bonds is conducive to the cytotoxic activity, which in the present case is applicable to the substituents in the 1, 3 nitrogen atoms of thiadiazinoacridine moiety. Thus it may be deduced that the orientation of atoms in the substituents (R) in the nitrogen atoms at the positions 1 and 3 influences the activity of thiadiazinoacridines significantly.

Connolly's solvent accessible area, a steric descriptor, represents the surface area that is in contact with the solvent. The descriptor bears negative coefficient in the model, suggesting increase in the bulkiness of the substituents and molecular solvent accessible surface area is not conducive to the activity. This is in accordance with the reported work of Antonini *et. al*. [9] which states that bulky substituents at the terminal nitrogen atoms decrease the cytotoxicity.

The descriptor Ovality in the second model bears a negative coefficient thereby it represent the steric hindrance associated with the bulk of the substituents. The observation only reaffirms the conclusion drawn from the descriptor CAA in the model 1.

Stretch bend Energy, a Thermodynamic parameter, deals with the stretching and bending or one can say the conformational flexibility of the molecule. The descriptor in the second model bears a positive coefficient, indicating, substituents that increase the flexibility of thiadiazinoacridines will enhance the cytotoxic activity.

4 CONCLUSIONS

QSAR analysis was performed on a series of cytotoxic thiadiazinoacridines using molecular modeling program Chemoffice2001. QSAR models were proposed for cytotoxic activity of the thiadiazinoacridines using chemSAR descriptors employing sequential multiple regression analysis method. The selected models were checked for multicollinearity and autocorrelation with VIF values and DW statistics respectively. The predictive power of each model was estimated with boot strapping r^2 method and leave–one–out cross validation method. It was observed from the selected models that biological activity of acridine derivatives are governed by thermodynamic and steric properties of the molecules. The models also provide valuable insight into the mechanism of action of these compounds. The result of the study suggests involvement of dipole–dipole interaction in the mechanism of cytotoxic action of thiadiazinoacridines and bulky substituents are undesirable due to steric hindrance. Additionally, presence of groups contributing to the flexibility of the molecule will increase cytotoxic potency of thiadiazinoacridines. The finding of the study will be helpful in the design of potent cytotoxic analogs of thiadiazinoacridines.

Acknowledgment

One of the authors, Garvita Choudhary, likes to thank All India Council for Technical Education (AICTE) for providing fellowship. The authors wish to thank the Director, Shri G.S. Institute of Technology & Sciences for providing the necessary facilities for undertaking this research work.

5 REFERENCES

[1] P. S. Callery and P. M. Gannett, Cancer and cancer chemotherapy; in: *Foye's Principles of Medicinal Chemistry* Eds D .A. Williams and T. L. Lemke, Gopsons Papers Ltd., 2002, pp. 924–951.

[2] E. Mounetou, J. Legault, J. Laccroix and C. Gaudreault, Antimitotic Antitumor Agents: Synthesis, Structure–Activity Relationships, and Biological Characterization of *N*–Aryl–*N*'– (2–chloroethyl) ureas as New Selective Alkylating Agents, *J. Med. Chem.* **2001**, *44*, 694–702

[3] D .B. Capps, J. Dunbar, S .R. Kestan, J. Shillis, and L. Werbil, 2–(Amino alkyl)–5–nitropyrazolo [3,4,5–kl] acridines, A New Class Of Anticancer Agents, *J. Med. Chem.* **1992**, *35,* 4770–4778

[4] A. A. Adeji, Current Status of Pyrazaloacridines as anticancer agents, *Invest. New Drugs*. **1999**, *17*, 43–48.

[5] Disubstituted Pyrimido [5,6,1–de]acridines, a Novel Group of Anticancer Agents Active on a Multidrug Resistant Cell Line**,** *J. Med. Chem.* **1995**, *38,* 3282–3286.

[6] I. Antonini, P. Polucci, L. Kelland, E. Menta, N. Pescalli, and S. Martelli, 2,3–Dihydro–1*H*, 7H–pyrimido[5,6,1–*de*]acridine–1,3,7–trione Derivatives, a Class of Cytotoxic Agents Active on Multidrug–Resistant Cell Lines: Synthesis, Biological Evaluation, and Structure–Activity Relationships, *J. Med. Chem*. **1999**, *42*, 2535–2541.

[7] I. Antonini, P. Polucci, A. Magnano and S. Martelli, Synthesis, Antitumor Cytotoxicity, and DNA–Binding of Novel *N*–5, 2–Di (ω–amino alkyl)–2,6–dihydropyrazolo[3,4,5–*kl*]acridine–5–carboxamides, *J. Med. Chem.* **2001,** *44***,** 3329–3333.

[8] I. Antonini, P. Polucci, A. Magnano, B. Gatto, M. Palumbo, E. Menta, N. Pescalli and S. Martelli, 2,6–Di(ω–aminoalkyl)–2,5,6,7–tetrahydropyrazolo[3,4,5–*mn*]pyrimido[5,6,1–*de*]acridine–5,7–diones: Novel, Potent, Cytotoxic, and DNA–Binding Agents**,** *J. Med. Chem.* **2002**, *45*, 696–702.

[9] I. Antonini, P. Polucci, A. Magnano, D. Cacciamani, M. T. Konieczny, J. P–Lukowicz and S. Martelli, Rational Design, Synthesis and Biological Evaluation of Thiadiazinoacridines: A New Class of Antitumour Agents, *Biorg. Med. Chem.* **2003**, *11*, 399–405.

[10] A. K. Gupta, M. A. Babu and S.G. Kaskhedikar, VALSTAT: Validation Program for Quantitative Structure Activity Relationship studies, *Indian J. Pharm. Sci*. **2004**, *66*, 396–402.

[11] *SYSTAT* **10.2 version***, supplied* by SYSTAT SOFTWARE INC.

[12] D. H. Cho, S. K. Lee, B.T. Kim, and K. T. No, Quantitative Structure–Activity Relationship (QSAR) Study of New Fluorovinyloxyacetamides, *Bull. Korean Chem. Soc*. **2001**, *22*, 388–394.

[13] T. Ivanciuc and O. Ivanciuc, Bone Resorption Inhibition Quantitative Structure–Activity Relationships for Aryl–Substituted Bisphosphonates, *Internet Electron. J. Mol. Des.* **2003**, *2*, 403–412, http://www.biochempress.com.

[14] J. Durbin and G. S. Watson, Testing for serial correlation in regression analysis I, *Biometrika.* **1950**, *37*, 409–428.

[15] J. Durbin and G. S. Watson, Testing for serial correlation in regression analysis II, *Biometrika*. **1951**, *38*, 159–178.